Nature Conservation

Nature Conservation 53: 1–16 (2023)
DOI: 10.3897/natureconservation.53.97873

Research Article

Effect of ecological restoration on topsoil phosphorus following afforestation on abandoned ponds in northern Chaohu Lake, China

Gang Li[1,2], Shengming Dong[1], Hao Wang[1], Yanmei Guan[3,4], Patrick Tyler Deja[5], Wei Nie[1]

1 *School of Architecture and Urban Planning, Anhui Jianzhu University, Hefei, Anhui, 230022, China*
2 *Everglades Wetland Research Park, The Water School, Florida Gulf Coast University, Naples, Florida, 34112, USA*
3 *School of Materials and Chemical Engineering, Anhui Jianzhu University, Hefei, Anhui, 230022, China*
4 *Anhui Key Laboratory of Advanced Building Materials, Anhui Jianzhu University, Hefei, Anhui, 230022, China*
5 *Education and Visitor Experience Department, Naples Botanical Garden, Naples, Florida, 34112, USA*
Corresponding author: Wei Nie (archway@qq.com)

Academic editor: Mathias Scholz
Received: 22 November 2022
Accepted: 7 May 2023
Published: 16 June 2023

ZooBank: https://zoobank.org/A44BE0A2-4626-4BF8-AE7F-D8E7F3B9798B

Citation: Li G, Dong S, Wang H, Guan Y, Deja PT, Nie W (2023) Effect of ecological restoration on topsoil phosphorus following afforestation on abandoned ponds in northern Chaohu Lake, China. Nature Conservation 53: 1–16. https://doi.org/10.3897/natureconservation.53.97873

Abstract

Afforestation is an approach for ecological restoration. Soil total phosphorus is one of the important ecological and evolutionary elements for carbon cycles and plant growth following afforestation. However, studies on soil total phosphorus of afforestation on abandoned ponds with different slopes are still lacking. Soil total phosphorus and other soil properties from afforestation sites with different slopes were investigated. Soil total phosphorus, total nitrogen, bulk density, soil water contents and pH of poplar (*Populus* spp.) plantation sites (Slope 1) with a steep slope and pond cypress (*Taxodium* spp.) plantation sites (Slope 2) with a flat slope were determined. Soil total nitrogen stocks, soil total phosphorus stocks and the ratio of soil total nitrogen to total phosphorus (N:P) were calculated. Results showed that soil bulk density, soil water content, total phosphorus, total phosphorus stocks and total nitrogen stocks of three soil layers at Slope 1 were significantly lower than those of Slope 2. N:P of Slope 1 was significantly higher, but no significant difference of total nitrogen and pH were found between the two sampling sites. Soil bulk density, soil water content and total nitrogen had significant positive relationships with both total phosphorus and total phosphorus stocks. No obvious correlation was found between pH and total phosphorus or total phosphorus stocks. Redundancy analysis (RDA analysis) suggested that soil water content and bulk density had the most important individual effect on total phosphorus and total phosphorus stocks with values at 59.3% and 59.5%, respectively. It is recommended that afforestation on a flat or gentle slope rather than on a steep gradient could be helpful for accumulation of soil total phosphorus and phosphorus stocks and could decrease the risk of soil phosphorus loss, when afforestation is used for ecological restoration.

Key words: ecological engineer, nitrogen and phosphorus stocks, plantation, slope, soil water content, wetlands

Introduction

Ecological restoration is defined by the Society for Ecological Restoration (SER) as the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed (Wortley et al. 2013). Theoretically, there are four principles for restoration: ecological integrity, long-term sustainability, societal benefits and engagement and informed by past and future (Suding et al. 2015). However, ecological restoration can be costly and cost-effective approaches should be considered for a better integration between economic and ecological components (Gong et al. 2012; Kimball et al. 2015). As an important ecological restoration approach, afforestation has been widely used for ecological system management and to improve soil quality. Soil total phosphorus (TP) is one of the important ecological and evolutionary elements and the supply and cycles of TP play an important role in carbon emission and uptake (Nottingham et al. 2015; Reed et al. 2015; Zhao et al. 2015) and could impact the carbon uptake and mineralisation due to being a potentially limiting nutrient for plant growth (Wang et al. 2015; Deng et al. 2017; Alewell et al. 2020; Guo et al. 2021). As afforestation with plants can have an effect on soil phosphorus and has been considered as an effective ecological restoration approach to mitigate carbon emissions and increase carbon sequestration in both the Eastern and Western world (Zhao et al. 2015; Lu et al. 2018; Yang et al. 2022), it is necessary to quantify soil TP following afforestation in order to sustain forest management and have better climate change mitigation (Karamian and Hosseini 2015; Liu et al. 2016; Deng et al. 2017).

Vegetation recovery such as afforestation has been a good practice to mitigate soil erosion risk, including soil phosphorus loss (Teng et al. 2019; Chen et al. 2021; Xu et al. 2022). Soil TP loss could result from surface runoff and lead to eutrophication of adjacent freshwater systems (He et al. 2018; He et al. 2019; McDowell et al. 2020). For example, the global soil TP loss from agricultural land reached 6.3 Tg yr^{-1} (Alewell et al. 2020) and the total loss of colloidal phosphorus accounted for 64.3% of surface runoff (He et al. 2019). Afforestation could be one of the alternative restoration approaches for bare and degraded lands (IPCC 2014) and this ecological practice has been implemented in China since the 1970s (Li et al. 2012). Previous results of soil phosphorus following afforestation varied according to different studies. According to some studies, topsoil TP tended to increase from afforestation of bare and degraded lands (Chen et al. 2016; Deng et al. 2017) and farmland (Zhang et al. 2018a). However, some other previous studies argued that TP stocks decreased in both regional and global scales following afforestation, ranging from 7% to 15% in temperate, arid and semi-arid areas, with the exception of afforestation of barren lands (Deng et al. 2017; Li et al. 2019). Additionally, another global systematic analysis reported that afforestation had no overall impacts on soil TP (Guo et al. 2021) nor altered soil phosphorus stocks (Zou et al. 2015). Unfortunately, many previous studies focused on afforestation of agricultural lands (Temesgen et al. 2016; Zhang et al. 2018a), grasslands (Chen et al. 2021), wetlands (Howson et al. 2022) and barren lands (Deng et al. 2017). Few studies on afforestation of abandoned ponds were reported, which could limit our understanding of the soil TP cycle following afforestation.

In-situ conditions, including hydrology, topography, soil and their interactions, may lead to limitations in our understanding of soil phosphorus following afforestation (MacDonald et al. 2012; Sohrt et al. 2017). For example, soil water status could impact soil TP directly or indirectly. Soil water status could directly impact soil TP as the decomposition of phosphorus declined with the increase in rainfall in tropical forests (Sun et al. 2020), while phosphorus losses in runoff could increase with the increase in soil water repellence (McDowell et al. 2020). Indirectly, it could have an impact because soil water status has an effect on slope erosion and hydrology, which could have a positive relationship with phosphorus loss (Chen et al. 2013; Keshavarzi et al. 2015). Previous studies showed that increases in soil moisture and rainfall could decrease the desorption and release of phosphorus from topsoil (Ford et al. 2018; He et al. 2018; Sun et al. 2020). Though higher water repellence (McDowell et al. 2020) could increase phosphorus loss in runoff, other studies suggested that lower precipitation and prolonged drought led to less TP depletion and decreased soil phosphorus release, due to limitation of phosphorus enzymatic activities in the forest (Li et al. 2019; Asensio et al. 2021).

Furthermore, previous studies showed that slope erosion could lead to phosphorus loss (Chen et al. 2013; Keshavarzi et al. 2015) and soil phosphorus content varied in position on the same slope, with the lower position having more phosphorus stocks and available phosphorus (Ide et al. 2007; Amiotti et al. 2013; Zou et al. 2015). However, previous simulation experiments showed that slope had an effect on soil phosphorus loss for different rainfall intensities (Wang et al. 2013; Ramos et al. 2019). For example, soil phosphorus loss of afforested land with a slope of 17% was higher for relatively low rainfall intensity (22 mm h^{-1}), compared to a natural forest with a slope of 19% (Ramos et al. 2019). Much of the soil phosphorus loss was found at the lower slope treatment for heavier rainfall intensity (65 mm h^{-1}). Overall, the complexity of *in-situ* conditions impacting soil TP following afforestation could be greater than our expectations and further study should be given to this. Unfortunately, studies on soil phosphorus and afforestation looking at different *in-situ* conditions, such as different soil water properties and slopes, is still lacking. As a global scale study could be limited for management guidance (Li et al. 2019), the *in-situ* studies on soil phosphorus following afforestation could be necessary for sustainable soil management.

In this study, soil TP and other soil properties (i.e. bulk density, pH, soil water content and total nitrogen) were carried out following afforestation of abandoned ponds. The hypothesis is that micro-topography of different slopes could have an effect on soil TP. The aims are: (1) to investigate the distribution of soil TP in the top 30 cm soil layer with different slopes following afforestation; (2) to estimate the effects of soil properties of different slopes on soil TP following afforestation; and (3) to provide a reference for ecological restoration by afforestation on abandoned lands.

Methods

Study sites

Experiments were carried out in Hefei, Anhui, China from late September to early October 2021. The study area is located in the northern Chaohu Lake area (117.36°E–117.43°E; 31.70°N–31.40°N, Fig. 1), where the afforestation of abandoned ponds by poplar (*Populus* spp.) and pond cypress (*Taxodium distichum* and *Taxodium distichum* var. *imbricatum*) has been undertaken. The soil type is yellow-brown and this area belongs to the northern subtropical monsoon climate with a mean annual temperature of 15.7 °C and mean annual precipitation of 995.4 mm (Huai-Jing 2018). At the poplar site (Slope 1), there are a lot of ditches with a very steep slope (Fig. 1). Poplar trees were planted on the top of the ditches in 2002 with a stand density of about 1066 ha^{-1}. At the pond cypress site (Slope 2), there are no ditches and a flat slope. Pond cypresses were planted on flat ground in 2018 with

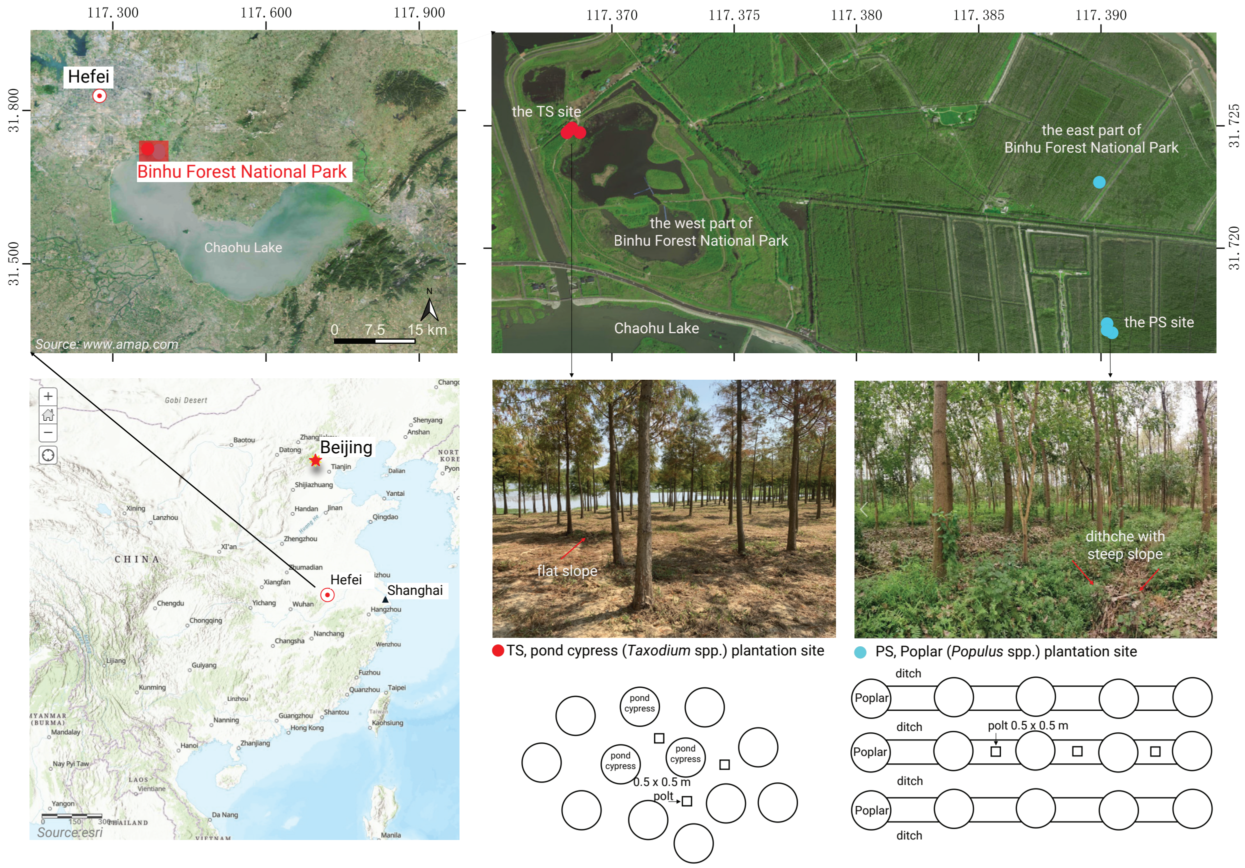


Figure 1. Location of the study area and sampling sites.

a stand density of about 2250 ha^{-1}. These two tree species both belong to FACW (facultative wetland; species are the most reliable vegetation indicators of wetland and many wetlands are characterised by these species), according to the Wetland Indicator Standards (Tiner 1988, 1993). More details are listed in Table 1.

Soil sampling and analysis

For both Slope 1 and Slope 2, there were four sampling sites with three plots as replications. The sampling sites were set randomly and a plot of 0.5 × 0.5 m was set in the area between two adjacent poplar trees or pond cypress trees. Three soil layers (0–10, 10–20 and 20–30 cm) were collected from each plot for analysis of soil physical and chemical properties of each soil layer, in line with previous studies: soil bulk density (BD) was collected by a cutting ring (100 cm^3); total phosphorus (TP), total nitrogen (TN), soil water content (SWC) and soil pH were collected from three soil layers using a corer (Li et al. 2018). Bulk density was measured by oven-dried soil mass (Yang et al. 2007); soil water content was mea-

Table 1. General situation of the sampling sites.

Sites	Mico-topography	Stand density ha^{-1}	Age (yr)	Dominant tree species	Dominant landcover species
Poplar	an obvious and steep slope with ditch	1066	19	*Populus* spp.	*Cyclosorus interruptus*, *Solidago decurrens*, and *Liriope spicata*
Pond cypress	a flat gradient with non-ditch	2250	4	*Taxodium* spp.	*Erigeron annuus*, *Lindernia crustacea*, and *Stellaria media*

sured gravimetrically at 105 °C for 24 h (Shang et al. 2013); pH was determined by a pH analyser (PHS-3C PH METER, Shanghai Puchun Measure Instrument Co., Ltd.). TP was determined by the Alkali Fusion-Mo-Sb Anti-spectrophotometric Method (China 2011.12.16) and total nitrogen was measured by the Kjeldahl Method (China 2015.01.01), according to the Chinese National Standards.

Statistical statistics and analyses

Soil total phosphorus stock (TPS) and total nitrogen stock (TNS) of each soil layer and the average TPS or TNS of three soil layers were calculated as follows (Li et al. 2018):

$$Stock_{ij} = Content_{ij} \times BD_{ij} \times D \times 100 \tag{1}$$

where, *Stock* is the soil total phosphorus stock (kg ha^{-1}) or total nitrogen stock (kg ha^{-1}) of each soil layer; *Content* is the soil TP content (g kg^{-1}) or total nitrogen content (g kg^{-1}) and BD is the soil bulk density (g cm^{-3}) of each soil layer; D is the soil sampling depth (m); *i* = TP or total nitrogen; *j* = 0–10, 10–20 or 20–30 cm of soil layer.

Data statistics

Differences of soil property contents between the two sampling sites (Slope1 and Slope 2) and three soil layers were tested by the Kruskal-Wallis Test and an independent Samples Wilcoxon Test (Wezel et al. 2000) with ‘*ggplog2*’ package, respectively (Fig. 2). Regression between TP or total phosphorus stocks and other soil properties, including bulk density, pH, soil water content and total nitrogen were analysed by a Linear Regression Model (GLM, Fig. 3) with ‘*ggplog2*’ package (Li et al. 2018). The hierarchical partitioning method was used to determine the contribution of each soil property to soil organic carbon with the Redundancy analysis (RDA method, Table 2 and Suppl. material 1) with ‘*rdacca.hp*’ package (Lai et al. 2022). Only $p < 0.05$ was considered statistically significant. Figures and tables were created by R (ver. 4.1.2) language and QGIS (ver. 3.22).

Results

Soil physical and chemical properties

For the same sampling site, only bulk density was found to have a significant difference amongst the three soil layers at both Slope 1 and Slope 2 and bulk density increased significantly with increases in depth ($p < 0.05$, Fig. 2). For the two different sampling sites, bulk density, soil water content and TP of the three soil layers at Slope 1 were significantly lower than those of Slope 2 where there was a flat slope ($p < 0.01$, Fig. 2). For example, the average bulk density at Slope 1 was 1.104 ± 0.040 g cm^{-3} (Mean ± S.E.), which was 78.5% of the value of Slope 2. Similarly, the average soil water content at Slope 1 was 47.4% less than that of Slope 2 with a value of 22.521 ± 0.892%. Additionally, the average TP of Slope 1 was 0.177 ± 0.016 g kg^{-1} with each of the three soil layers measuring 0.190 ± 0.028, 0.158 ± 0.018 and 0.182 ± 0.035 g kg^{-1}, respectively. The average TP of

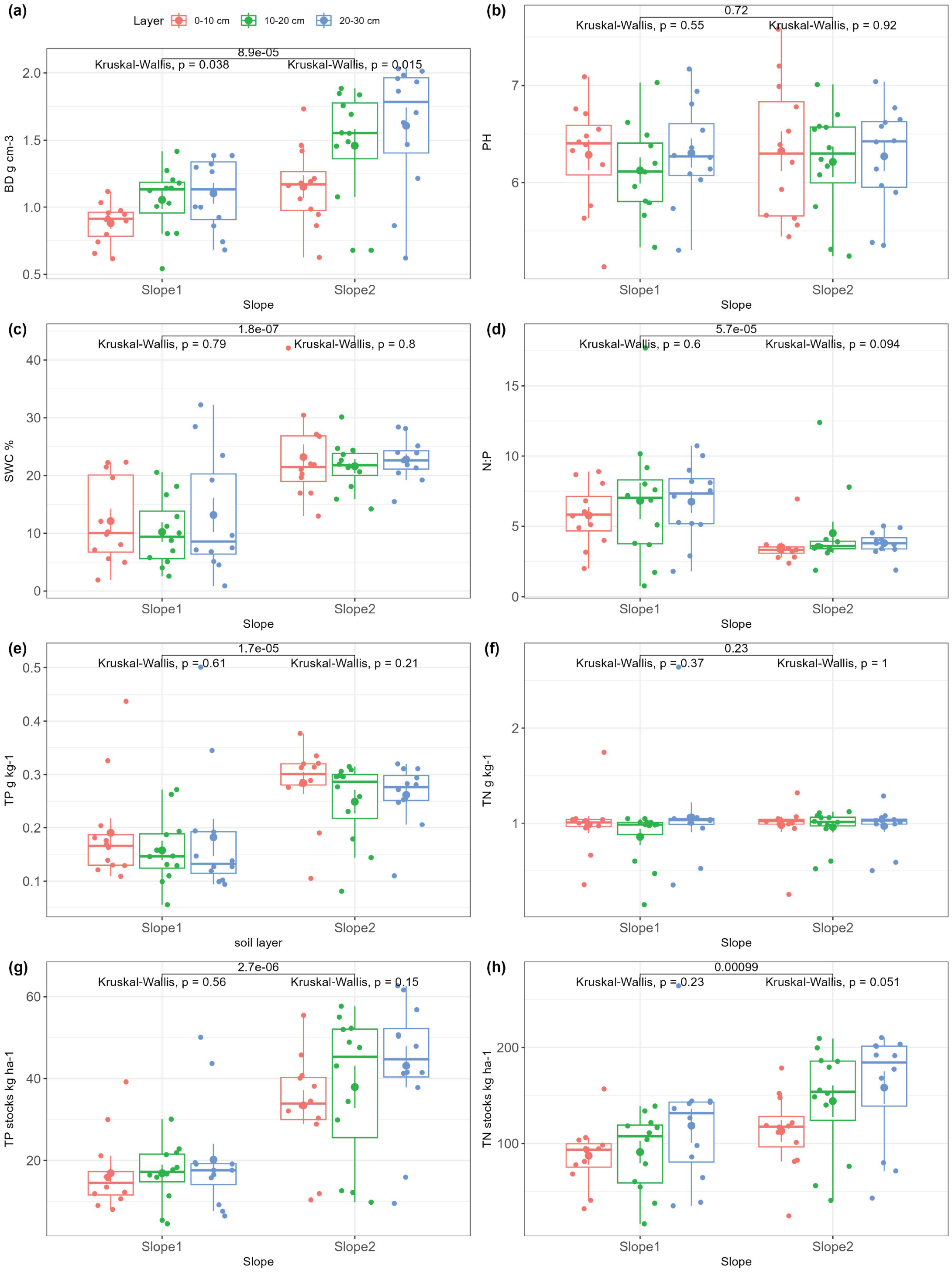


Figure 2. Soil properties of three soil sampling layers. Notes: BD, bulk density; pH, soil pH value; SWC, soil water content; N:P, soil nitrogen to phosphorus ratio; TP, soil total phosphorus; TPS, soil phosphorus stocks; TN, soil total nitrogen; TNS, soil nitrogen stocks; a solid line of each box is the Median value; a solid dot on the bar is the Mean value; the value on top of a solid line at the top of each graph refers to significance between Slope 1 and Slope 2 by Wilcoxon Test; Kruskal-Wallis refers to significance amongst three soil layers at the same Slope site; *n* = 12 for each soil layer and *n* = 72 for two sampling sites.

Slope 2 was 0.265 ± 0.011 g kg^{-1} with each of the three soil layers measuring 0.284 ± 0.021, 0.250 ± 0.022 and 0.262 ± 0.017 g kg^{-1}, respectively. However, N:P of each soil layer at Slope 1 (5.779 ± 0.612, 6.817 ± 1.30 and 6.755 ± 0.792 g kg^{-1}) was higher than that of Slope 2 (3.520 ± 0.330, 4.517 ± 0.819 and 3.810 ±

Table 2. Results of RDA analysis of both soil TP and TP stocks in this study.

Variables	TP model					TPS model				
	SWC %	TN g kg^{-1}	BD g cm^{-3}	pH	Total	SWC %	TN g kg^{-1}	BD g cm^{-3}	pH	Total
VIF [a]	1.550	1.023	1.567	1.609	—	1.550	1.023	1.567	1.609	—
Unique	0.253	0.133	-0.004	0.007	0.389	0.096	0.037	0.250	0.004	0.388
Average.shared [b]	0.063	0.017	0.064	-0.001	0.144	0.124	0.016	0.213	0.038	0.392
Individual importance	0.316	0.150	0.060	0.007	0.533	0.220	0.053	0.463	0.043	0.780
I.perc (%)[c]	59.340	28.110	11.310	1.220	100	28.250	6.850	59.470	5.480	100
p-values [d]	0.001	0.001	0.001	0.529	—	0.001	0.001	0.001	0.002	—
F	37.820	27.496	19.298	0.399	—	30.558	29.209	183.667	11.607	—
	Df	Variance	F	Pr (>F)		Df	Variance	F	Pr(>F)	
Model	4	0.005	21.253	0.001		4	219.979	63.760	0.001	
Residual	67	0.004				67	57.789			

Note: TP model, total phosphorus predictive model; TPS model, total phosphorus stocks predictive model; VIF, values of variables used to develop predictive model; TN, soil total nitrogen; BD, bulk density; pH, soil pH value; SWC, soil water content; sampling sites with *a*, variance inflation factor (VIF); *b*, total average shared effects with other predictors; *c*, Individual effect divided by total adjusted R^2 found in column Individual importance; *d*, *p*-values based on permutation test based on 999 randomizations; *n* = 72; Pr (> F), probability of obtaining a value larger than the F-test value.

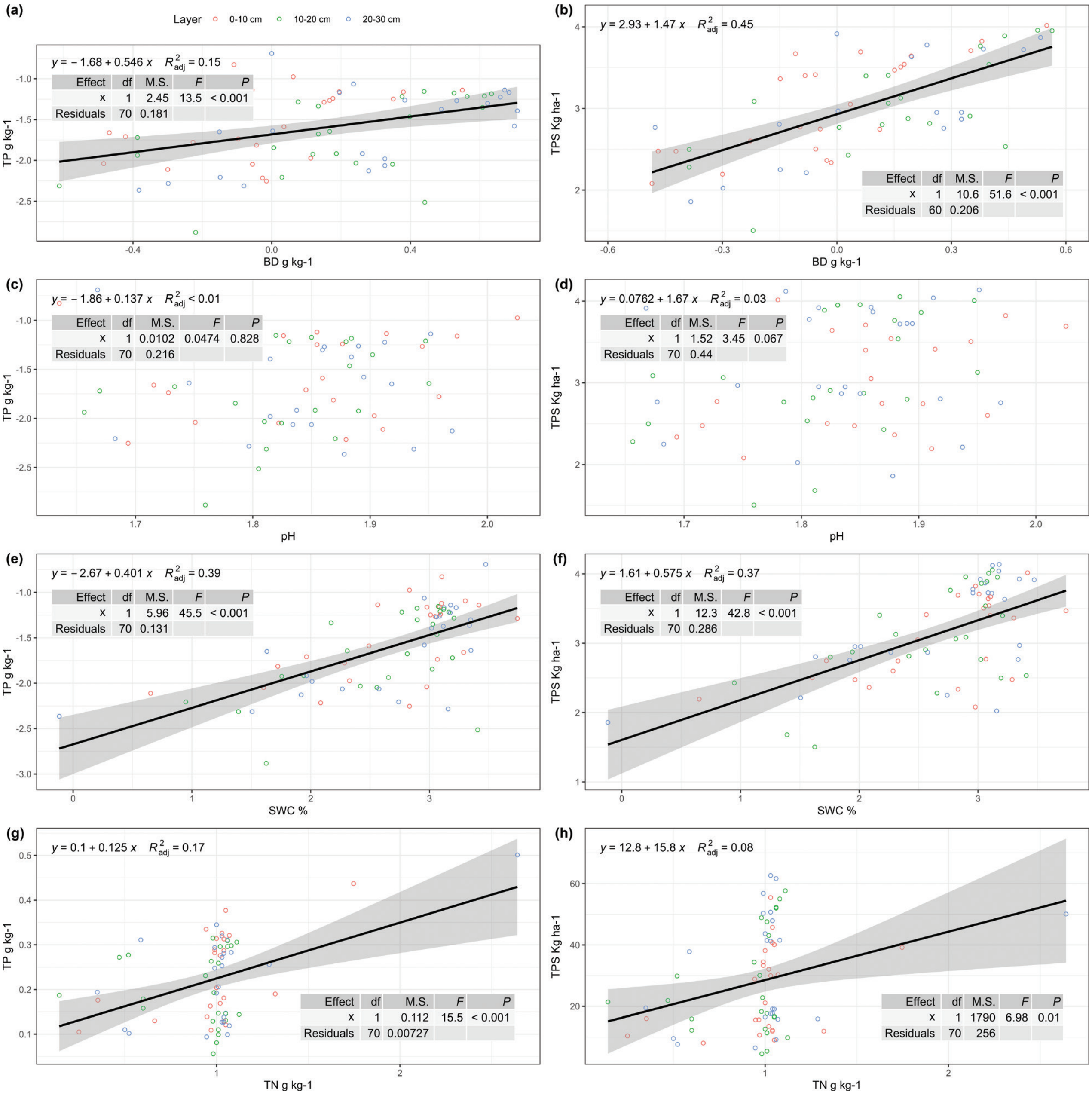
0.242 g kg^{-1}). No significant differences of pH and total nitrogen were found between Slope 1 and Slope 2. The average pHs of the three soil layers were 6.286 ± 0.157, 6.125 ± 0.134 and 6.304 ± 0.150 for Slope 1, compared to 6.325 ± 0.204, 6.213 ± 0.157 and 6.271 ± 0.154 of Slope 2. The averages of total nitrogen for the three soil layers were 0.986 ± 0.091, 0.855 ± 0.0840 and 1.062 ± 0.158 g kg^{-1} for Slope 1 and 0.979 ± 0.071, 0.959 ± 0.056 and 0.970 ± 0.062 g kg^{-1} for Slope 2.

Soil total phosphorus and total nitrogen stocks

The averages of total phosphorus stocks and total nitrogen stocks of Slope 1 were both significantly lower than those of Slope 2 ($p < 0.01$, Fig. 2). For Slope 1, the average total phosphorus stock was 17.973 ± 1.681 kg ha^{-1} and the values for the three soil layers were 16.883 ± 2.656, 16.868 ± 2.088 and 20.170 ± 3.860 kg ha^{-1}. For Slope 2, the average total phosphorus stock was 38.183 ± 2.644 kg ha^{-1} and the values for the three soil layers were 33.466 ± 3.680, 37.949 ± 5.151 and 43.134 ± 4.732 kg ha^{-1}, respectively. Additionally, the average total nitrogen stock at Slope 1 was 98.917 ± 7.863 kg ha^{-1} (87.223 ± 9.290, 91.040 ± 11.625 and 118.489 ± 17.773 kg ha^{-1} for each of the three soil layers, respectively), compared to 138.382 ± 54.328 kg ha^{-1} at Slope 2 (112.905 ± 11.363, 144.085 ± 16.354 and 158.156 ± 16.986 kg ha^{-1} for each of the three soil layers, respectively). There were no significant differences for both total phosphorus stocks and total nitrogen stocks amongst the three soil layers at the same sampling site.

Regression analysis

Bulk density, soil water content and total nitrogen had significant positive relationships with both TP and total phosphorus stocks ($p < 0.01$, Fig. 3). For example, TP and total phosphorus stocks both increased sharply with the increase of



(a) $y = -1.68 + 0.546\,x \quad R^2_{adj} = 0.15$

Effect	df	M.S.	F	P
x	1	2.45	13.5	< 0.001
Residuals	70	0.181		

(b) $y = 2.93 + 1.47\,x \quad R^2_{adj} = 0.45$

Effect	df	M.S.	F	P
x	1	10.6	51.6	< 0.001
Residuals	60	0.206		

(c) $y = -1.86 + 0.137\,x \quad R^2_{adj} < 0.01$

Effect	df	M.S.	F	P
x	1	0.0102	0.0474	0.828
Residuals	70	0.216		

(d) $y = 0.0762 + 1.67\,x \quad R^2_{adj} = 0.03$

Effect	df	M.S.	F	P
x	1	1.52	3.45	0.067
Residuals	70	0.44		

(e) $y = -2.67 + 0.401\,x \quad R^2_{adj} = 0.39$

Effect	df	M.S.	F	P
x	1	5.96	45.5	< 0.001
Residuals	70	0.131		

(f) $y = 1.61 + 0.575\,x \quad R^2_{adj} = 0.37$

Effect	df	M.S.	F	P
x	1	12.3	42.8	< 0.001
Residuals	70	0.286		

(g) $y = 0.1 + 0.125\,x \quad R^2_{adj} = 0.17$

Effect	df	M.S.	F	P
x	1	0.112	15.5	< 0.001
Residuals	70	0.00727		

(h) $y = 12.8 + 15.8\,x \quad R^2_{adj} = 0.08$

Effect	df	M.S.	F	P
x	1	1790	6.98	0.01
Residuals	70	256		

Figure 3. Regression analysis between soil total phosphorus and other soil properties. Note: BD, bulk density; pH, soil pH value; SWC, soil water content; TN, soil total nitrogen; TP, soil total phosphorus; TPS, soil phosphorus stocks; *n* = 72. Values were converted to logarithmic values for (a), (b), (c), (d), (e) and (f).

soil water contents with the value of R^2_{adj} at 0.39 and 0.37, respectively. Similarly, total phosphorus stocks had a significant correlation with bulk density with 0.45 of the R^2_{adj} compared to the R^2_{adj} value 0.15 for TP. However, no obvious correlation was found between pH and TP or total phosphorus stocks.

Results of RDA analysis of soil TP

The variance inflation factor (VIF) of all soil parameters showed in Table 2 were less than 2, indicating a very low possibility of multicollinearity amongst soil water content, total nitrogen contents, bulk density and pH. For soil TP, soil water content had the highest individual importance (59.3% of the total R^2,

Table 2). The total unique contribution of predictor was 53.3% (38.9% + 14.4% = 53.3%). The order from high to low was soil water content (25.3%), total nitrogen (13.3%), pH (0.7%) and bulk density (-0.4%). The F value of the TP predictive model was 21.25 ($p < 0.001$). For total phosphorus stocks, bulk density had the highest individual importance (59.5% of the total R^2), followed by soil water content at 28.3%, total nitrogen at 6.9% and pH at 5.5% (Table 2). The total unique contribution of predictor was relatively high (78.0%, 38.8% + 39.2% = 78.0%). In order from high to low was bulk density (25.0%), soil water content (9.6%), total nitrogen (3.7%) and pH (0.4%). The F value of the total phosphorus stocks predictive model was 63.76 ($p < 0.001$).

Discussion

In this study, both soil TP and total phosphorus stocks of the two sampling sites were significantly different with the value of Slope 2 much higher than that of Slope 1 where the slope was higher. One possible reason could be attributed to different soil water content, as soil water content had obvious relationships with soil TP and total phosphorus stocks in this study. This was similar to previous studies on grassland where soil water content had a significant and positive relationship with soil TP (He et al. 2018) and strongly impacted the soil phosphorus pool in the sandy acid sulphate soil (Mayakaduwage et al. 2021). What's more, this was consistent with the RDA analysis that soil water content had the highest individual effect on soil TP compared to other soil properties. Another possible reason could be bulk density, which is an index of soil compaction, as soil bulk density had significant correlation with both soil TP and total phosphorus stocks. This was consistent with a previous study on continental monsoon climate grassland ecosystems (He et al. 2018) and our RDA analysis that bulk density had the highest individual effect on soil total phosphorus stocks followed by soil water content.

The deep soil was excavated to create ditches with a two-sided steep slope before afforestation on the abandoned ponds at Slope 1, where TP and total phosphorus stocks were both lower. This indicated that the higher slope could cause low soil phosphorus, which was consistent with previous studies in the forest system (Karamian and Hosseini 2015; Kumar et al. 2021), agriculture system (He et al. 2019; He et al. 2020b) and simulation experiments in rocky slope protection (Chen et al. 2013). One possible reason could be that it is much harder for water to absorb into a steep slope as soil phosphorus loss increased with increases in slope gradients (He et al. 2020a; He et al. 2020b; Hou et al. 2022). The higher slope increased soil erosion risk and soil phosphorus losses with surface runoff, leading to less soil phosphorus accumulation (Zhang et al. 2018b; Xu et al. 2022). Furthermore, the bulk density of Slope 1 was much lower, which could be attributed to less soil water content. Soil could be less compact under dry rather than moist conditions. Hence, the lower soil water content and bulk density of Slope 1 could be a result from a steeper slope, leading to less TP and total phosphorus stocks accumulation.

Compared to similar studies, TP of Slope 1 was lower than that of West Donting Lake and Songnen Plain study sites where there were ditches with steep slopes (Suppl. material 2). The plantation of poplar in this study was larger than that in Donting Lake, indicating that soil TP might decrease with the increasing of plantation age at a steep slope site. However, data in this field are still lacking. It is suggested that future studies are needed on soil TP afforestation on different slopes.

A previous study suggested that afforestation enhanced soil TP accumulation by vegetation restoration (Zhao et al. 2015) and the authors agree with this argument. However, our result highlighted that soil water content could be another vital factor of afforestation and high slope gradients may offset the effect of afforestation/vegetation restoration on soil TP accumulation. Additionally, N:P at Slope 1 was much higher than that of Slope 2, indicating there could be a phosphorus limitation that hinders tree growth because phosphorus is one of a necessary elements for plants (Elser 2012). Hence, phosphorus limitation could have a negative effect on TP accumulation by vegetation restoration. It is recommended that afforestation on flat rather than steep-sloped lands and avoiding drainage could help to decrease a potential risk of terrestrial soil phosphorus loss and eutrophication from afforestation systems to other ecosystems.

Overall, our results confirmed that low soil water content and steep slopes could be attributed to the low soil TP and may have contributed to the higher soil phosphorus loss risk at Slope 1. Hence, it could be necessary to improve soil water properties by afforesting on flat gradient land or decreasing the steepness of the slope before afforestation, which could be an approach to decrease soil phosphorus loss risks and should be suggested for afforestation on lands similar to Slope 1.

There was no significant difference of soil total nitrogen between the two sites. However, our results confirmed that soil total nitrogen could affect phosphorus cycling, as an obviously positive relationship was found between soil total nitrogen and TP in the present study, which was similar with previous studies on a forest ecosystem where available phosphorus was positively correlated with soil total nitrogen (Lemanowicz 2018) and on warm-humid subtropical monsoon vegetation communities that soil TP on the surface of stone significantly increased with total nitrogen increasing (Wu et al. 2022). Additionally, our results showed that soil total phosphorus stocks increased with soil total nitrogen increases. This was consistent with previous studies where soil phosphorus stocks increased noticeably with soil nitrogen stock increases amongst both different plantation ages up to 50 years (Zhang et al. 2018a; Smal et al. 2019) and soil depth down to 100 cm following afforestation (Zhao et al. 2015). This indicated that soil phosphorus could increase if nitrogen addition was carried out. However, this could lead to a potential soil phosphorus loss risk like at Slope 1, as the higher slope and lower soil water content mentioned above. Hence, it might not be good practice to carry out nitrogen addition at Slope 1 where there was a steep slope. Additionally, it is suggested that any nitrogen addition for the forest management should assess the soil phosphorus loss risk, especially for lands with steep slopes.

Conclusions

Soil bulk density, soil water content, TP, N:P, total nitrogen stocks and total phosphorus stocks all showed significant differences between the two micro-topography sampling sites with different slopes. Higher soil bulk density, soil water content and TP were found at Slope 2 with the flat slope gradient. Both soil TP and total phosphorus stocks strongly correlated with soil bulk density, soil water content and total nitrogen. Soil water content and bulk density had the most important individual effect values at 59.3% for soil TP and 59.4% for total phosphorus stocks, respectively. It is recommended that afforestation on a flat or gentle slope rather than on steep gradient land could be helpful for soil TP or total phosphorus stocks accumulation and decrease soil phosphorus loss risk, when afforestation is used for ecological restoration.

Acknowledgements

We appreciate Dr. Li Zhang's comments and suggestions, who is an assistant Director of Everglades Wetland Research Park, The Water School, Florida Gulf Coast University; Mr. Renhai Wei and Mr. Daxing Ma who both work in Binhu National Forest Park, Binhu Impression Tourism Investment Development Corporation, Ltd. Hefei, Anhui, China; Mr. Xiang Ma and Mr. Xin Ye who both work in Qing Ya Landscape Architecture Design., Ltd, Hefei, Anhui, China and Mr. Kunshan Li for coordinating the filed works. Additionally, we also thank M.D. Minzhou, Dr. Jun Yang, Dr. Weihua Li, Ms. Yuan Wang and Mr. Yunfa Wu for coordinating the samples processing. This work was supported by the University Admission of Talents (Ph.D.) Scientific Research Start-up Fund of Anhui Jianzhu University (Nos. 2019QDZ58), the Outstanding Talent Nurturing Grant for Universities of the Department of Education of Anhui Province (Nos. gxyq2021195) and China Scholarship Council (Nos. CSC NO. 202008340054).

Additional information

Conflict of interest

No conflict of interest was declared.

Ethical statement

No ethical statement was reported.

Funding

The University Admission of Talents (Ph.D.) Scientific Research Start-up Fund of Anhui Jianzhu University (Nos. 2019QDZ58); the Outstanding Talent NurturingGrant for Universities of the Department of Education of Anhui Province (Nos.gxyq2021195); and the China Scholarship Council (Nos. CSC NO. 202008340054).

Author contributions

Gang Li: Conceptualisation, Methodology, Investigation, Data curation, Visualisation, Writing-Original draft preparation, Revision; Project administration, Funding acquisition. Shengming Dong: samples. Hao Wang: samples. Yanmei Guan: Samples. Patrick Tyler Deja: Editing and polishing. Wei Nie: Supervisor.

Author ORCIDs

Gang Li https://orcid.org/0000-0001-9097-4105
Shengming Dong https://orcid.org/0000-0002-7371-5790
Hao Wang https://orcid.org/0009-0007-5878-1312
Yanmei Guan https://orcid.org/0000-0001-5436-3307
Patrick Tyler Deja https://orcid.org/0009-0007-4482-5521
Wei Nie https://orcid.org/0009-0002-5130-7945

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

References

Alewell C, Ringeval B, Ballabio C, Robinson DA, Panagos P, Borrelli P (2020) Global phosphorus shortage will be aggravated by soil erosion. Nature Communications 11(1): 4546. https://doi.org/10.1038/s41467-020-18326-7

Amiotti NM, Zalba P, Ares A, Rossi JM (2013) Coniferous afforestation increases soil carbon in maritime sand dunes. Archives of Agronomy and Soil Science 59(2): 289–304. https://doi.org/10.1080/03650340.2011.627326

Asensio D, Zuccarini P, Ogaya R, Marañón-Jiménez S, Sardans J, Peñuelas J (2021) Simulated climate change and seasonal drought increase carbon and phosphorus demand in Mediterranean forest soils. Soil Biology & Biochemistry 163: 108424. https://doi.org/10.1016/j.soilbio.2021.108424

Chen Z, Yang L, Jiang Z, Li C, Hu X, Pang L, Li S, Sun H (2013) Runoff-driven nitrogen and phosphorus dynamics of substrate material for rocky slope eco-engineering. Ecological Engineering 51: 123–132. https://doi.org/10.1016/j.ecoleng.2012.12.074

Chen L-F, He Z-B, Zhu X, Du J, Yang J-J, Li J (2016) Impacts of afforestation on plant diversity, soil properties, and soil organic carbon storage in a semi-arid grassland of northwestern China. Catena 147: 300–307. https://doi.org/10.1016/j.catena.2016.07.009

Chen X, Condron LM, Dunfield KE, Wakelin SA, Chen L (2021) Impact of grassland afforestation with contrasting tree species on soil phosphorus fractions and alkaline phosphatase gene communities. Soil Biology & Biochemistry 159: 108274. https://doi.org/10.1016/j.soilbio.2021.108274

China (2011) Soil. Determination of Total Phosphorus by alkali fusion. Mo-Sb Anti spectrophotometric method. Ministry of Ecology and Environmental of the Peoples's Republic of China (Chines National Standard), Beijing, China, HJ 632-2011: 10P, A14.

China (2015) Soil quality — Determination of total nitrogen — Modified Kjeldahl method. Ministry of Ecology and Environmental of the Peoples's Republic of China (Chines National Standard), Beijing, China.

Deng Q, McMahon DE, Xiang Y, Yu C-L, Jackson RB, Hui D (2017) A global meta-analysis of soil phosphorus dynamics after afforestation. The New Phytologist 213(1): 181–192. https://doi.org/10.1111/nph.14119

Elser JJ (2012) Phosphorus: A limiting nutrient for humanity? Current Opinion in Biotechnology 23(6): 833–838. https://doi.org/10.1016/j.copbio.2012.03.001

Ford W, Williams MR, Young MB, King KW, Fischer E (2018) Assessing intra-event phosphorus dynamics in drainage water using phosphate stable oxygen isotopes. Transactions of the ASABE 61(4): 1379–1392. https://doi.org/10.13031/trans.12804

Gong C, Xu C, Chen L, Cao S (2012) Cost-effective compensation payments: A model based on buying green cover to sustain ecological restoration. Forest Policy and Economics 14(1): 143–147. https://doi.org/10.1016/j.forpol.2011.08.007

Guo Y, Abdalla M, Espenberg M, Hastings A, Hallett P, Smith P (2021) A systematic analysis and review of the impacts of afforestation on soil quality indicators as modified by climate zone, forest type and age. The Science of the Total Environment 757: 143824. https://doi.org/10.1016/j.scitotenv.2020.143824

He J, Su D, Lv S, Diao Z, Bu H, Wo Q (2018) Analysis of factors controlling soil phosphorus loss with surface runoff in Huihe National Nature Reserve by principal component and path analysis methods. Environmental Science and Pollution Research International 25(3): 2320–2330. https://doi.org/10.1007/s11356-017-0570-5

He X, Zheng Z, Li T, He S, Zhang X, Wang Y, Huang H, Yu H, Liu T, Lin C (2019) Transport of colloidal phosphorus in runoff and sediment on sloping farmland in the purple soil area of south-western China. Environmental Science and Pollution Research International 26(23): 24088–24098. https://doi.org/10.1007/s11356-019-05735-5

He J, Diao Z, Zheng Z, Su D, Lyu S (2020a) Laboratory investigation of phosphorus loss with snowmelt and rainfall runoff from a Steppe wetland catchment. Chemosphere 241: 125137. https://doi.org/10.1016/j.chemosphere.2019.125137

He X, Zheng Z, Li T, He S (2020b) Effect of slope gradient on phosphorus loss from a sloping land of purple soil under simulated rainfall. Polish Journal of Environmental Studies 29(2): 1637–1647. https://doi.org/10.15244/pjoes/109722

Hou G, Zheng J, Cui X, He F, Zhang Y, Wang Y, Li X, Fan C, Tan B (2022) Suitable coverage and slope guided by soil and water conservation can prevent non-point source pollution diffusion: A case study of grassland. Ecotoxicology and Environmental Safety 241: 113804. https://doi.org/10.1016/j.ecoenv.2022.113804

Howson TR, Chapman PJ, Holden J, Shah N, Anderson R (2022) A comparison of peat properties in intact, afforested and restored raised and blanket bogs. Soil Use and Management 39(1): 104–121. https://doi.org/10.1111/sum.12826

Huai-Jing R (2018) A Preliminary Study on Improvement of Vegetation Landscape Quality in Hefei Binhu Lake National Forest Park. Journal of Hefei University.

Ide J-i, Nagafuchi O, Chiwa M, Kume A, Otsuki K, Ogawa S (2007) Effects of discharge level on the load of dissolved and particulate components of stream nitrogen and phosphorus from a small afforested watershed of Japanese cypress (*Chamaecyparis obtusa*). Journal of Forest Research 12(1): 45–56. https://doi.org/10.1007/s10310-006-0250-7

IPCC (2014) Climatic Change: 2013. [The physical science basis: Working Group I contribution to the Fifth assessment report of the Intergovernmental Panel on Climate Change, Cambridge university press]

Karamian M, Hosseini V (2015) Effect of position and slope aspect on organic carbon, total nitrogen and available phosphorus in forest soils (case study: The forest of Ilam province, Dalab). Journal of Science and Technology of Agriculture and Natural Resources 19(71): 109–117. https://doi.org/10.18869/acadpub.jstnar.19.71.109

Keshavarzi A, Sarmadian F, Omran E-SE, Iqbal M (2015) A neural network model for estimating soil phosphorus using terrain analysis. The Egyptian Journal of Remote Sensing and Space Sciences 18(2): 127–135. https://doi.org/10.1016/j.ejrs.2015.06.004

Kimball S, Lulow M, Sorenson Q, Balazs K, Fang Y-C, Davis SJ, O'Connell M, Huxman TE (2015) Cost-effective ecological restoration. Restoration Ecology 23(6): 800–810. https://doi.org/10.1111/rec.12261

Kumar R, Bhardwaj AK, Rao BK, Vishwakarma AK, Kakade V, Dinesh D, Singh G, Kumar G, Pande VC, Bhatnagar PR, Bagdi GL (2021) Soil loss hinders the restoration potential of tree plantations on highly eroded ravine slopes. Journal of Soils and Sediments 21(2): 1232–1242. https://doi.org/10.1007/s11368-020-02833-7

Lai J, Zou Y, Zhang J, Peres-Neto PR (2022) Generalizing hierarchical and variation partitioning in multiple regression and canonical analyses using the rdacca.hp R package. Methods in Ecology and Evolution 13(4): 782–788. https://doi.org/10.1111/2041-210X.13800

Lemanowicz J (2018) Dynamics of phosphorus content and the activity of phosphatase in forest soil in the sustained nitrogen compounds emissions zone. Environmental Science and Pollution Research International 25(33): 33773–33782. https://doi.org/10.1007/s11356-018-3348-5

Li M, Liu A, Zou C, Xu W, Shimizu H, Wang K-y (2012) An overview of the "Three-North" Shelterbelt project in China. Forestry Studies in China 14(1): 70–79. https://doi.org/10.1007/s11632-012-0108-3

Li G, Zhang Z, Shi L, Zhou Y, Yang M, Cao J, Wu S, Lei G (2018) Effects of Different Grazing Intensities on Soil C, N, and P in an Alpine Meadow on the Qinghai—Tibetan Plateau, China. International Journal of Environmental Research and Public Health 15(11): 2584. https://doi.org/10.3390/ijerph15112584

Li X, Li Y, Peng S, Chen Y, Cao Y (2019) Changes in soil phosphorus and its influencing factors following afforestation in Northern China. Land Degradation & Development 30(14): 1655–1666. https://doi.org/10.1002/ldr.3345

Liu N, Wang Y, Wang Y, Zhao Z, Zhao Y (2016) Tree species composition rather than biodiversity impacts forest soil organic carbon of Three Gorges, southwestern China. Nature Conservation 14: 7–24. https://doi.org/10.3897/natureconservation.14.6486

Lu F, Hu H, Sun W, Zhu J, Liu G, Zhou W, Zhang Q, Shi P, Liu X, Wu X, Zhang L, Wei X, Dai L, Zhang K, Sun Y, Xue S, Zhang W, Xiong D, Deng L, Liu B, Zhou L, Zhang C, Zheng X, Cao J, Huang Y, He N, Zhou G, Bai Y, Xie Z, Tang Z, Wu B, Fang J, Liu G, Yu G (2018) Effects of national ecological restoration projects on carbon sequestration in China from 2001 to 2010. Proceedings of the National Academy of Sciences of the United States of America 115(16): 4039–4044. https://doi.org/10.1073/pnas.1700294115

MacDonald GK, Bennett EM, Taranu ZE (2012) The influence of time, soil characteristics, and land-use history on soil phosphorus legacies: A global meta-analysis. Global Change Biology 18(6): 1904–1917. https://doi.org/10.1111/j.1365-2486.2012.02653.x

Mayakaduwage S, Mosley LM, Marschner P (2021) Phosphorus pools in acid sulfate soil are influenced by soil water content and form in which P is added. Geoderma 381: 114692. https://doi.org/10.1016/j.geoderma.2020.114692

McDowell RW, Catto W, McDowell NLS (2020) The mitigation of phosphorus losses from a water-repellent soil used for grazed dairy farming. Geoderma 362: 114125. https://doi.org/10.1016/j.geoderma.2019.114125

Nottingham AT, Turner BL, Stott AW, Tanner EVJ (2015) Nitrogen and phosphorus constrain labile and stable carbon turnover in lowland tropical forest soils. Soil Biology & Biochemistry 80: 26–33. https://doi.org/10.1016/j.soilbio.2014.09.012

Ramos MC, Lizaga I, Gaspar L, Quijano L, Navas A (2019) Effects of rainfall intensity and slope on sediment, nitrogen and phosphorous losses in soils with different use and soil hydrological properties. Agricultural Water Management 226: 105789. https://doi.org/10.1016/j.agwat.2019.105789

Reed SC, Yang X, Thornton PE (2015) Incorporating phosphorus cycling into global modeling efforts: A worthwhile, tractable endeavor. The New Phytologist 208(2): 324–329. https://doi.org/10.1111/nph.13521

Shang ZH, Feng QS, Wu GL, Ren GH, Long RJ (2013) Grasslandification has significant impacts on soil carbon, nitrogen and phosphorus of alpine wetlands on the Tibetan Plateau. Ecological Engineering 58: 170–179. https://doi.org/10.1016/j.ecoleng.2013.06.035

Smal H, Ligęza S, Pranagal J, Urban D, Pietruczyk-Popławska D (2019) Changes in the stocks of soil organic carbon, total nitrogen and phosphorus following afforestation of post-arable soils: A chronosequence study. Forest Ecology and Management 451: 117536. https://doi.org/10.1016/j.foreco.2019.117536

Sohrt J, Lang F, Weiler M (2017) Quantifying components of the phosphorus cycle in temperate forests. WIREs. Water 4(6): e1243. https://doi.org/10.1002/wat2.1243

Suding K, Higgs E, Palmer M, Callicott JB, Anderson CB, Baker M, Gutrich JJ, Hondula KL, LaFevor MC, Larson BMH, Randall A, Ruhl JB, Schwartz KZS (2015) Committing to ecological restoration. Science 348(6235): 638–640. https://doi.org/10.1126/science.aaa4216

Sun F, Song C, Wang M, Lai DYF, Tariq A, Zeng F, Zhong Q, Wang F, Li Z, Peng C (2020) Long-term increase in rainfall decreases soil organic phosphorus decomposition in tropical forests. Soil Biology & Biochemistry 151: 108056. https://doi.org/10.1016/j.soilbio.2020.108056

Temesgen D, Gonzálo J, Turrión MB (2016) Effects of short-rotation Eucalyptus plantations on soil quality attributes in highly acidic soils of the central highlands of Ethiopia. Soil Use and Management 32(2): 210–219. https://doi.org/10.1111/sum.12257

Teng M, Huang C, Wang P, Zeng L, Zhou Z, Xiao W, Huang Z, Liu C (2019) Impacts of forest restoration on soil erosion in the Three Gorges Reservoir area, China. The Science of the Total Environment 697: 134164. https://doi.org/10.1016/j.scitotenv.2019.134164

Tiner RW (1988) Field Guide to Nontidal Wetland Identification. Maryland Department of Natural Resources, Water Resources Administration.

Tiner RW (1993) The primary indicators method—A practical approach to wetland recognition and delineation in the United States. Wetlands 13(1): 50–64. https://doi.org/10.1007/BF03160865

Wang L, Liang T, Zhang Q (2013) Laboratory experiments of phosphorus loss with surface runoff during simulated rainfall. Environmental Earth Sciences 70(6): 2839–2846. https://doi.org/10.1007/s12665-013-2344-9

Wang Y-P, Zhang Q, Pitman AJ, Dai Y (2015) Nitrogen and phosphorous limitation reduces the effects of land use change on land carbon uptake or emission. Environmental Research Letters 10(1): 014001. https://doi.org/10.1088/1748-9326/10/1/014001

Wezel A, Rajot JL, Herbrig C (2000) Influence of shrubs on soil characteristics and their function in Sahelian agro-ecosystems in semi-arid Niger. Journal of Arid Environments 44(4): 383–398. https://doi.org/10.1006/jare.1999.0609

Wortley L, Hero J-M, Howes M (2013) Evaluating ecological restoration success: a review of the literature. Restoration Ecology 21(5): 537–543. https://doi.org/10.1111/rec.12028

Wu F, Yang W, Sun B, Yang T, Chen X, Xu Z, Song H (2022) Soil C, N and P stocks and stoichiometry under different vegetation on the surface of the Leshan Giant Buddha. Soil Ecology Letters 4(1): 69–77. https://doi.org/10.1007/s42832-020-0061-3

Xu J, Zhang Y, Huang C, Zeng L, Teng M, Wang P, Xiao W (2022) Forest restoration shows uneven impacts on soil erosion, net primary productivity and livelihoods of local households. Ecological Indicators 134: 108462. https://doi.org/10.1016/j.ecolind.2021.108462

Yang Y, Mohammat A, Feng J, Zhou R, Fang J (2007) Storage, patterns and environmental controls of soil organic carbon in China. Biogeochemistry 84(2): 131–141. https://doi.org/10.1007/s10533-007-9109-z

Yang Y, Shi Y, Sun W, Chang J, Zhu J, Chen L, Wang X, Guo Y, Zhang H, Yu L, Zhao S, Xu K, Zhu J, Shen H, Wang Y, Peng Y, Zhao X, Wang X, Hu H, Chen S, Huang M, Wen X, Wang S, Zhu B, Niu S, Tang Z, Liu L, Fang J (2022) Terrestrial carbon sinks in China and around the world and their contribution to carbon neutrality. Science China. Life Sciences 65(5): 861–895. https://doi.org/10.1007/s11427-021-2045-5

Zhang W, Qiao W, Gao D, Dai Y, Deng J, Yang G, Han X, Ren G (2018a) Relationship between soil nutrient properties and biological activities along a restoration chronosequence of Pinus tabulaeformis plantation forests in the Ziwuling Mountains, China. Catena 161: 85–95. https://doi.org/10.1016/j.catena.2017.10.021

Zhang X, Hu M, Guo X, Yang H, Zhang Z, Zhang K (2018b) Effects of topographic factors on runoff and soil loss in Southwest China. Catena 160: 394–402. https://doi.org/10.1016/j.catena.2017.10.013

Zhao F, Kang D, Han X, Yang G, Yang G, Feng Y, Ren G (2015) Soil stoichiometry and carbon storage in long-term afforestation soil affected by understory vegetation diversity. Ecological Engineering 74: 415–422. https://doi.org/10.1016/j.ecoleng.2014.11.010

Zou L-Q, Chen F-S, Duncan DS, Fang X-M, Wang H (2015) Reforestation and slope-position effects on nitrogen, phosphorus pools, and carbon stability of various soil aggregates in a red soil hilly land of subtropical China. Canadian Journal of Forest Research 45(1): 26–35. https://doi.org/10.1139/cjfr-2014-0275

Supplementary material 1

Results of RDA analysis graphic of both soil TP and TP stocks in this study

Authors: Gang Li

Data type: image (Word file)

Explanation note: (a) TP model, total phosphorus model; (b) TPS model, total phosphorus stocks model; TP, soil total phosphorus; BD, bulk density; pH, soil pH value; SWC, soil water content; Individual effect was divided by the adjusted R^2 of each variable found in column Individual importance; $n = 72$.

Link: https://doi.org/10.3897/natureconservation.53.97873.suppl1

Supplementary material 2

Literature based soil TP at different slopes following poplar and pond cypress afforestation

Authors: Gang Li

Data type: table (Word file)

Explanation note: Lat., latitude; AT, annual temperature; AP, annual precipitation; PA, plantation age; PD, plantation density; SL, soil layer; TN, soil total nitrogen; TP, soil total phosphorus; Ref., reference.

Link: https://doi.org/10.3897/natureconservation.53.97873.suppl2

Nature Conservation

Nature Conservation 53: 17–38 (2023)
DOI: 10.3897/natureconservation.53.99313

Research Article

Promoting private forests for biodiversity conservation and ecosystems restoration in the Sahel region

Karafa Bognini[1,2], Loyapin Bondé[3], Sié Sylvestre Da[2], Abisha Mapendembe[4], Roch Yao Gnabeli[1]

1 *Université Félix Houphouët Boigny, UFR Biosciences, Côte d'Ivoire, 22 BP 582 Abidjan 22, Cote d'Ivoire*
2 *West African Science Service Center on Climate Change and Adapted Land Use (WASCAL), Competence center, Avenue Mouammar Kadhafi Ouaga 2000 06BP9507, Ouagadougou, Burkina Faso*
3 *Université Joseph Ki-Zerbo, Laboratory of Plant Biology and Ecology, 03 BP 7021, Ouagadougou 03, Burkina Faso*
4 *UN Environment Programme World Conservation Monitoring Centre, 219 Huntingdon Rd, Cambridge CB3 0DL, UK*
Corresponding author: Karafa Bognini (kalifabognini75@gmail.com)

OPEN ACCESS

Academic editor: Stefano Chelli
Received: 27 December 2022
Accepted: 9 May 2023
Published: 16 June 2023

ZooBank: https://zoobank.org/3E967729-56E6-4F1B-AE1E-F8B9A20794A9

Citation: Bognini K, Bondé L, Da SS, Mapendembe A, Gnabeli RY (2023) Promoting private forests for biodiversity conservation and ecosystems restoration in the Sahel region. Nature Conservation 53: 17–38. https://doi.org/10.3897/natureconservation.53.99313

Abstract

Private forests have the potential to mitigate biodiversity loss and improve community livelihoods. However, information on the socio-ecological factors that drive their establishment and long-term management are limited. This study aimed to narrow this gap by assessing the potential of privately-owned forests in conserving biodiversity and supporting the livelihoods of communities in northern Burkina Faso. Floristic data were collected within 26 plots (900 m^2 each) equally distributed between private Gourga forest, established in 1980) and its adjacent communal areas. Sixty-three (63) private landowners were interviewed in order to underpin their motivations and associated traditional knowledge and a stakeholder's workshop was conducted to develop conservation models for private forests and participatory implementation roadmap. Findings revealed that species richness was 132 in the Gourga forest and 85 in the communal areas, highlighting the importance of private forest in species conservation. Local communities recognized the provisioning (36.46%), regulating (28.46%) and supporting (22.48%) of ecosystem services provided by the Gourga forest as motivating factors. The main barriers to their establishment and management include lack of financial resources (35%), scarce lands (26%) and human pressures (8%). The implementation of private forests will need to be supported by the enactment of a secure land tenure policy, as well as payment for ecosystem services (PES) policies, incentivizing locals. We suggest decision makers mainstream privately-owned lands into national conservation strategies and design incentives policies to motivate local communities' engagement.

Key words: Burkina Faso, conservation, ecosystem services, Gourga forest, private forest, private land, species diversity

Introduction

The degradation of ecosystems and biodiversity loss are still on the rise, with severe impacts on people around the world (Ceballos et al. 2015). To halt and reverse the decline of biodiversity, there have been calls for national governments to expand the coverage of protected areas and ensure the conservation of 30% of the Earth's land and sea areas by 2030 (Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES 2018); Tsioumani 2020). Indeed, protected areas (PAs) are an important strategy for conserving biodiversity and improving human well-being through ecosystem goods and services (Bonet-Gracia et al. 2015; Adams et al. 2018). In addition, they constitute an essential tool to boost local economies and combat climate change (Watson et al. 2014). However, population growth and other related variables such as habitat destruction and the negative effects of climate change undermine the capacity of protected areas to achieve their conservation outcomes (Fousseni et al. 2012). In West Africa, natural ecosystems are undergoing fast land-use changes due to rapid human population growth and increasing agricultural (cash-crop) production, infrastructural development, rangeland expansion and forest product harvesting (Wittig et al. 2007; Ouédraogo et al. 2010). The pressures on natural ecosystems jeopardize their sustainability. For instance, in Burkina Faso, federal protected areas considered as the main vehicles for biodiversity conservation are under threat from croplands expansion and illegal trees logging, resulting in the loss of some protected areas (Ouoba 2006). This indicates that state-owned protected areas have limited capacity to ensure biodiversity conservation. In addition, while there is a willingness to increase conservation areas network, Lambin et al. (2011) reported the scarcity of public domains to establish new protected areas.

In this context, additional initiatives need to be promoted to compensate for the limits of state protected areas. Thus, governments and conservation planners are increasingly exploring privately-owned conservation areas (PCAs) as a bottom-up approach to achieve national and global conservation goals (Stolton et al. 2014). To that end, the World Commission on Protected Areas (WCPA) raised the international profile of private land conservation in conservation strategies (Stolton et al. 2014), spurring the emergence of privately-owned lands as biodiversity conservation instruments (Juffe-Bignoli et al. 2014). Recent works stated that privately-owned land is an innovative and effective approach to strengthen conservation efforts (Cortés Capano 2021). These new conservation areas have the potential to complement the existing protected area networks, and eventually play a key role in mitigating global biodiversity loss and climate change (Kamal et al. 2015). The mainstreaming of privately-owned lands into national biodiversity conservation strategies has piqued the interest of policy makers and key stakeholders, such that several governments have recently established private land conservation (PLC) mechanisms (International Land Conservation Network 2016). To support decision making, various types of research have been conducted globally to assess the ecological, economic, and social benefits of forestry and the motivations behind the establishment of private conservation areas (Gooden et al. 2019). Recent studies have revealed that most of the research on private land conservation has been carried out in the United States of America, Australia, South Africa, and Canada (Cortés Capano et al. 2019). Spatial variability of climatic conditions, ecosystems, plant diversity and socio-economic activities affects biodiversity use, management, and conservation practices (Levers et al. 2018), suggesting that finer scale information on conservation strategies is greatly needed to achieve global conservation goals. In Burkina Faso, socio-ecological information supporting the establishment and management of private forests, as well as associated challenges and constraints, are poorly addressed, limiting the integration of private forests in the conservation policies of the country. The objectives of this study, therefore, were to: (i) assess the contribution of private forests in plant species conservation in northern Burkina Faso; (ii) assess the key ecosystem services provided by private forests to local communities; (iii) identify barriers to the establishment and management of private forests, and (iv) propose sustainable community-based measures for the sustainability of biodiversity conservation on private lands. Three research questions were addressed in this study:

(i) Is private forest efficient in threatened species' conservation?
(ii) Do local communities perceive the role of private forests in the support of their livelihoods?
(iii) Do local people have the willingness to establish private forests?

Materials and methods

Description of the study area

This study was carried out in the municipality of Ouahigouya where the Gourga forest is geographically located at 13.35°N, 2.30°W. This private Gourga forest covers an area of 28 ha (Fig. 1). The Gourga forest is located in the Sudano-sahelian zone, characterized by a short rainy season from June to October (4–5 months) and a long dry season (7–8 months) Dipama (2010). Average annual rainfall varies from 600 to 900 mm, and average monthly temperatures vary from 20 to 30 °C. The vegetation is characterized by desert and Sahelian species and the common species encountered are *Vachellia seyal* Delile, *Combretum glutinosum* Perr. ex DC., *Balanites aegyptiaca* (L.) Delile, *Cassia sieberiana* DC., *Combretum micranthum* G.Don, and *Combretum nigricans* Lepr. ex Guill. & Perr. (Sambaré et al. 2011) including *Andropogon gayanus* Kunth and *Zornia glochidiata* Rchb. ex DC., which are the representative grasses (Maisharou 2014). Pressure on land use is extremely high in this area due to intensive livestock production. Farming methods are still traditional and are mainly food crops, predominantly sorghum and millet. Soils are generally degraded in the study area (Fig. 2a). Therefore, some traditional agricultural practices such as semi-circular dikes and *zaï* are undertaken by farmers for soil restoration and fertilization before planting. Rainfall irregularities, as well as deforestation and livestock grazing, lead to vegetation loss and land degradation, which are the main challenges for sustainable development in the region. The Gourga forest was created in 1980 and owned by Mr. Yacouba SAWADOGO, a farmer and right livelihood award winner known as "*the man who stopped the desert*". The overall objective of creating the forest was to build local resilience to the adverse impacts of climate change experienced in the drought 1970s period in the Sahel, resulting in the loss of many woody species. The ecological landscape of the forest is completely different to its communal areas (Fig. 2b).

Ecological data collection

Floristic data were collected in 26 individual plots equally distributed in two land use types, including the Gourga forest, which is privately owned, and the communal areas used as control areas in the framework of this study. In both areas main plots of individual surface of 900 m^2 (30 m × 30 m) were installed for the inventory of woody species (Nacoulma et al. 2011; Samandoulgou et al.

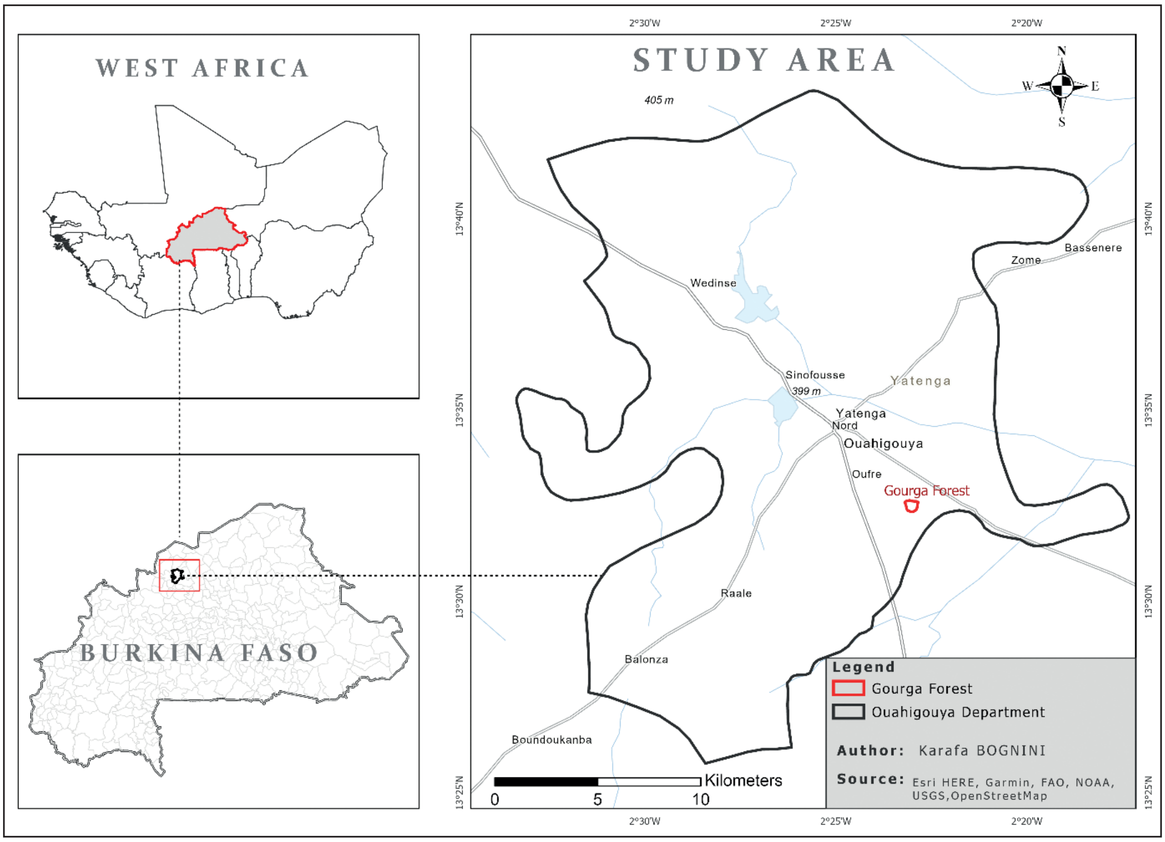


Figure 1. Location of the study area.

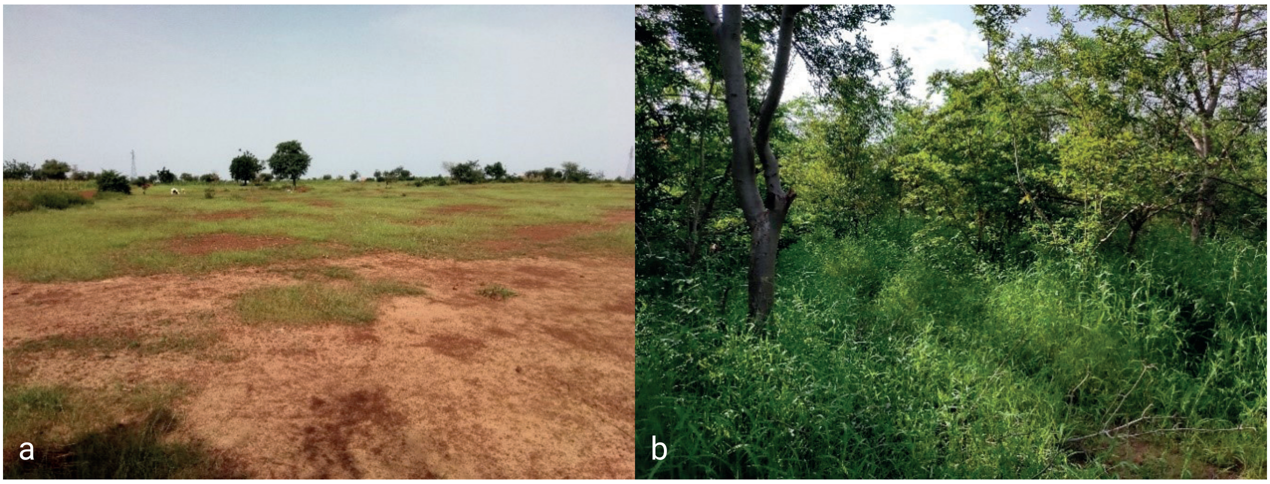


Figure 2. Overview of the study area during data collection period (**a**) communal area and (**b**) Gourga forest.

2019). Within each main plot, a subplot of 100 m^2 (10 m × 10 m) was installed for the inventory of herbaceous species. The inventory consisted of systematic counting and listing of all plant species present in each specific plot. All species were directly recorded with their scientific names. For species unidentified on the field, representative samples were collected and brought to the Laboratory of Plant Biology and Ecology of Université Joseph KI-ZERBO for identification. All species were listed using the nomenclatures of Angiosperm Phylogeny Group (APG) IV and Kyalangalilwa et al. (2013).

Social data collection

Social data related to private forest was collected in three nearest villages (Gourga, Saye, and Somiaga) to the Gourga forest. Based on their proximity and daily interaction with the forest, people living close to it could probably provide better information on its ecosystem services that they benefited from (N'Da et al. 2008; Oldekop et al. 2015). On this point, the surveys mainly targeted men, who according to social practices are the only landowners (Coulibaly-Lingani et al. 2009). Individual semi-structured interviews were performed with the Gourga forest owner and landowners from three different villages (Ouédraogo et al. 2020). In total, 63 landowners aged from 24 to 70 with 21 landowners per sampled village were interviewed. An interview was conducted with the Gourga forest owner (Mr. Yacouba SAWADOGO) to collect data on the benefits and constraints of forest management. For landowners, the main information collected concerned their perception of the key ecosystem services provided by the Gourga forest, their willingness to establish a private forest and related main constraints, as well as their recommendations to boost the private forest sector in Burkina Faso.

Stakeholder's workshop

Based on the barriers to private land conservation perceived by the communities, a stakeholder's consultative workshop was convened in the municipality of Ouahigouya using the qualitative Delphi method (Peter et al. 2021). The workshop aimed to bring together various stakeholders and determine a private land conservation model suitable to the national context. Indeed, the workshop gathered conservation-based NGOs, landowners, researchers from biodiversity areas, and experts from land and natural resource managing government institutions. The main question of the workshop was "*what is the appropriate private land conservation model for Burkina Faso and its implementation measures*?" In addition, participants were asked to rate the recommendations formulated by the communities and express their insights on conservation on private land. Recommendations were scored on a four-point scale (0–3), which correspond to: 1 = very important; 2 = less important and 3 = not important.

Data analysis

The ecological data were synthesized to constitute a floristic database arranged according to the taxonomic hierarchy (family, genus, and species). Plant diversity was described at family and species levels. At family level, the relative diversity of family (RDF) was calculated for each family in both land use types using equation 1.

$$\text{RDF} = (\text{number of species in a family} / \text{total number of species}) \times 100 \quad (1)$$

At species level, the common metrics widely used for assessing plant community diversity were calculated: species richness (SR), mean species richness per plot (MSR), Shannon index (H) and Pielou index (E) (Gnoumou et al. 2011; Bondé et al. 2013). SR is estimated by the total number of species recorded in an area. Hence, it refers to the number of taxa found in an area, without assessing their frequency or abundance. Therefore, it is not a meaningful measure for comparing community diversity. For this reason, H and E, which consider both the relative abundance of species and the total specific richness, are used to characterize the floristic diversity of environments. H quantifies the heterogeneity of the specific diversity of an environment, while E evaluates the equitability of all individuals among all species in the environment. PC-ORD 6.0.4 was used to calculate the number of species per plot, Shannon's diversity and Pielou's equitability indices. To assess the impact of land use on the species diversity, a non-parametric test (Wilcoxon test) at the 5% threshold was performed for each diversity index. For social data, we performed a generalized linear model (GLM) with Poisson error distribution to compare counting data (number of threats and number of ecosystem services) according to respondent age and locality (distance to the studied private forest). All statistical tests were generated using R software 4.0.3.

The stakeholders scored the recommendations formulated by the landholders as a way to motivate them to involve conservation and prioritized them following their importance. Further on, participants discussed the private land conservation model suitable for the socio-economic context of the country and agreed on its implementation measures. Graphical representation of the degree of consensus per recommendation was generated.

Results

Species diversity and land use pattern

In the whole study area, the surveys revealed a floristic richness of 217 herbaceous and woody species. Of these, the flora of the Gourga counted for 132 species (Appendix 1) whilst the communal areas counted for 85 species (Appendix 2). In general, the GF recorded higher values of relative diversity for all species families (Fig. 3). Families with one species (i.e., family relative diversity = 0.46%) are represented by other in the Fig. 3. The mean species richness and Shannon index based on wood species were significantly influenced by land use with higher values in the GF (Table 1). However, for the Pielou equitability index values, no difference was observed between the Gourga forest and the communal areas.

Particularly protected woody species encountered in the study area

By cross checking the species surveyed in the forest with the red list of the International Union for Conservation of Nature (IUCN) and the list of species under special protection in Burkina Faso, it was possible to establish the list of both threatened and those highly protected in Burkina Faso (Table 2). Overall, the finding revealed 46 woody species with special status according to the IUCN Red List of Threatened Species in the study area. 26 of them were only found in the Gourga Forest and 2 in the communal areas whilst 18 species were common to both areas. Among these species, 41 were in the category of least concern (LC), one specie is near threatened (*Dalbergia melanoxylon* Guill. & Perr.) and two are threatened species (*Adansonia digitata* L. and *Vitellaria paradoxa* C. F. Gaertn.). Furthermore, the study identified 12 woody species with special protection status in Burkina Faso with 11 species growing in the Gourga forest and 1 species in communal areas.

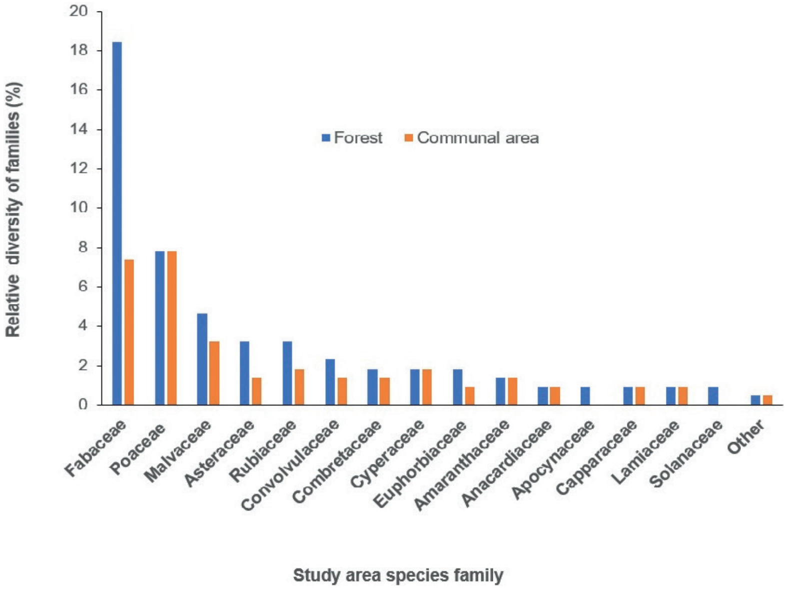


Figure 3. Relative diversity of families according to land use.

Table 1. Summary of diversity indexes of the woody species according to land use type.

	Total species richness	Mean species richness	Shannon index	Pielou index
Gourga forest	55	10.15 ± 4.16a	1.85 ± 0.56a	0.82 ± 0.11a
Communal area	27	4.46 ± 4.17b	1.07 ± 0.43b	0.84 ± 0.14a

Mean ± SD. Indexes with the same letter are statistically not different at 5% level.

Communities’ perception of the private land conservation

Key ecosystem services of the Gourga forest

Statistical analyses revealed no influence of both the respondent’s age and location (distance to the forest) on their perception regarding ecosystem services. In general, the results showed that communities of the three surveyed villages clearly perceived the key ecosystem services from the Gourga forest. Actually, the communities identified 17 goods and services (Fig. 4). provided by the Gourga forest. The findings showed that the provisioning of services accounts for 36.46% of the total services while the regulating of services accounts for 28.46%. Further on, the findings highlighted that a supporting service was fairly provided by the forest (22.48%). The category of cultural services was rarely mentioned (12.83%) by the population.

Certainly, concerning the category of provisioning services, the respondents highlighted that the forest plays a crucial role in traditional medicine (79.36%), supply of fruits (36.5%), and forage for livestock (9.52%). The cultural services mentioned include shade production (15.87%), village reputation (9.52%), and tourism value (6.34%). The three services (aesthetics, research, and education) are sparsely cited with a rate of 3.17% per service. Finally, respondents cited human well-being (1.58%) and social cohesion (1.58%). In terms of regulatory services, the respondents mentioned soil fertilization (36.5%), improved rainfall (26.98%), air purification (23.8%)

Table 2. Species with particular status observed in the whole study area.

		Occurrence			
Family	**Species**	**Forest**	**Communal area**	**IUCN's status**	**National protection status**
Olacaceae	*Ximenia americana* L.	+	-	LC	PP
Fabaceae	*Senegalia ataxacantha* DC.	+	-	LC	
Fabaceae	*Vachellia nilotica* subsp. *Leiocarpa*	+	-	LC	
Fabaceae	*Senegalia senegal* (L.) Willd	+	-		PP
Malvaceae	*Adansonia digitata* L.	+	+	VU	PP
Annonaceae	*Annona senegalensis* Pers.	+	-	LC	
Combretaceae	*Anogeisus leiocarpa* (DC.) Guill. & Perr.	+	-		PP
Meliaceae	*Azadirachta indica* A.Juss.	+	+	LC	
Zygophyllaceae	*Balanites aegyptiaca* (L.) Del.	+	+	LC	
Fabaceae	*Senna sieberiana* DC.	+	+	LC	
Fabaceae	*Boscia senegalensis* Lam	+	+	LC	
Malvaceae	*Ceiba pentandra* (L.) Gaertn.	+	-	LC	PP
Fabaceae	*Combretum aculeatum* Vent.	+	-	LC	
Fabaceae	*Combretum fragans* F.Hoffm.	+	-	LC	
Fabaceae	*Combretum glutinosum* Perr. ex DC.	+	+	LC	
Fabaceae	*Combretum marginatum* Engl. & Diels	+	-	LC	
Fabaceae	*Combretum micranthum* G. Don	+	+	LC	
Fabaceae	*Combretum molle* R. Br.ex G. Don	+	-	LC	
Fabaceae	*Combretum nigricans* Lepr. Ex Guill. & Perr.	+	-	LC	
Fabaceae	*Dalbergia melanoxylon* Guill. & Perr (African Blackwood)	+	-	NT	PP
Ebenaceae	*Diospyros mespiliformis* Hochst. ex A. DC.	+	+	LC	
Fabaceae	*Faidherbia albida* (Del.) A. Chev	+	+	LC	PP
Moraceae	*Ficus platyphylla* Del.	+	-	LC	
Phyllanthaceae	*Flueggea virosa* (Roxb. Ex Willd.)	+	-	LC	
Rubiaceae	*Gardenia ternifolia* Schumach & Thonn. subsp. *Ternifolia*	+	-	LC	
Malvaceae	*Grewia bicolor Juss.*	+	-	LC	
Apocynaceae	*Holarrhena floribunda* (G. Don) T. Durand. & Schinz	+	-	LC	
Euphorbiaceae	*Jatropha gossypiifolia* L.	+	+	LC	
Anacardiaceae	*Lannea microcarpa* Engl. & K. Krause	+	+	LC	
Capparaceae	*Maerua angolensis* DC.	+	-	LC	
Capparaceae	*Maerua crassifolia* Forssk	+	-	LC	
Fabaceae	*Parkia biglobosa* (Jacq.) Benth.	+	-	LC	PP
Fabaceae	*Prosopis africana* (Guill. & Perr.) Taub.	+	+	LC	PP
Fabaceae	*Pterocarpus lucens* Lepr. ex Guill. & Perr.	+	-	LC	PP
Fabaceae	*Senegalia dudgeoni* Craib ex Holl.	+	-	LC	
Fabaceae	*Senegalia macrostachya* (Rchb. Ex DC.) Kyal. & Boatwr	+	-	LC	
Bignoniaceae	*Stereospermum kunthianum* Cham.	+	-	LC	
Combretaceae	*Terminalia avicennioides* Guill. & Perr.	+	-	LC	
Fabaceae	*Vachellia seyal* (Delile.) P.J.H. Hurter	+	+	LC	
Combretaceae	*Guiera senegalensis* J.F. Gmel.	+	+	LC	

		Occurrence			
Fabaceae	*Vachellia sieberiana* DC.	+	+	LC	
Sapotaceae	*Vitellaria paradoxa* C. F. Gaertn.	+	+	VU	PP
Rhamnaceae	*Ziziphus mauritiana* Lam.	+	+	LC	
Fabaceae	*Senegalia dudgeoni* Craib ex Holl.	-	+	LC	
Fabaceae	*Faidherbia albida* (Del.) A. Chev.	+	+	LC	
Fabaceae	*Tamarindus indica* L.	-	+	LC	PP

Legend: LC: Least concerned; PP: Particular protection; VU: Vulnerable; NT: Nearly threatened; +: Present in the area; -: Not present in the area.

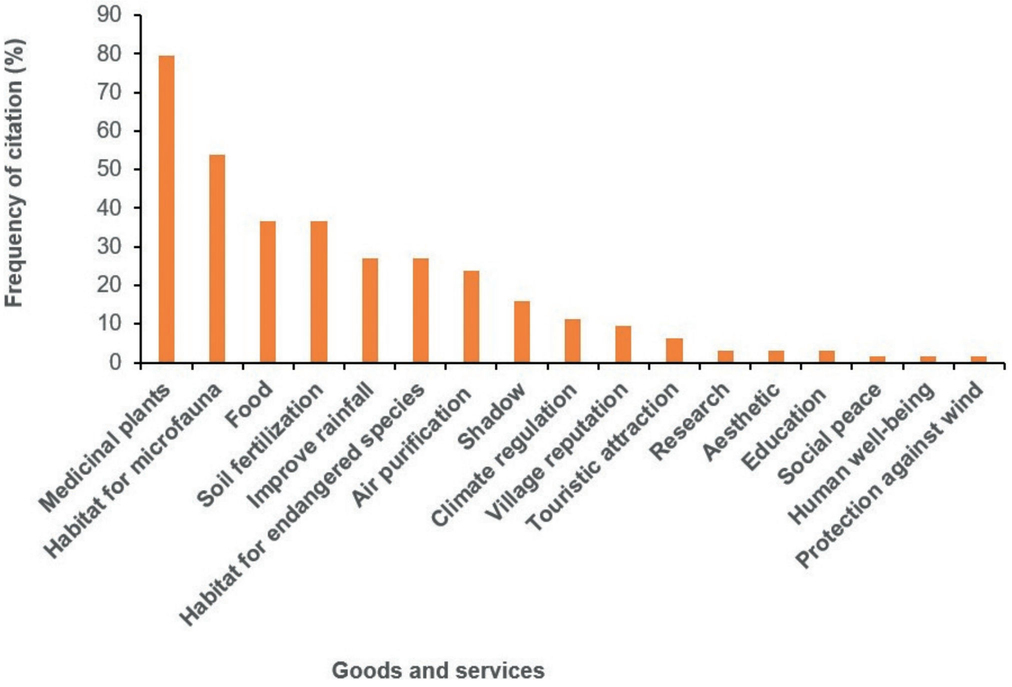


Figure 4. Species goods and services of the Gourga forest based on communities' perception.

and improved climate (11.11%). As for the category of support services, the respondents cited among others the refuge of plant species (26.98%), the refuge of animal species (53.96%) and, finally, the protection against winds (1.58%).

Communities' perception on private forests establishment constraints

General linear model (GLM) showed that there was no significant relationship between the respondents' location and age on the constraints to private forests forest establishment. Respondents identified 11 key constraints preventing them from establishing their own forests (Fig. 5). Findings revealed that land scarcity and lack of financial resources were the constraints most perceived by the communities. Despite these constraints, 100% of the surveyed landowners expressed a willingness to establish their own private forests. Therefore, they suggested 11 recommendations to motivate private landowners to engage in biodiversity conservation. These recommendations include financial support, capacity building, trees, and seedling, working materials, land security, parcel of land, water, sensitization, monitoring, conservation agreements, and promotion of green jobs (Fig. 6).

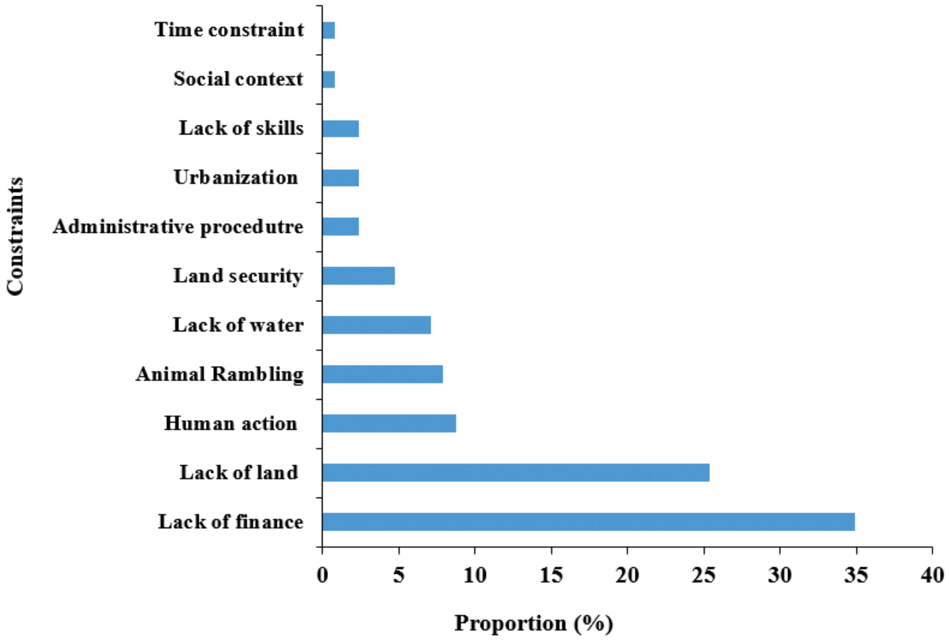


Figure 5. Communities' perception on private forest establishment constraints.

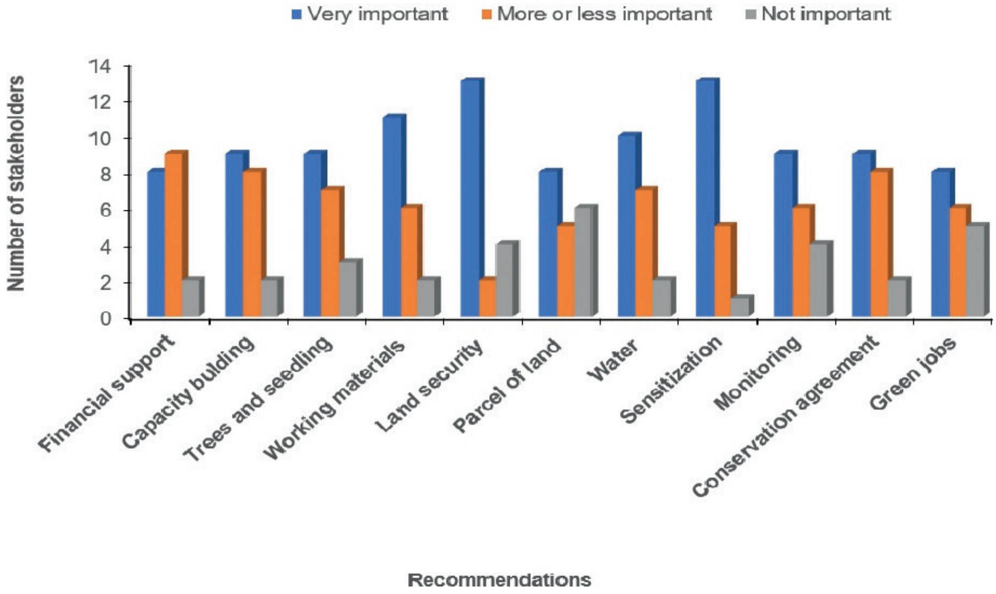


Figure 6. Stakeholder ranking of private land conservation enforcement measures.

Stakeholders' perception of incentive measures for private land conservation

The stakeholders' workshop revealed the social acceptability of private land conservation by different stakeholders. The findings showed that the stakeholders had different perceptions of the eleven recommendations addressed by landowners. Thus, upon deep discussions on these recommendations, the stakeholders ranked them based on their efficiency (Fig. 6). Results indicated that stakeholders perceived that land tenure security and landowners' awareness raising as key pathways to promote private land conservation in Burkina Faso. Furthermore, all stakeholders viewed the provision of working materials such as barrows, grating, conservation agreements between landholders and decision makers, and water as very pressing for promoting private land conservation. Nevertheless, the participants mentioned financial support as a more or less important measure.

The stakeholders, upon discussing the recommendations formulated by the landowners, indicated that the provision of financial resources in advance is not necessary for the promotion of private land conservation. Nevertheless, they proposed the development of some financial incentive instruments that could motivate landowners to engage in conservation programs. In this respect, all stakeholders identified the payment for ecosystem services as a conservation scheme.

Stakeholders stressed that the legal framework for land management is not suitable for the national social context. They reported that current land legislation lacks the capacity to secure privately-owned lands. However, the workshop identified a few measures that could support the integration of privately-owned lands in biodiversity conservation strategies. These measures include legal security of privately-owned land and easing regulations on the exploitation of natural resources in private forests.

Discussion

Species diversity and land use

The survey findings highlighted a significant floristic richness of the Gourga forest compared to the communal areas. This high species richness of the Gourga forest and the other diversity indexes could be linked to the sustainable land management practices and daily monitoring actions by the forest owner. These practices include semi-circular dikes, zai plantation pits, stone/vegetation dikes, composting, farmer-assisted natural regeneration, small-scale dams, stone cordons, and village irrigation systems. The forest also is roughly sheltered from human activities. These findings corroborate those observed by Bondé et al. (2013), who showed that protected areas are subject to less anthropogenic pressure than fallow lands which occur most of the time on communal areas and explain therefore the species richness of protected areas.

By way of contrast, the findings highlighted a low floristic diversity in the communal areas. This low species richness may be linked to anthropogenic actions combined with grazing activities, which are highly developed in the area. These findings support those of Soulama et al. (2015) who reported that vegetation in unprotected areas (fallows, grazing areas) is the most degraded due to exacerbation of anthropogenic pressure. In similar research, Kouassi et al. (2012) observed a similar trend in Côte d’Ivoire where work proved that savannah formations are the most affected by anthropogenic activities.

The largest families in both sites were the Fabaceae with 27% of forest species and 37% of communal area species and the Combretaceae with 20% of forest species and 11% of communal area species. This high prevalence of these families could be explained by the resilience capacity of these species. The findings are in line with those reported by Ouédraogo et al. (2020) who found in a similar study the prevalence of those two families in eastern Burkina Faso. This could be linked by their capacity to resist ecological and human constraints as highlighted by Bognounou et al. (2009) and Savadogo et al. (2016). Indeed, their findings indicated that these species are resilient to water stress and rainfall deficiency.

Communities' perception of the Gourga forest priority ecosystem services

The GLM analysis showed that there was no significant difference between both age and distance on the respondents' perception of the forest ecosystem services. Therefore, respondents from all age groups and villages regardless of distance perceive the ecosystem services of the forest. This robust perception of the ecosystem services of the forest could be related to the fact that both old and young people nowadays have a better awareness of the importance of natural resources on their livelihoods and well-being. The finding revealed that the Gourga forest supports the riparian communities with eighteen goods and services. These benefits refer to the classification provided by the (Millennium Environmental Assessment 2005) which recognized four main categories of ecosystem services (provisioning, regulating, supporting and cultural) with the prevalence of provisioning service. Therefore, of the total provided ecosystem services, the provisioning services were more perceived by the respondents. These findings support those observed by the Ouédraogo et al. (2020). Indeed, in their work on local people's perceptions of ecosystem services in PAs in eastern Burkina Faso, they found that people living around a PA were familiar with these four categories of ecosystem services. Despite the private ownership of the Gourga forest, the communities have recognized its range of services and benefits. Indeed, 79.36% of the respondents recognized the medicinal value of the forest whilst of them 36% indicated that the forest produces fruits. These results imply that private land conservation could be a complementary approach to improve the community's livelihood through its multiple benefits similar to those of a public forest. Further on, 26.99% of the respondents indicated that the forest contributes to the improvement of local rainfall while 23.8% of them think that it improves air purification. These findings show the potential of the forest to mitigate climate change effects. This implies that private land conservation could be an effective approach to support communities' resilience in a world threatened by climate change impact (Raymond et al. 2015).

Sustainability of private land conservation strategy

During social surveys, people suggested recommendations to promote private land conservation in Burkina Faso. Following the workshop, the stakeholders agreed on individual private land conservation as the appropriate model. For this purpose, the stakeholders prioritized the recommendations made by the populations according to their level of importance. The workshop insights show that securing land tenure and sensitizing landowners are more urgent than financial support. The results of the workshop are in line with the conclusions of Silva et al. (2021) who in a very recent study in Brazil underlined the crucial role of land tenure security in the promotion of private forestry. Indeed, the applicable land tenure laws do not guarantee sustainable investments on private land. As a follow-up to the stakeholders' insights on sustainable private land conservation in Burkina

Faso, we suggest the further promotion of the tripartite contracts between landowners, the central government and any local government hosting a private forest.

Further, to ensure that private forests are conserved in the long term, stakeholders highlighted the need for conservation agreements between landowners and the government. In particular, stakeholders stressed that land security alone does not guarantee the perpetuity of conservation activities on the privately-owned land. The finding supported the conclusions of the work of Kamal et al. (2015) who found that conservation agreements are effective legal instruments under which, ownership of the land (habisus) is conferred to a legal entity or government agency, while use rights (fructus and usus) revert to the landowner by right. We assume that this approach could serve as a window for private forestry in Burkina Faso.

Conclusion

This study, which assessed the potential of private forests, confirmed their effectiveness in conserving biodiversity and supporting local communities' livelihood if several conditions or factors are met. Therefore, findings indicated the potential of private forests as an option to increase the national protected areas network. Furthermore, this study made it possible to understand the motivations behind the establishment of the forest and the key constraints to private land conservation. Indeed, the study combined a mixed method to assess the floristic richness of the Gourga forest and to understand people's perception of private land conservation. The findings highlighted that the forest vegetation was more diverse than the communal areas. The ethnobotanical surveys provided information on forest management practices and economic management strategies. The study revealed that the Gourga forest is a source of income for the landowner and a livelihood for his family. Nevertheless, the forest owner reported some management constraints. The second phase of the ethnobotanical study identified the key ecosystem services provided to the communities despite the forest private ownership. Regarding the benefits associated with the establishment of a private forest, communities have expressed their willingness to initiate their creation. However, the research identified a few constraints that would prevent people from engaging in biodiversity conservation. Finally, the stakeholders' workshop explored the plausible future of private forests and their implementation strategies.

Acknowledgements

This work was conducted within the framework of the West African Biodiversity and Ecosystem Services (WABES) project, funded by the international climate initiative (IKI) of the German Federal Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection (BMUV). (Grant No.: 17_IV _072_West Africa_IPBES Support). We are also grateful to the Ministry of Environment of Burkina Faso which funded the workshop organization through the project "Plan d'Investissement Forestier (PIF)". We further thank Mr. Yacouba Sawadogo for allowing us to conduct our research in his forest. We also thank Dr. Blaise Kabré and Dr. Alhassane Zaré for field assistance and the local communities of Ouahigouya for their active participation during data collection, and Bossila Séraphin Hien for his support in data processing and analysis. We finally thank Dr. Isimemen Osemwegie for editing the English language.

Additional information

Conflict of interest

No conflict of interest was declared.

Ethical statement

No ethical statement was reported.

Funding

This work was funded by International climate initiative (IKI) of the German Federal Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection (Grant No.: 17_IV _072_West Africa_IPBES Support). Ministry of Environment of Burkina Faso funded the workshop organization through the project "Plan d'Investissement Forestier (PIF)".

Author contributions

Karafa Bognini as the main author, he conceptualized the work, collected data and prepare a first draft of this paper. Loyapin Bondé as part of the supervision team, designed the methodology and reviewed and edited this paper. Sié Sylvestre Da, Abisha Mapendembe supervised the research implementation. Roch Yao Gnabeli was the main supervisor during data collection.

Author ORCIDs

Karafa Bognini https://orcid.org/0000-0002-3243-3957
Loyapin Bondé https://orcid.org/0000-0002-9399-8644
Sié Sylvestre Da https://orcid.org/0000-0001-8284-4589

Data availability

All of the data that support the findings of this study are available in the main text.

References

Adams VM, Mills M, Weeks R, Segan DB, Pressey RL, Gurney GG, Álvarez-Romero JG (2018) Implementation strategies for systematic conservation planning. Ambio 48(2): 139–152. https://doi.org/10.1007/s13280-018-1067-2

Bognounou F, Thiombiano A, Savadogo P, Boussim JI, Odén PC, Guinko S (2009) Woody vegetation structure and composition at four sites along a latitudinal gradient in Western Burkina Faso. Bois et Forêts des Tropiques 300(2): 29–44. https://doi.org/10.19182/bft2009.300.a20412

Bondé L (2019) Distribution, production fruitière et potentiel socio-économique de *Tamarindus indica* L. et de *Vitellaria paradoxa* C.F.Gaertn au Burkina Faso. PhD These, Université Joseph KI-ZERBO, Burkina Faso, 158 pp.

Bondé L, Ouédraogo O, Kagambèga F, Joseph I, Boussim JI (2013) Impact des gradients topographique et anthropique sur la diversité des formations ligneuses soudaniennes. Bois et Forêts des Tropiques 318(4): 15–25. https://doi.org/10.19182/bft2013.318.a20514

Bonet-García FJ, Pérez-Luque AJ, Moreno-Llorca RA, Pérez-Pérez R, Puerta-Piñero C, Zamora R (2015) Protected areas as elicitors of human well-being in a developed region: A new synthetic (socioeconomic) approach. Biological Conservation 187: 221–229. https://doi.org/10.1016/j.biocon.2015.04.027

Chirwa PW, Mahamane L (2017) Overview of restoration and management practices in the degraded landscapes of the Sahelian and dryland forests and woodlands of East and southern Africa. Southern Forests 79(2): 87–94. https://doi.org/10.2989/20702620.2016.1255419

Cortés Capano G (2021) Private land conservation policies: navigating from global gaps to local perceptions and needs. PhD thesis, Faculty of Science of the University of Helsinki, Finland, 172 pp.

Ceballos G, Ehrlich PR, Barnosky AD, Garcia A, Pringle RM, Palmer TM (2015) Accelerated modern human-induced species losses: Entering the sixth mass extinction. Science Advances 1(5): e1400253–e1400253. https://doi.org/10.1126/sciadv.1400253

Cortés Capano G, Toivonen T, Soutullo A, Di Minin E (2019) The emergence of private land conservation in scientific literature: A review. Biological Conservation 237: 191–199. https://doi.org/10.1016/j.biocon.2019.07.010

Coulibaly-Lingani P, Tigabu M, Savadogo P, Oden P, Ouadba JM (2009) Determinants of access to forest products in southern Burkina Faso. Forest Policy and Economics 11(7): 516–524. https://doi.org/10.1016/j.forpol.2009.06.002

da Silva JMC, Pinto LP, Scarano FR (2021) Toward integrating private conservation lands into national protected area systems: Lessons from a megadiversity country. Conservation Science and Practice 3(7): 1–8. https://doi.org/10.1111/csp2.433

Dipama JM (2010) Climate. In: Thiombiano A, Kampmann D (Eds) Biodiversity Atlas of West Africa. Ouagadougou, Burkina Faso; Frankfurt/Main, Germany, 122–124.

Fousseni F, Marra D, Wala K, Batawila K, Zhang C, Zhao X, Akpagan K (2012) Assessment and impact of anthropogenic disturbances in protected areas of northern Togo. Forestry Studies in China 14(3): 216–223. https://doi.org/10.1007/s11632-012-0308-x

Gnoumou A, Bognounou F, Hahn K, Thiombiono (2011) Woodybplatndiversity and stand structure in the Comoe-Leraba reserve, Southwestern Burkina Faso (West Africa). Journal of Biological Science 2: 111–123. https://doi.org/10.3923/jbs.2011.111.123

Gooden J, ‘t Sas-Rolfes M (2019) A review of critical perspectives on private land conservation in academic literature. Ambio 49(5): 1019–1034. https://doi.org/10.1007/s13280-019-01258-y

IPBES (2018) The IPBES assessment report on land degradation and restoration. Zenodo. https://doi.org/10.5281/zenodo.3237393

International Land Conservation Network (2016) Celebrating the Derecho Real de Conservacio’n, Newsletter of the International Land Conservation Network.

Juffe-Bignoli D, Burgess ND, Bingham H, Belle EMS, de Lima MG, Deguignet M, Bertzky B, Milam AN, Martinez-Lopez J, Lewis E, Eassom A, Wicander S, Geldmann J, van Soesbergen A, Arnell AP, O’Connor B, Park S, Shi YN, Danks FS, MacSharry B, Kingston N (2014) Protected. The Planetary Report 2014. UNEP-WCMC, Cambridge.

Kamal S, Grodzinska-Jurczak M, Kaszynska AP (2015) Challenges and opportunities in biodiversity conservation on private land: An institutional perspective from Central Europe and North America. Biodiversity and Conservation 24(5): 1271–1292. https://doi.org/10.1007/s10531-014-0857-5

Kouassi AM, Kouamé KF, Ahoussi KE, Oularé S, Biemi J (2012) Impacts conjugués des changements climatiques et des pressions anthropiques sur les modifications de la couverture végétale dans le bassin versant du N’zi-Bandama (Côte d’Ivoire). Revue Ivoirienne des Sciences et Technologie 20: 124–146.

Kyalangalilwa B, Boatwright JS, Daru BH, Maurin O, van der Bank M (2013) Phylogenetic position and revised classification of *Acacia* s.l. (Fabaceae: Mimosoideae) in Africa, including new combinations in *Vachellia* and *Senegalia*. Botanical Journal of the Linnean Society 172(4): 500–523. https://doi.org/10.1111/boj.12047

Lambin EF, Meyfroidt P (2011) Global land use change, economic globalization, and the looming land scarcity. Proceedings of the National Academy of Sciences of the United States of America 108(9): 3465–3472. https://doi.org/10.1073/pnas.1100480108

Levers C, Schneider M, Prishchepov AV, Estel S, Kuemmerle T (2018) Spatial variation in determinants of agricultural land abandonment in Europe. The Science of the Total Environment 664: 95–111. https://doi.org/10.1016/j.scitotenv.2018.06.326

Maisharou A (2014) Practices, Techniques and Technologies for Restoring Degraded Landscapes in the Sahel. African Forest Forum, Working Paper Series (Vol. (2)3), 42 pp.

N'Da HD, Adou YCY, N'Guessan KE, Koné M, Sagne YC (2008) Analyse de la diversité floristique du parc national de la Marahoué, Centre-Ouest de la Côte d'Ivoire. Afrique Science: Revue Internationale des Sciences et Technologie 4(3): 552–579. https://doi.org/10.4314/afsci.v4i3.61700

Nacoulma BMI, Schumann K, Traoré S, Bernhardt-Römermann M, Hahn K, Wittig R, Thiombiano A (2011) Impacts of land-use on West African savanna vegetation: A comparison between protected and communal area in Burkina Faso. Biodiversity and Conservation 20(14): 3341–3362. https://doi.org/10.1007/s10531-011-0114-0

Oldekop JA, Holmes G, Harris WE, Evans KL (2015) A global assessment of the social and conservation outcomes of protected areas. Conservation Biology 30(1): 133–141. https://doi.org/10.1111/cobi.12568

Ouédraogo I, Tigabu M, Savadogo P, Compaoré H, Odén PC, Ouadba JM (2010) Land cover change and its relation with population dynamics in Burkina Faso, West Africa. Land Degradation & Development 21(5): 453–462. https://doi.org/10.1002/ldr.981

Ouédraogo I, Sambare O, Savadogo S, Thiombiano A (2020) Local perceptions of ecosystem services in protected areas in Eastern Burkina Faso. Ethnobotany Research and Applications 20: 1–18. https://doi.org/10.32859/era.20.13.1-18

Ouoba P (2006) Flore et végétation de la forêt classée de Niangoloko, sud-ouest du Burkina Faso. PhD Thesis, Université de Ouagadougou, Burkina Faso, 140 pp.

Peter S, Le Provost G, Mehring M, Müller, Manning P (2021) Cultural worldviews consistently explain bundles of ecosystem service prioritisation across rural Germany. People and Nature 4(1): 218–230. https://doi.org/10.1002/pan3.10277

Raymond CM, Lechner AM, Lockwood M, Carter O, Harris RMB, Gilfedder L (2015) Private land manager capacity to conserve threatened communities under climate change. Journal of Environmental Management 159: 235–244. https://doi.org/10.1016/j.jenvman.2015.04.048

Samandoulgou Y, Compaore H, Zoundi SJ, Zoungrana-Kabore Y (2019) Evaluation de la productivité des herbacées fourragères des forêts sacrées de Koupéla dans le Centre Ouest du Burkina Faso. International Journal of Biological and Chemical Sciences 13(1): 99–109. https://doi.org/10.4314/ijbcs.v13i1.9

Sambaré O, Bognounou F, Wittig R, Thiombiano A (2011) Woody species composition, diversity and structure of riparian forests of four watercourse types in Burkina Faso. Journal of Forestry Research 22(2): 145–158. https://doi.org/10.1007/s11676-011-0143-2

Savadogo OM, Ouattara K, Pare S, Ouedraogo I, Sawadogo-Kaboré S, Barron J, Nabsanna PZ (2016) Structure, composition spécifique et diversité des ligneux dans deux zones contrastées en zone Sahélienne du Burkina Faso. https://doi.org/10.4000/vertigo.17282 [Accessed on November 11, 2021]

Soulama S, Kadeba A, Nacoulma B, Traore S, Bachmann Y, Thiombiano A (2015) Impact des activités anthropiques sur la dynamique de la végétation de la réserve partielle de faune de Pama et de ses périphéries (sud-est du Burkina Faso) dans un contexte de variabilité climatique. Journal of Applied Biosciences 87(1): 8047–8064. https://doi.org/10.4314/jab.v87i1.6

Stolton S, Redford KH, Dudley N (2014) The futures of privately protected areas. IUCN, Gland, Switzerland.

Tsioumani E (2020) Convention on Biological Diversity: A Review of the Post-2020 Global Biodiversity Framework Working Group Negotiations. Environmental Policy and Law 50(1–2): 1–2. [55–59.] https://doi.org/10.3233/EPL-200207

Watson J, Dudley N, Segan D (2014) The performance and potential of protected areas. Nature 515: 67–73. https://doi.org/10.1038/nature13947

Wittig R, Konig K, Scmidt M (2007) A study of climate change and anthropogenic impacts in West Africa. Environmental Science and Pollution Research International 14(3): 182–189. https://doi.org/10.1065/espr2007.02.388

Appendix 1

Table A1. Full species recorded in the private forest Gourga.

N°	Family	Species
1	Olacaceae	*Ximenia americana* L.
2	Fabaceae	*Senegalia ataxacantha* DC.
3	Fabaceae	*Vachellia nilotica* subsp. *Leiocarpa*
4	Fabaceae	*Senegalia senegal* (L.) Willd
5	Malvaceae	*Adansonia digitata* L.
6	Annonaceae	*Anona senegalensis* Pers.
7	Combretaceae	*Anogeisus leiocarpa* (DC.) Guill. & Perr.
8	Meliaceae	*Azadirachta indica* A.Juss.
9	Zygophyllaceae	*Balanites aegyptiaca* (L.) Del.
10	Fabaceae	*Senna sieberiana* DC.
11	Fabaceae	*Boscia senegalensis* Lam
12	Fabaceae	*Ceiba pentandra* (L.) Gaerth.
13	Fabaceae	*Combretum aculeatum* Vent.
14	Fabaceae	*Combretum fragans* F.Hoffm.
15	Fabaceae	*Combretum glutinosum* Perr. ex DC.
16	Fabaceae	*Combretum marginatum* Engl. & Diels
17	Fabaceae	*Combretum micranthum* G. Don
18	Fabaceae	*Combretum molle* R. Br.ex G. Don
19	Fabaceae	*Combretum nigricans* Lepr. Ex Guill. & Perr.
20	Fabaceae	*Dalbergia melanoxylon* (African Blackwood)
21	Fabaceae	*Dichrostachys cinerea* (L.) Wight & Arn.
22	Ebenaceae	*Diospyros mespiliformis* Hochst. ex A. DC.
23	Fabaceae	*Faidherbia albida* (Del.) A. Chev.
24	Rubiaceae	*Feretia apodanthera* Del.
25	Moraceae	*Ficus platyphylla* Del.
26	Phyllanthaceae	*Flueggea virosa* (Roxb. Ex Willd.)
27	Rubiaceae	*Gardenia ternifolia* Schumach & Thonn. subsp. *Ternifolia*

N°	Family	Species
28	Rubiaceae	*Gardenia sokotenis* Hutch.
29	Malvaceae	*Grewia bicolor* Juss.
30	Malvaceae	*Grewia lasiodiscus* K. Schum.
31	Apocynaceae	*Holarrhena floribunda* (G. Don) T. Durand. & Schinz
32	Euphorbiaceae	*Jatropha gossypiifolia* L.
33	Anacardiaceae	*Lannea microcarpa* Engl. & K. Krause
34	Asclepiadaceae	*Leptadenia hastata* (Pers.) Decne.
35	Capparaceae	*Maerua angolensis* DC.
36	Capparaceae	*Maerua crassifolia* Forssk
37	Moringaceae	*Moringa oleifera* Lam
38	Fabaceae	*Parkia biglobosa* (Jacq.) Benth.
39	Fabaceae	*Piliostigma reticulatum* (DC.) Hochst.
40	Fabaceae	*Prosopis africana* (Guill. & Perr.) Taub.
41	Fabaceae	*Pterocarpus lucens* Lepr. ex Guill. & Perr
42	Polygaceae	*Securidaca longipedunculata* Fresen.
43	Apocynaceae	*Saba senegalensis* var. *glabriflora* (Hua) Pichon
44	Anacardiaceae	*Sclerocarya birrea* (A. Rich.) Hochst. Subsp. Birrea
45	Fabaceae	*Senegalia dudgeoni* Craib ex Holl.
46	Fabaceae	*Senegalia macrostachya* (Rchb. Ex DC.) Kyal. & Boatwr
47	Bignoniaceae	*Stereospermum kunthianum* Cham.
48	Loranthaceae	*Tapinanthus globiferus* var. *glabriflora* (Hua) Pichon
49	Combretaceae	*Terminalia avicennioides* Guill. & Perr.
50	Combretaceae	*Terminalia macroptera* Guill. & Perr.
51	Fabaceae	*Vachellia seyal* (Delile.) P.J.H. Hurter
52	Combretaceae	*Guiera senegalensis* J.F. Gmel.
53	Fabaceae	*Vachellia sieberiana* DC.
54	Sapotaceae	*Vitellaria paradoxa* C. F. Gaertn.
55	Rhamnaceae	*Ziziphus mauritiana* Lam.
56	Malvaceae	*Waltheria indica* L.
57	Fabaceae	*Senna obtusifolia* (L.) H. S. Irwin & Barneby
58	Poaceae (Gramineae)	*Pennisetum pedicellatum* Trin.
59	Poaceae (Gramineae)	*Aristida kerstingii* Pilg.
60	Poaceae (Gramineae)	*Setaria pumila* (Poir.) Roem. & Schult.
61	Asteraceae	*Aspilia africana* (Pers.) C.A. Adams
62	Convolvulaceae	*Ipomoea argentaurata* Hall. f.
63	Acanthaceae	*Blepharis maderaspatensis* (L.) B. Heyne ex Roth
64	Rubiaceae	*Spermococe intricans* (Hepper) H.M.Burkill
65	Asteraceae	*Bidens engleri* O.E. Schultz
66	Fabaceae	*Chamaecrista mimosoides* (L.) Greene
67	Poaceae (Gramineae)	*Brachiaria lata* (Schumach.) C.E. Hubbard
68	Fabaceae	*Alysicarpus ovalifolius* (Schum.) J. Léonard
69	Rubiaceae	*Spermacoce ruelliae* DC.
70	Fabaceae	*Zornia glochidiata* Reichb. ex DC.
71	Amaranthaceae	*Pandiaka heudelotii* (Moq.) Hiern

N°	Family	Species
72	Fabaceae	*Desmodium adscendens* (Sw.) DC. var. *adscendens*
73	Fabaceae	*Cassia absus* L.
74	Poaceae (Gramineae)	*Microchloa* sp.
75	Malvaceae	*Sida ovata* Forssk
76	Fabaceae	*Desmodium ospriostreblum* Chiov.
77	Rubiaceae	*Spermacoce filifolia* (Schumach. & Thonn.) J.-P. Lebrun & Stork
78	Poaceae (Gramineae)	*Brachiaria villosa* (Lam.) A. Camus
79	Fabaceae	*Stylosantes erecta* P.Beauv.
80	Poaceae (Gramineae)	*Microchloa indica* (L. f.) P. Beauv.
81	Malvaceae	*Sida alba* L.
82	Poaceae (Gramineae)	*Aristida adscensionis* L.
83	Solanaceae	*Physalis micrantha* L.
84	Malvaceae	*Corchorus olitorius* L.
85	Fabaceae	*Crotalaria retusa* L.
86	Rubiaceae	*Spermacoce verticillata* L.
87	Asteraceae	*Aspilia bussei* O. Hoffm. & Muschler
88	Fabaceae	*Calopogonium mucunoides* Desv.
89	Malvaceae	*Triumfetta rhomboidea* Jacq.
90	Poaceae (Gramineae)	*Elionurus elegans* Kunth
91	Fabaceae	*Indigofera senegalensis* Lam.
92	Lamiaceae	*Leucas martinicensis* (Jacq.) R. Br.
93	Poaceae (Gramineae)	*Rottboellia cochinchinensis* (Lour.) Clayton
94	Fabaceae	*Desmodium gangeticum* (L.) DC.
95	Poaceae (Gramineae)	*Digitaria horizontalis* Willd.
96	Malvaceae	*Wissadula amplissima* (L.) R.E. Pries var. *rostrata* (Schumach. & Thonn.)
97	Euphorbiaceae	*Acalypha ciliata* Forssk.
98	Solanaceae	*Physalis angulata* L.
99	Poaceae (Gramineae)	*Hackelochloa granularis* (L.) Kuntze
100	Asteraceae	*Acanthospermum hispidum* DC.
101	Euphorbiaceae	*Euphorbia hirta* L.
102	Amaranthaceae	*Achyranthes aspera* L.
103	Cyperaceae	*Kyllinga pumila* Michx.
104	Asteraceae	*Chrysanthellum indicum* DC. subsp. *Afroamericanum* B. L. Turner
105	Poaceae (Gramineae)	*Dactyloctenium aegyptium* (L.) Willd.
106	Poaceae (Gramineae)	*Eragrostis gangetica* (Roxb.) Steud.
107	Cyperaceae	*Cyperus difformis* L.
108	Convolvulaceae	*Ipomoea coscinosperma* Hochst.
109	Nyctaginaceae	*Boerhavia erecta* L.
110	Cyperaceae	*Bulbostylis* sp.
111	Vitaceae	*Cissus* sp.
112	Cyperaceae	*Fimbristylis ferruginea* (L.) Vahl.
113	Euphorbiaceae	*Euphorbia dregeana* E.Mey. Ex Boss.
114	Asteraceae	*Tridax procumbens* L.
115	Caryophyllaceae	*Polycarpaea corymbosa* (L.) Lam. subsp. *Corymbosa*

N°	Family	Species
116	Amaranthaceae	*Pupalia lappacea* (L.) Juss.
117	Fabaceae	*Indigofera erecta* Thunb.
118	Poaceae (Gramineae)	*Panicum laetum* Kunth
119	Lamiaceae	*Ocimum americanum* L.
120	Sapindaceae	*Cardiospermum halicacarbum* L.
121	Polygalaceaa	*Polygala arenaria* Willd.
122	Fabaceae	*Cassia nigricans* Vahl
123	Fabaceae	*Cassia absus* L.
124	Asteraceae	*Aspilia kotschyi* (Sch.Bip. Ex Hochst.)
125	Convolvulaceae	*Ipomoea ochracea* (Lindl.) G. Don
126	Poaceae (Gramineae)	*Panicum maximum* Jacq.
127	Convolvulaceae	*Ipomoea asarifolia* (Desr.) Roem. & Schult.
128	Fabaceae	*Vigna racemosa* (G. Don) Hutch. & Dalziel
129	Poaceae (Gramineae)	*Schoenefeldia gracilis* Kunth
130	Malvaceae	*Corchorus tridens* L.
131	Convolvulaceae	*Evolvulus alsinoides* (L.) L.
132	Scrophulariaceae	*Striga hermonthica* (Del.) Benth.

Appendix 2

Table A2. Full species recorded in the communal area.

N°	Families	Species
1	Meliaceae	*Azadirachta indica* A. Juss.
2	Myrtaceae	*Eucalyptus camaldulensis* Dehnh. [cult.]ptus globulus
3	Anacardiaceae	*Sclerocarya birrea* (A. Rich.) Hochst. subsp. *Birrea*
4	Fabaceae	*Tamarindus indica* L.
5	Ebenaceae	*Diospyros mespiliformis* Hochst. ex A. DC.
6	Anacardiaceae	*Lannea microcarpa* Engl. & K. Krause
7	Fabaceae	*Vachellia sieberiana* (DC.)
8	Fabaceae	*Acacia sieberiana* DC.
9	Zygophyllacea	*Balanites aegyptiaca* (L.) Del.
10	Combretaceae	*Combretum micranthum* G. Don
11	Combretaceae	*Guiera senegalensis* J.F. Gmel.
12	Rhamnaceae	*Ziziphus mauritiana* Lam.
13	Fabaceae	*Cassia sieberiana* DC.
14	Fabaceae	*Piliostigma reticulatum* (DC.) Hochst.
15	Fabaceae	*Vachellia seyal* (Del.) P.J.H. Hurter
16	Fabaceae	*Faidherbia albida* (Del.) A. Chev.
17	Fabaceae	*Prosopis africana* (Guill. & Perr.) Taub.
18	Euphorbiaceae	*Jatropha gossypiifolia* L.
19	Malvaceae	*Adansonia digitata* L.
20	Asclepiadaceae	*Leptadenia hastata* (Pers.) Decne.
21	Fabaceae	*Bauhinia refescens* Lam.

N°	Families	Species
22	Capparaceae	*Maerua angolensis* DC.
23	Sapotaceae	*Vitellaria paradoxa* C. F. Gaertn.
24	Mimosaceae	*Acacia dudgeoni* Craib ex Holl.
25	Combretaceae	*Combretum glutinosum* Perr. ex DC.
26	Capparaceae	*Boscia senegalensis* (Pers.) Lam. Ex Poir.
27	Asclepiadaceae	*Calotropis procera* (WILLD) R. Br.
28	Fabaceae	*Senna obtusifolia* (L.) H. S. Irwin & Barneby
29	Convolvulaceae	*Ipomea coscinosperma* Hochst.
30	Fabaceae	*Zornia glochidiata* Reichb. ex DC.
31	Malvaceae	*Corchorus tridens* L.
32	Poaceae (Gramineae)	*Dactyloctenium aegyptium* (L.) Willd.
33	Poaceae (Gramineae)	*Eragrostis tenella* (L.) Roem. & Schult.
34	Poaceae (Gramineae)	*Digitaria horizontalis* Willd.
35	Poaceae (Gramineae)	*Setaria pumila* (Poir.) Roem. & Schult
36	Rubiaceae	*Spermacoce verticillata* L.
37	Malvaceae	*Sida alba* L.
38	Asteraceae	*Chrysanthellum indicum* DC. subsp. *Afroamericanum* B. L. Turner
39	Asteraceae	*Acanthospermum hispidum* DC.
40	Lamiaceae	*Hyptis spicigera* Lam.
41	Amaranthaceae	*Gomphrena celosioides* Mart.
42	Onagraceae	*Ludwigia abyssinica* A. Rich.
43	Rubiaceae	*Mitracarpus villosus* (Sw.) DC.
44	Cyperaceae	*Cyperus difformis* L.
45	Cyperaceae	*Cyperus rotundus* L.
46	Fabaceae	*Alysicarpus ovalifolius* (Schumach.) J. Léonard
47	Amaranthaceae	*Alternanthera sessilis* (L.) DC.
48	Poaceae (Gramineae)	*Hackelelochloa granularis* (L.) Kuntze
49	Cyperaceae	*Cyperus sphacelatus* Rottb.
50	Fabaceae	*Chamaecrista mimosoides (L.) Greene*
51	Malvaceae	*Melochia corchorifolia* L.
52	Poaceae (Gramineae)	*Pennisetum pedicellatum* Trin.
53	Solanaceae	*Physalis micrantha* Link
54	Euphorbiaceae	*Euphorbia hirta* L.
55	Malvaceae	*Abelmoschus esculentus* (L.) Moench
56	Amaranthaceae	*Amaranthus spinosus* L.
57	Poaceae (Gramineae)	*Panicum laetum* Kunth
58	Lamiaceae	*Leucas martinicensis* (Jacq.) Ait.f.
59	Commelinaceae	*Commelina benghalensis* L.
60	Fabaceae	*Indigofera senegalensis* Lam.
61	Scrophulariaceae	*Striga hermonthica* (Del.) Benth.
62	Sterculiaceae	*Waltheria indica* L.
63	Malvaceae	*Triumfetta rhomboidea* Jacq.
64	Fabaceae	*Arachis hypogaea* L.
65	Poaceae (Gramineae)	*Eragrostis tremula* Hochst. ex Steud.

N°	Families	Species
66	Poaceae (Gramineae)	*Eragrostis gangetica* (Roxb.) Steud.
67	Cyperaceae	*Fimbristylis hispidula* (Vahl) Kunth subsp. *bachyphylla* (Cherm.) Napper
68	Rubiaceae	*Spermacoce radiata* (DC.) Hiern
69	Poaceae (Gramineae)	*Andropogon gayanus* Kunth
70	Asclepiadaceae	*Leptadenia hastata* (Pers.) Decne.
71	Poaceae (Gramineae)	*Microchloa indica* (L. f.) P. Beauv.
72	Poaceae (Gramineae)	*Aristida adscensionis* L.
73	Acanthaceae	*Blepharis maderaspatensis* (L.) B.Heyne ex Roth
74	Asteraceae	*Bidens engleri* O.E. Schultz
75	Malvaceae	*Sida ovata* Forssk.
76	Poaceae (Gramineae)	*Brachiaria villosa* (*Lam.*) A. Camus
77	Convolvulaceae	*Evolvulus alsinoides* (L.)
78	Poaceae (Gramineae)	*Panicum maximum* Jacq.
79	Convolvulaceae	*Ipomoea eriocarpa* R. Br.
80	Poaceae (Gramineae)	*Schoenefeldia gracilis* Kunth
81	Poaceae (Gramineae)	*Elionurus elegans* Kunth
82	Rubiaceae	*Spermacoce filifolia* (Schumach. & Thonn.) J.-P. Lebrun & Stork
83	Polygalaceae	*Polygala arenaria* Willd.
84	Poaceae (Gramineae)	*Diheteropogon amplectens* (Nees) Clayton
85	Nyctaginaceae	*Boerhavia diffusa* L.

Nature Conservation

Nature Conservation 53: 39–59 (2023)
DOI: 10.3897/natureconservation.53.104243

Research Article

First exhaustive distribution and habitat modelling of *Morimus asper* (Sulzer, 1776) *sensu lato* (Coleoptera, Cerambycidae) in Bulgaria

Rumyana Kostova[1], Rostislav Bekchiev[2], Georgi Popgeorgiev[2], Yurii V. Kornilev[2,3,4]

1 *Department of Zoology and Anthropology, Faculty of Biology, Sofia University, 8 Dragan Tsankov blvd., Sofia, Bulgaria*
2 *National Museum of Natural History, Bulgarian Academy of Sciences, 1 Tsar Osvoboditel blvd., 1000 Sofia, Bulgaria*
3 *Unit of Integrative Zoology, Department of Evolutionary Biology, Faculty of Life Sciences, University of Vienna, Djerassiplatz 1, 1030 Vienna, Austria*
4 *Institute of Zoology, University of Natural Resources and Life Sciences (BOKU), Vienna, Austria*
Corresponding author: Rumyana Kostova (rkostova@biofac.uni-sofia.bg)

Abstract

Although *Morimus asper*, in particular the ssp. *funereus*, is considered as widespread throughout Bulgaria, a current national mapping of the species is missing. Thus, here we present the first exhaustive study on the distribution of *M. asper* in Bulgaria. Our research combined 967 georeferenced presence records from scientific publications, from the museum collection of the National Museum of Natural History-BAS, as well as authors' and citizen scientists' field observations. An Ecological Niche Model (ENM) was generated using software MaxEnt to identify the potential distribution of the species based on niche suitability. The potentially suitable area for the species was 26% of Bulgaria (29 059 km^2). The main predictor variables in *M. asper*'s ENM assessed by a Jackknife test were the distance to mixed Fagus-Carpinus forests, the mean forest age, the mean tree height, the maximal temperature during the hottest month and the altitude. The percentage contribution to the model of the first two variables was also the largest – respectively 40% and 11%. The remaining variables contributed less than 10% each. Furthermore, we recommend some changes to the current species monitoring methodology to the National Biodiversity Monitoring System.

Key words: Cerambycidae, conservation, ecological niche model, Natura 2000, saproxylic

Academic editor: Bela Tóthmérész
Received: 29 March 2023
Accepted: 6 June 2023
Published: 7 July 2023

ZooBank: https://zoobank.org/1489CABF-567C-4776-8BAE-450A5899E766

Citation: Kostova R, Bekchiev R, Popgeorgiev G, Kornilev YV (2023) First exhaustive distribution and habitat modelling of *Morimus asper* (Sulzer, 1776) *sensu lato* (Coleoptera, Cerambycidae) in Bulgaria. Nature Conservation 53: 39–59. https://doi.org/10.3897/natureconservation.53.104243

Introduction

The genus *Morimus* Brullé, 1832 is represented in Bulgaria by *M. asper funereus* Mulsant, 1862, *M. orientalis* Reitter, 1894 and *M. verecundus bulgaricus* Danilevsky et al. 2016 (Danilevsky 2023). But there is uncertainty about the taxonomic status of those taxa and the correct identification of the specimens based on morphology due to the existence of "transitional" forms (Solano et al. 2013). Based on COI and ITS2 gene sequences all European and Turkish populations of *Morimus* should be referred to a single polymorphic and a polytypic species, *M. asper* (Sulzer, 1776) Solano et al. (2013). All other taxa on that territory have to be elevated to infraspecific rank and are subspecies or simple colour morphs (Solano et al. 2013). Therefore, although we provide citations and information for all the taxa as they were presented by the original authors, in this paper we consider *Morimus asper sensu lato*.

The first records of *M. asper funereus* (as *Morimus funereus*) (Fig. 1) from Bulgaria were reported by Bulgarian naturalists in the early 19th century (Yoakimoff 1899; Markovich 1904, 1909; Yoakimov 1904; Kovachev 1905; Nedelkov 1906, 1909a, b; Netolitzky 1912). Since then, occurrence data have been published in at least 33 papers (Heyrovský 1931; Roubal 1931; Chorbadzhiev 1932; Kantardjiewa-Minkova 1934; Csiki 1943; Anguélov 1960; Angelov 1964, 1967; Palm 1966; Bringmann 1996; Kodzhabashev and Penev 1998; Samuelian 1998; Hubenov et al. 2000; Guéorguiev 2001, 2018; Guéorguiev et al. 2003; Bechev and Stojanova 2004; Migliaccio et al. 2004; Georgiev et al. 2005a, b, 2006, 2015, 2018, 2019; Siering and Beier 2005; Georgiev and Hubenov 2006; Migliaccio et al. 2007; Rapuzzi and Georgiev 2007; Guéorguiev and Ljubomirov 2009; Topalov et al. 2014; Danilevsky et al. 2016; Doychev et al. 2017; Doychev et al. 2018; Kostova et al. 2019). The species was known from multiple localities, distributed throughout the country. It was recorded mainly from the foothills (Predbalkan, Kraishte) and the mountains (Stara Planina, Vitosha, Sredna Gora, Rila, Maleshevska Planina, Western and Central Rhodopes, Belasitsa and Slavyanka), as well from the Ludogorie plateau. Although there is a large amount of published and unpublished data, in general the information is occasional. *Morimus asper funereus* is a protected species included in Annex II of the EU Habitats Directive (92/43/EEC) (as *M. funereus*) and has been assessed as "Vulnerable" in the IUCN Red List (World Conservation Monitoring Centre 1996). It is also

Figure 1. *Morimus asper funereus* in its microhabitat, Sredna gora Mountains.

a protected species under the Bulgarian Biodiversity Act (09.08.2002, latest amendment, State Gazette N°102/23.12.2022). However, the species was not included in the latest editions of the European Red List of Saproxylic Beetles (Nieto and Alexander 2010; Cálix et al. 2018). The populations of the subspecies *M. asper funereus* are distributed in Slovakia, Hungary, Romania, Moldova, Ukraine and the whole Balkan Peninsula (Hardersen et al. 2017a).

The first records for *Morimus orientalis* Reitter, 1894 in Bulgaria were from Stara Zagora, Burgas and Varna by Nedelkov (1909a) not confirmed since then. Bringmann (1996) considered those findings accidental, probably transferred by transported wood from Strandzha Mts. Confirmed localities were only from Malko Tarnovo and Sinemorets (Strandzha Mts.) by Bringmann (1996). Later new localities were reported, all in Strandzha Mts. (Georgiev et al. 2015; Georgiev et al. 2018).

The first records of *Morimus verecundus bulgaricus* came from two close localities: the botanical gardens of Sofia University in Balchik and Varna (Danilevsky et al. 2016). These are the only known localities to date.

Morimus asper is a stenotopic, silvicolous, xylodetriticolous, xylophagous and saproxylic species according to its ecological requirements (Jurc et al. 2008). It inhabits predominantly deciduous and mixed forests or well-structured woodlands, often dominated by oak (*Quercus* spp.) or by beech (*Fagus* spp.), with a medium to high density of dead wood. Additionally, this beetle species is often found in coppiced stands, characterised by the presence of old stumps and decaying wood on the ground (Hardersen et al. 2017a, b; Jugovic et al. 2022). Preferred microhabitats include the leaf litter decomposition layer, moist stumps, fallen wood, the soil surface and trunks with remaining bark of trees. The species is extremely polyphagous and has emerged from the wood of *Abies*, *Picea*, *Acer*, *Alnus*, *Castanea*, *Platanus*, *Juglans*, *Populus*, *Prunus*, *Quercus*, *Salix*, *Ulmus*, *Tilia* and *Fagus* (Hardersen et al. 2017b; Leonarduzzi et al. 2017). Reported host plants for *M. asper funereus* in Bulgaria were *Populus ×canadensis* Moench, *Pseudotsuga menziesii* (Mirb.) Franco, *Fagus sylvatica* L., *Quercus pubescens* Willd., *Quercus dalechampii* Ten., another *Quercus* sp., *Salix caprea* L. (Georgiev et al. 2013; Doychev et al. 2017, 2018). In Romania favourable habitats of *M. asper funereus* were found to be *Tilia tomentosa* Moench and *Quercus petraea* (Matt.) Liebl woodlands, which are semi-shaded and semi-open habitats, characteristic of mature, old, deciduous forests (Manu et al. 2017; Manu et al. 2019). According to Bringmann (1996), *M. orientalis* develops predominantly in oak wood.

The conservation status of the Bulgarian populations of *M. asper* is favourable for all parameters (FV) in all biogeographical regions, according to the reporting under Art. 17 of the Habitats Directive 92/43/EEC in 2013 (for the period 2007–2012) and in 2019 (for the period 2013–2018) with the exception of Unfavourable-Unsatisfactory status (U1) for the perspectives and overall assessment in the Continental region in the 2013 report. The following major threats to the species were identified: use of plant protection chemicals in forestry: forest fires, felling, and the removal of dead wood. The species has been included as potentially occurring in the Standard Data Forms from 159 Natura 2000 zones in Bulgaria, according to the latest EIONET Central Data Repository (2023), but occurrence has not been registered for all of them.

Regardless of many publications providing data on the habitat preferences of *M. asper*, to date little is known about the complexity of factors determining its distribution. Environmental niche models are a useful tool for estimating the real and potential species habitats. Such models for *M. asper* were made only for Slovenia and Italy (Vrezec et al. 2014; Redolfi De Zan et al. 2023). For Slovenia, the obtained habitat suitability model using the maximum entropy approach was assessed as unreliable due to lack of sufficiently good data for the habitats of this species. Redolfi De Zan et al. (2023) used more diverse and more precise environmental data for their models as well as presence data based on citizen science using four different approaches: generalized linear model (GLM), boosted regression tree (BRT), random forest (RF) and maximum entropy (MaxEnt). RF and Max Ent models provided the best discriminant ability for the species.

In that context, our research aimed to update and for the first time to provide resolution to the current distribution of *M. asper* in Bulgaria and the main factors for its presence at a given locality in Bulgaria based on a MaxEnt's Ecological Niche Model.

Materials and methods

Study area

The territory of Bulgaria (43°N, 25°E) covers ca. 111 000 km^2 and encompasses heterogenous eco-physiographic conditions and habitats. Elevation ranges from 0 to 2 925 m asl., with highly diverse relief, stretching from extensive plains and lowlands to subalpine and alpine mountains. Five hypsometric belts consist of lowlands (0–200 m, 31.4% of the territory), hills (200–600 m, 41%), low mountains (600–1 000 m, 15.3%), mountains of average height (1 000–1 600 m, 9.8%) and high mountains (> 1 600 m, 2.5%) (Simeonov and Totzev 1997). The climate is dominated by Mediterranean, Oceanic and Continental influences, combining 12 Köppen-Geiger climate classes (Beck et al. 2018). Bulgaria is a country with a great variation in natural conditions, from the East-Mediterranean coast and steppes to the highest elevations of the Balkan mountain ranges. Broadleaved forests prevail (68.9%) above conifers (31.1%). The most widespread tree species are *Quercus* sp. (32.6%) and *Fagus* (16.1%), followed by *Pinus sylvestris* L. (16.0%) and *P. nigra* J.F. Arnold (8.5%). Nearly 80% of forests are in state ownership; the other 20% are in private or community ownership. At present, 5 780 km^2 (5.2%) of Bulgaria are under protection; of the total wooded area, 16.5% is protected in various ways. The deciduous and mixed forests dominated by *Fagus sylvatica* are the second most common forest type in Bulgaria. They form a continuous forest belt at altitudes ranging from 800 to 1 000 metres and 1 500 to 1 600 metres asl. In some places the beech forests reach lower altitudes. The forests of *Fagus orientalis* Lipsky have more limited distribution, preferring shady and humid locations, especially in places with climate inversions in Strandzha Mts. (Tzonev et al. 2006; Veen et al. 2010).

Presence data

To generate maps of the known current distribution and the predictive model of the distribution of *M. asper* subspecies in Bulgaria, we compiled into a georeferenced database the presence records from the following sources:

- published and available scientific literature encompassing 1904–2019 (110 records);

- the collection of the National Museum of Natural History – Bulgarian Academy of Sciences (89 specimens with exact location labelled, from 301);
- field data collected by the authors during 2012–2021 and by citizen science (voluntary provided data from amateurs, members of a Bulgarian Facebook group: The Insects and the Entomologists) incorporated in the SmartBirds.org database (Popgeorgiev et al. 2015) (768 records).

All available records were used to map the current distribution of *M. asper* on a 10 km × 10 km MGRS grid. Only data from the field studies and citizen science, which were with high level of accuracy (up to 20 m) were used in the Ecological Niche Modelling.

Ecological Niche Model

The Ecological Niche Model (**ENM**) was generated using software MaxEnt 3.4.1 (Phillips et al. 2017). A data matrix with biological and environmental variables for modelling was created using ArcGIS v.10.3 (ESRI, Redlands, CA, USA). We used continuous environmental variables of three types: climatic, topographic, and habitat. The climatic variables were the 19 bioclimatic parameters of the freely available WorldClim v.2, with original resolution ≈ 1 km^2 cell and averaged data from 1970–2000 (Fick and Hijmans 2017). Although slightly outdated, this is the best climatic database available for Bulgaria. Global Aridity Index and the Global Potential Evapotranspiration (CGIAR-CSI Global-Aridity and Global-PET Geospatial Database, Trabucco and Zomer (2019)) were used. We downscaled the original rasters to 40 m cells, using "cubic convulsion" resampling in ArcGIS. The topographic variables entered in the model were elevation (m above sea level), aspect (°) and slope (°), derived from a 40-m resolution Digital Elevation Model (DEM), and yearly solar radiation (kJ m-2 day-1). The habitat variables were derived from the Spatial Database of the Executive Forest Agency. These included forest types (dominated by *Fagus* sp. and *Carpinus* sp.; dominated by *Quercus* sp.; riparian forests; other deciduous forest types), forest age, average tree height, and average tree diameter. We converted the categorical data to continuous by calculating the Euclidian distance (ArcGIS tool) to each habitat type in the original vector layers and rasterizing the results to 40 m grids.

To minimize collinearity in the ENM, the correlated environmental factors and autocorrelated points were omitted from the model calculations. For that purpose, we generated 100 000 random points across the geographic scope and associated to them the corresponding values of all variables, and then tested them by Spearman Rank Order Correlations (Statistica v.10; StatSoft, Tulsa, OK, USA), setting |r| = 0.7 as a threshold. Then, we generated a preliminary model with 10 replicates, for which we included all environmental factors. For the set-up of the final ENM, factors were either kept or removed based on the following: the factor with the highest percent contribution (PC) from the preliminary model was retained and all its correlated factors were removed; of the remaining factors, the one with the second highest PC was retained and all the factors correlated with it were removed; and so on, until all factors were considered (Kornilev et al. 2017). After the correlation test, nine of the climatic variables remained in the analyses (Table 1). The slope and the annual solar radiation were removed from the topographic variables. The average tree diameter was removed as a

Table 1. Average Percent contribution (%) and Permutation importance (PI) of uncorrelated variables, estimated by MaxEnt's Ecological Niche Model of *Morimus asper* in Bulgaria.

Environmental variable (Unit)	Unit	Percent contribution		Permutation importance	
		Average	**Min-Max**	**Average**	**Min-Max**
Fagus-Carpinus forest, distance to	m	40.02	37.34–43.73	22.28	17.41–25.98
Average forest age	year	11.14	8.8–13.54	2.23	1.21–3.49
Other deciduous forest, distance to	m	8.23	7.49–9.03	8.29	1.35–2.27
Average tree height	m	6.78	3.85–9.73	5.18	3.86–6.90
Precipitation seasonality (Coefficient of Variation) (Bio15)	%	5.83	3.92–7.21	22.19	19.95–25.26
Elevation	m above sea level	5.48	4.23–6.89	4.86	3.88–6.07
Precipitation of coldest quarter (Bio19)	mm	4.20	3.16–5.25	2.34	1.66–3.29
Precipitation of wettest quarter (Bio16)	mm	3.77	1.90–5.25	11.49	9.21–13.88
Riparian forest, distance to	m	2.88	2.29–3.44	2.55	1.62–3.61
Min temperature of coldest month (Bio06)	°C	2.68	1.70–3.94	2.18	1.10–3.65
Mean temperature of driest quarter (Bio09)	°C	2.49	1.17–4.19	7.90	6.14–9.64
Quercus forest, distance to	m	1.54	1.09–2.16	1.81	6.48–9.77
Aspect	°	1.51	1.12–2.59	2.20	1.21–3.10
Precipitation of driest quarter (Bio17)	mm	1.32	0.10–2.58	0.36	0.01–0.67
Max temperature of warmest month (Bio05)	°C	1.01	0.42–3.98	1.80	0.47–4.07
Mean temperature of warmest quarter (Bio10)	°C	0.98	0.36–2.11	2.20	0.80–6.13
Mean diurnal range (Mean of monthly (max temp – min temp)) (Bio02)	°C	0.14	0.00–1.58	0.13	0.00–0.40

factor in the model as well due to its correlation with the average tree height. To avoid spatial autocorrelation, a buffer of 250 m around each point of species presence was set to remove all but one of the records. This value was chosen to reflect the maximum dispersal capacity of one *M. asper* individual.

Out of 768 points of *M. asper* presence, 546 remained for modelling. Removed points were either within 250 m of each other or within settlements. Although *M. asper* might occur naturally in settlements, especially those with old parks and old trees, we cannot be sure if the recorded beetles were not transported with cut wood, and thus we removed these records to avoid seriously distorting the model towards non-natural habitats. For all models, we used the following settings: logistic output to describe the probability of presence, calibrated using randomly 75% of the available records as training data, and the remaining 25% were used for model validation as test data; 100 000 randomly selected background points as pseudoabsence; maximum iteration was 500; replicated run type was cross-validation; 100 replicates were generated to get the average prediction. The outputs (in ASCII format) were processed and visualised using QGIS. We used the average result as a balance between a highly restricted and a highly inflated model, so that it can be optimally used for making management decisions.

The Jackknife procedure was used to indicate the most informative variables. Response curves to all variables in the model were obtained. The resultant "mean values" ENM was thresholded into unsuitable/suitable space using the "Maximum test sensitivity plus specificity logistic threshold" calculated by MaxEnt, as max SSS is one of the best threshold selection methods for presence/

absence data (Liu et al. 2005; Liu et al. 2015). The remaining data were assigned to three classes of suitability (Low, Medium, and High) for analysis, using Jenks Natural Breaks in ArcGIS. Areas were calculated from the obtained raster layer.

Descriptive statistics of the environmental factors with highest influence on the *M. asper* presence were calculated using software SigmaPlot 12 (Systat Software, San Jose, CA).

To validate the obtained ENM for *M. asper*, we collected 77 presence points available at GBIF (24 record with accuracy under 20 m) and field data collected in 2022 from the SmartBirds.org database (53 records) (Fig. 2). In cases of multiple observations within 250 m of each other, only a single one chosen randomly was removed. Thus, we used seventy points to assess the ENM. The distance between those records and the nearest pixel with suitable area according to the average ENM was calculated.

Results

We generated the first ENM for *Morimus asper s.l.* in Bulgaria as well as compiled the first mapping of its recent distribution in Bulgaria (Fig. 3), based on 546 and 967 observations, respectively. The occurrence was recorded in a total of 364 MGRS 10 km cells. The presence of *M. asper* was recorded for the first time in 291 MGRS cells, confirmed in 47 MGRS cells, and not confirmed by our study in 26 MGRS cells (Fig. 3).

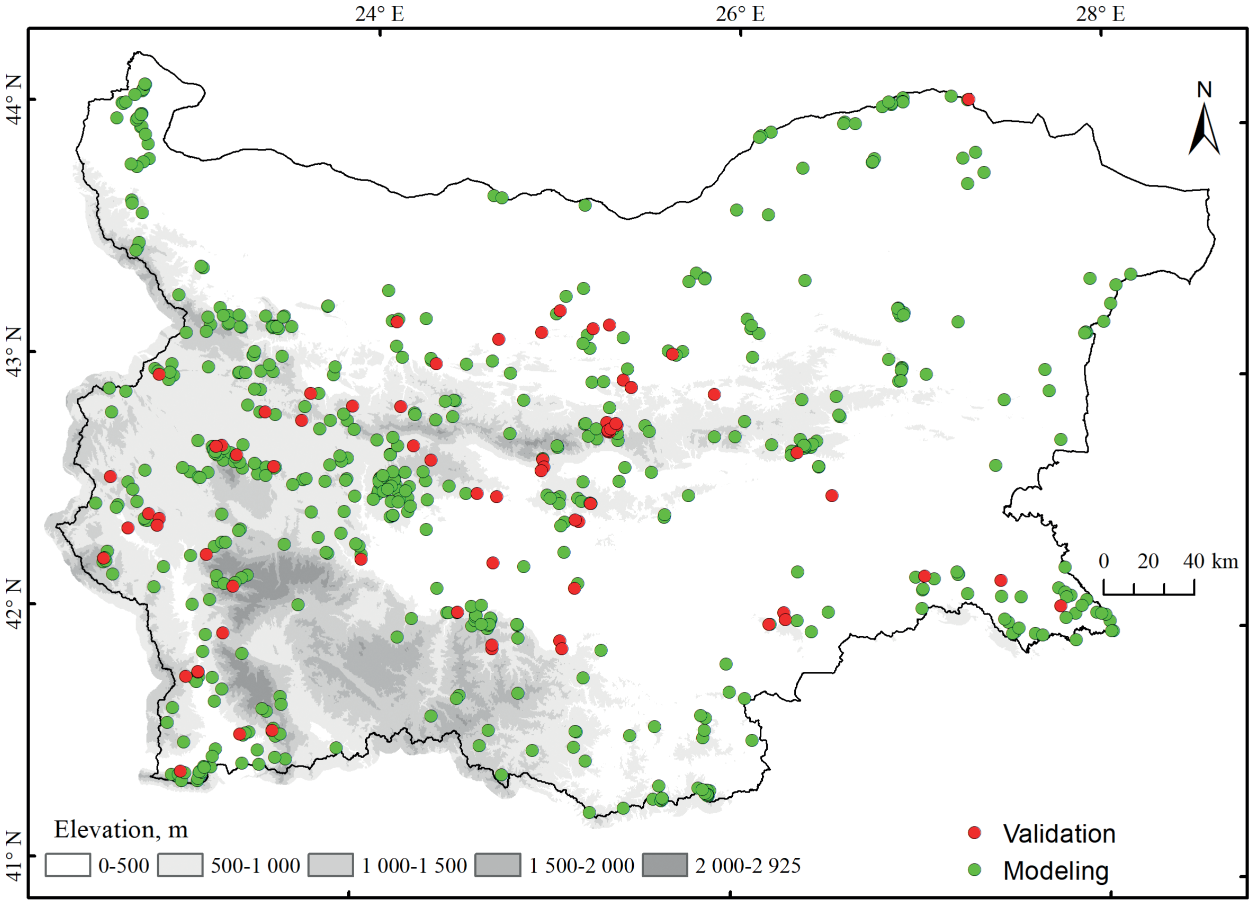


Figure 2. Map of the records used in generating and validating the environmental niche model of *Morimus asper* in Bulgaria.

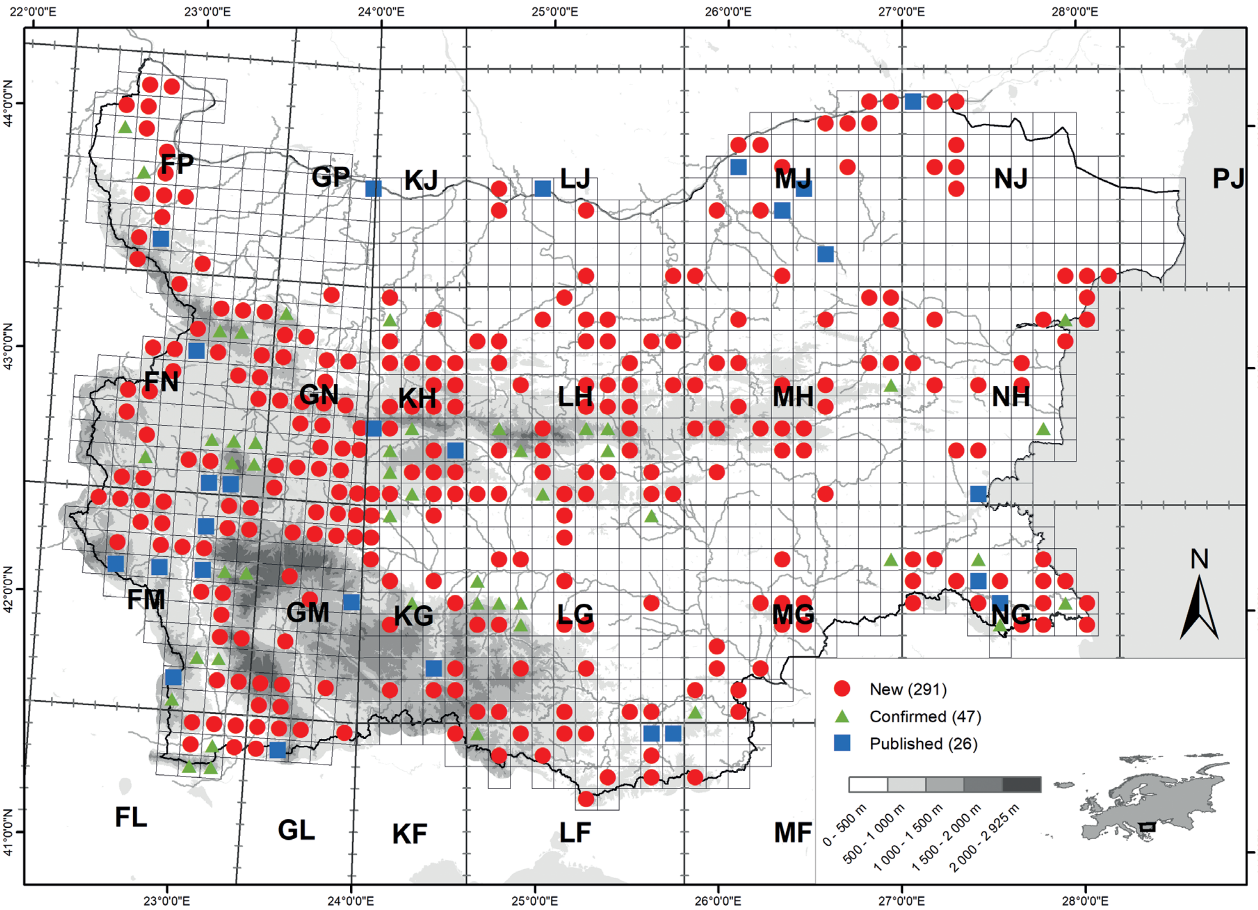


Figure 3. Distribution of the *Morimus asper* in Bulgaria based on a 10 km × 10 km MGRS grid.

Ecological Niche Model

The ENM predictive power was relatively high with AUC value (Area Under the Curve): 0.83 (SD = 0.06). The threshold for unsuitable/suitable area was 0.34.

Potentially suitable habitats for *M. asper* occupy 26% of Bulgaria (Fig. 4). The overall suitable area (29 059 km^2) was distributed into 5 573 km^2 of high suitability, 10 652 km^2 of medium suitability, and 12 834 km^2 of low suitability habitats. The most suitable areas were old mountain beech forests and lowland riparian forests. The predicted largest suitable habitats patches are the beech forest belt of Belasitsa Mts., the beech forest belt of Osogovo Mts., the deciduous forest belt of Pirin Mts., Rila Mts. and West Rhodopes Mts., high parts of Eastern Rhodopes, Vitosha Mts., Lozenska Mts., Sredna Gora Mts., the beech forest belt of Stara Planina Mts., Strandzha Mts., and the coastal floodplain forests. In northern Bulgaria the suitable areas are small and fragmented along the riparian forests. The suitable habitats along the Black Sea Coast are also highly fragmented; those are generally floodplain deciduous forests (longoz).

Overall, 50% of suitable territories, and 11% of the highest suitability habitat areas, fall within the terrestrial protected areas of the NATURA 2000 network in Bulgaria. An essential part of the territory of all National and Natural Parks in Bulgaria is suitable for *M. asper* presence according to the ENM (57% of their territory) which represents 9% of the total suitable area.

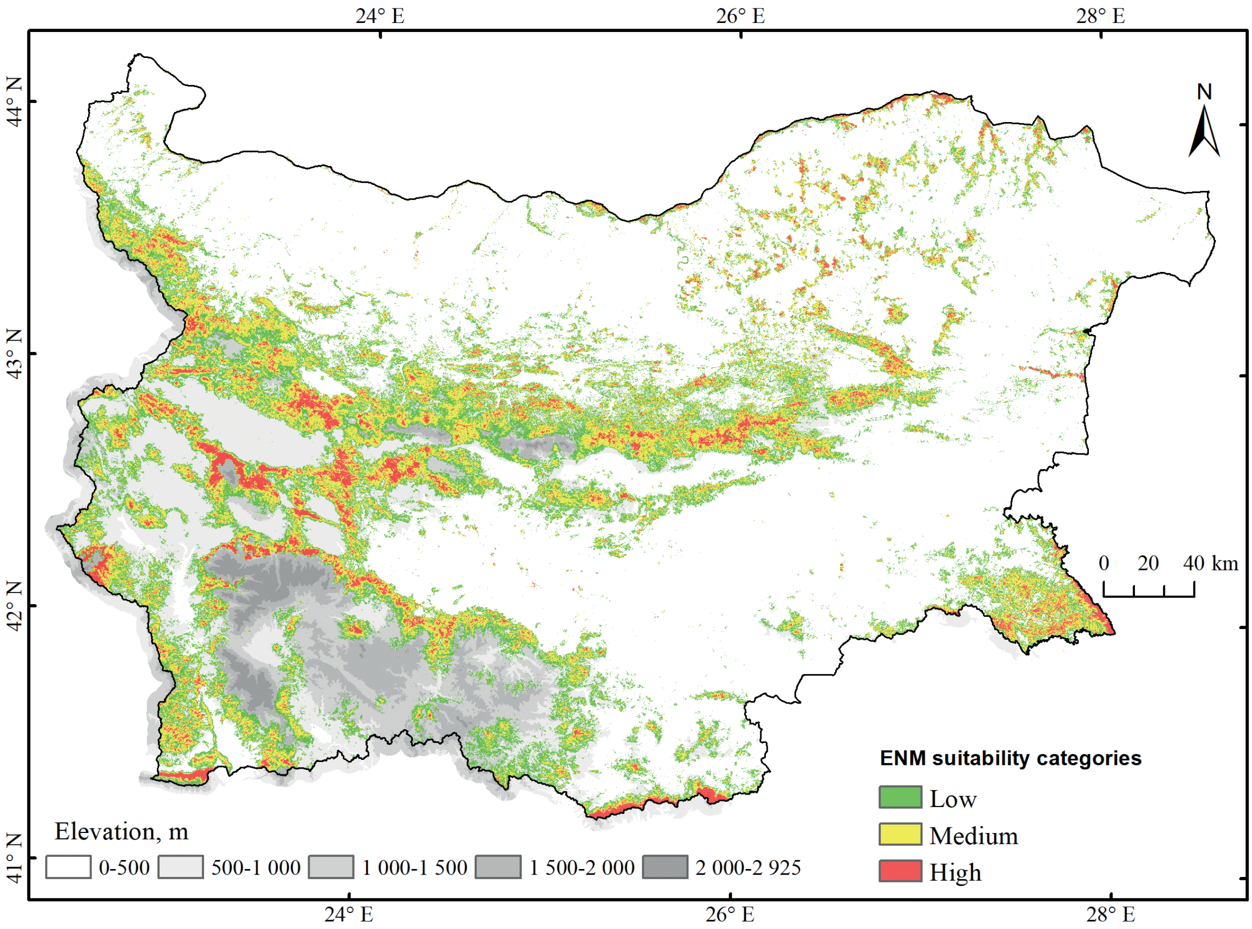


Figure 4. Map of the average Ecological Niche Model for *Morimus asper* in Bulgaria.

Estimates of the environmental variables' importance for the ENM

Both the percent contribution to the model and the Jackknife procedure were used in determining the importance of individual variables to the model. Both analyses showed as the most important predictors contributing to the model the distance to Fagus-Carpinus forest (40% contribution) and the average forest age (11% contribution). The remaining environmental factors contributed less than 10% each (Table 1). Although with much smaller contribution, according to the Jackknife test the next most important variables are the maximum temperature at the warmest month and elevation, and mean temperature at the warmest quarter (Table 1 and Fig. 5).

Main factors statistics

The mean values and variation in the main ecological factors contributing to the ENM of *M. asper* are shown in Table 2 and Fig. 6. The distances of precise observations to Fagus-Carpinus forests vary from 0 m to nearly 15 km, but the values' distribution is highly asymmetrical and most of the species' locations are indeed very close to the nearest pixel of the polygons – 50% of the species records are up to 57 m of the Fagus-Carpinus forest polygon and 90% of the records are up to 1.3 km. The average age of the forests (at which 90% of the *M. asper* records were located), was between 0–120 years, forming two peaks

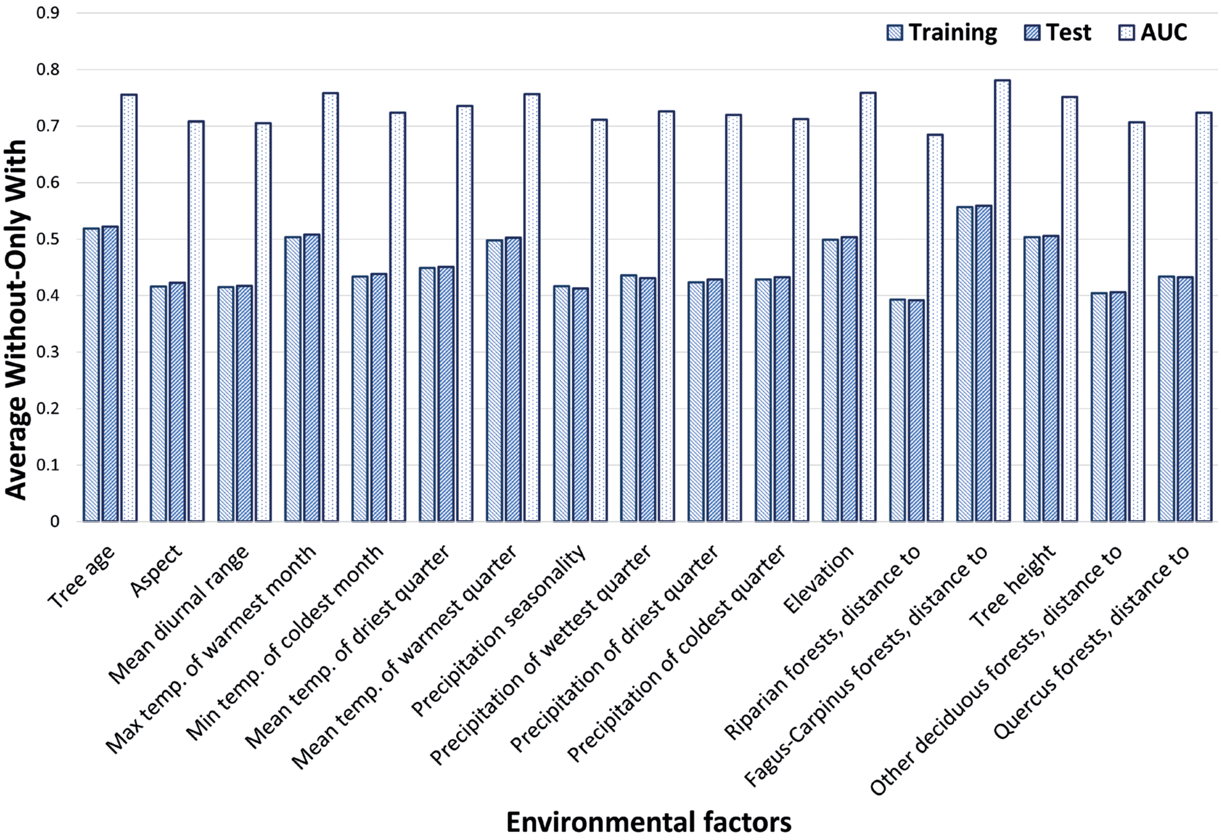


Figure 5. Jackknife results of MaxEnt ENM for *Morimus asper* in Bulgaria – average gain and area under the curve (AUC) on training and test data between models built without and only with a given variable. Variables that are more closely related to the distribution of the species are indicated by smaller differences in both gain and AUC.

Table 2. Descriptive statistics of the environmental variables with highest influence on the suitability of the habitats according to the ENM at the points of *Morimus asper* registrations.

Environmental variable (Unit)	Mean	Std Dev	Std. Error	Min	Max	Median
Fagus-Carpinus forests, distance to (m)	504.85	1286.66	66.71	0.00	14802.00	56.57
Average forest age (year)	54.42	46.11	2.39	0.00	170.00	55.00
Average tree height (m)	12.00	8.83	0.46	0.00	32.00	14.00
Maximum temperature at the warmest month (°C)	23.88	2.43	0.13	11.35	29.42	24.15
Elevation (m asl)	668.65	391.22	20.28	1.00	1663.0	645.00

– at 0 (records out of the forest stands) and at 40–70 years forests. The values of tree height were also asymmetrical, with a bimodal distribution forming one peak at 0 m and then between 15–20 m; most records (90%) are in forests with tree height up to 24 m. The frequencies' accumulation at zero values are due to many findings near the forests but not in them, normal for the beetle's dispersion during the mating season. The most frequent records (60%) of *Morimus* are at locations with maximum temperature in the warmest month between 22–26 °C, and 90% of them are at locations with maximum temperature in the warmest month up to 26.7 °C. The frequency distribution of the records related to the elevation is quite platykurtic, 70% were between 180 m and 1 000 m asl, and 90% were up to 1 200 m, which coincides with the deciduous forest belt. Still, the species reaches elevations of up to 1663 m.

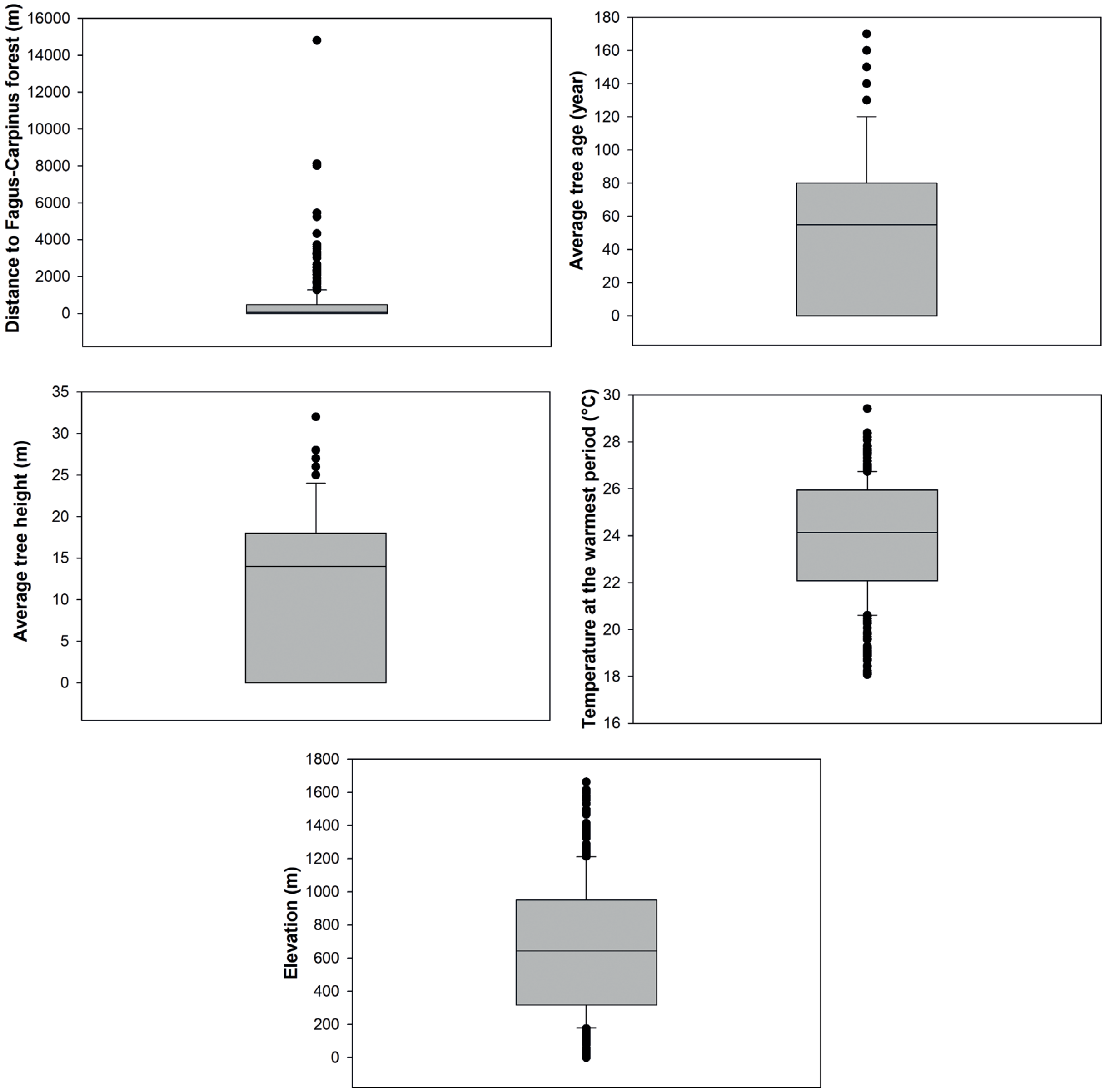


Figure 6. Variation of the main environmental factors contributing to the ENM of *Morimus asper* in Bulgaria.

The marginal response curves of the environmental factors showed that the probability of the species occurrence is maximal when the distance to Fagus-Carpinus forest is minimal, and sharply decreases with an increase in the distance. A positive relation between probability of occurrence and forest age and tree height was observed, as the probability sharply increases after 50–60 years forest age, after which the probability remains constant. The same tendency was observed for the average tree height factor – the probability increases abruptly up to 15 m, and more smoothly to 40 m, after which it remains constant. The probability of occurrence was maximal when maximum temperature of the warmest month ranges between 18–25 °C, most probable at 22 °C corresponding to the mountains, another probability maximum is at 30 °C corresponding to the lowland habitats. The response curve of the elevation showed that the probability of the species occurrence increases to 1 000 m, subsequently it gradually decreases, and after 2 000 m asl it is practically zero (Fig. 7).

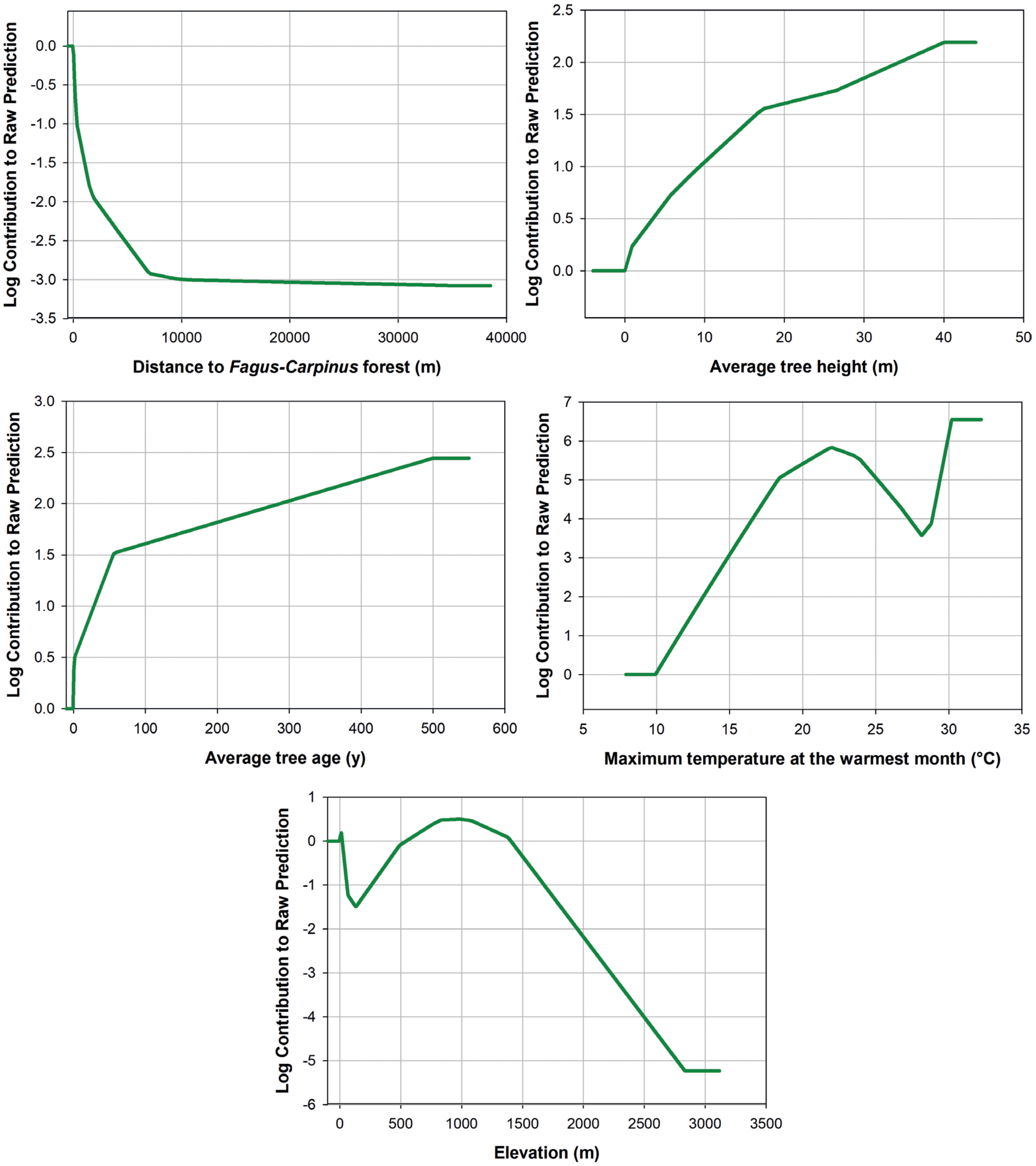


Figure 7. Marginal response curves of major variables contributing to the ENM of *Morimus asper* in Bulgaria.

Validation of the ENM

Overall, 84% of the records used for validating the ENM fall within predicted suitable habitats (57% of all) or were less than 250 m to the nearest (27% of all).

Discussion

The ENM of *M. asper* showed old growth forests dominated by *Fagus* sp. and *Carpinus* sp. with tall trees as the most suitable habitats for the species in Bulgaria, followed by other deciduous forests with much lower probability and riparian forests, in a broad altitudinal range (0–2 000 m asl). The highest probability of species occurrence was connected also with moderate air temperatures – maximum at 22 °C in the mountains, and 30 °C in the plains. The main predictors in the obtained ENM for *M. asper* in Bulgaria were different but in accordance with those obtained by Redolfi De Zan et al. (2023) in Italy, where the high autumn normalised difference vegetation index (correlated to biomass and canopy biophysical parameters such as photosynthetic activity), low autumn solar radiation and annual mean temperature between 7–13 °C were the main factors determining the species distribution. In our preliminary model, prior to the exclusion of this variable, contribution of the annual mean temperature in Bulgaria was 0.3%. However, comparing the response of the species to 'annual mean temperature (Bio01)' in Bulgaria and Italy, a common peak in the probability of occurrence (between 6–10 °C in Bulgaria) was observed, but the main difference is that in Bulgaria there is a second peak connected to much warmer territories, where the mean annual temperature is over 14 °C.

The generated ENM presented prominent ecological continuity of the suitable habitats in Central and Southwestern Bulgaria, and almost no continuity in northern and eastern Bulgaria with significant fragmentation of the suitable habitats. Special attention must be paid to the Black Sea Coast, where large patches of lowland and riparian forests still exist but are fragmented and under high threat because of anthropogenic pressures. The *M. asper* populations in those areas are quite isolated due to the low dispersal ability of the beetle (Rossi de Gasperis et al. 2016; Cateau et al. 2018).

The isolation of the suitable habitats in Strandzha Mts. according to the ENM gives a reasonable explanation for the distribution of the *M. asper orientalis* in Bulgaria, limited to that area. In addition, most of the records were from oriental beech woods in contrast to the statement of Bringmann (1996) that the species predominantly develops in oak wood.

The suitable habitats in the vicinity of the only known locality of *M. asper verecundus bulgaricus* are also highly isolated, possibly greatly limiting the subspecies' ability to disperse.

A large area of the potential optimal habitats falls within existing protected areas: National and Natural parks, as well as terrestrial NATURA 2000 sites, which theoretically provides sufficient capacity to protect *M. asper*. To ensure high genetic diversity and effective population size, however, their connectivity must be maintained through functioning, undisturbed biocorridors. Ensuring the continuity of optimal habitats for *M. asper* would allow the species to migrate and occupy new suitable habitats where it is not currently found. A measure in that direction was the proposed creation of "stepping stone" habitats for saproxylic beetles between larger conservation territories (parks, reserves, etc.), for example by retaining dead wood in managed forests along the designed dispersal routes (Bełcik et al. 2019). The protection of populations and habitats in the small suitable areas in northern Bulgaria and along rivers is of great importance for the preservation of the genetic diversity of *M. asper*. As many of these areas are poplar plantations that are cleared and then replanted, it is necessary to leave a certain percentage of dead wood in the felling, without disturbing the surrounding natural tree vegetation, before planting the new saplings. This measure would ensure a smoother and safer transition of the population from one habitat to another, given their relative low displacement capacity.

The favourable conservation status of *M. asper* populations in Bulgaria, assessed during the preparation of the specific reports under Art. 17 of the Habitat Directive in 2013 and 2019, should be maintained with the necessary measures to reduce the threats to the species, mainly concerning forest management practices. The availability of suitable dead wood is crucial for all saproxylic species and even a temporary lack could bring rapid population collapse (Seibold et al. 2015; Bełcik et al. 2019). The fragmentation of suitable habitats for *M. asper* has to be reduced to a minimum, maintaining well-connected natural and semi-natural forests with a diverse age structure, autochthonic deciduous tree species and sufficient number of mature and decaying old trees in various stages of aging. The recommended amount of dead wood has to be above 20–35 m^3/ha (or 3–8% of the total volume of available wood) and all dead trees larger than 22–50 cm in breast-height diameter not to be removed after felling, allowing more dead wood in advanced stages of decomposition to develop, as well as designating strict forest reserves with exceptionally high amounts of dead wood, that would serve as a refuge for and sources of saproxylic specialists such as *M. asper* (Gossner et al. 2013; Della Rocca et al. 2014).

An important step for the protection of suitable habitats for *Morimus* and the sustainable use of the forest resources in Bulgaria that has been taken is to FSC certify all state forestries. Unfortunately, the actual implementation of the measures related to this certificate are still not fully implemented, a fact that we have observed often during our field work. Another problem observed so far is the carrying out of the necessary monitoring activities of the insect species included as criteria in this certification, as well as the activities of carrying out the mandatory regular monitoring and reporting under Art.17 of the Habitat Directive to the EC. The monitoring activities conducted to date have not been regular and comprehensive. One of the main reasons for that is mainly the lack of capacity and human resources, and the resulting excessive engagements of scientists and forest workers, another is the still poorly developed citizen science in Bulgaria. Citizen science is a useful approach to solving this problem, accelerating the process of gathering occurrence data (Zapponi et al. 2017; Redolfi De Zan et al. 2023). It is especially suitable for relatively easy observation and identification of species such as *Morimus asper*.

In addition, we recommend changes in the monitoring scheme for *M. asper* of the National Biodiversity Monitoring System in Bulgaria. They include a shift from quantitative to qualitative monitoring with bigger sample size. The current methodology consists of walking along a transect (1 km long, 5 m wide) and counting the number of live individuals of the species for the given transect (Chehlarov 2014). The effectiveness of such monitoring would benefit from increased field effort, which has so far proven impossible as mentioned above, due to lack of sufficient time for sampling, finances, and human capacity. A good knowledge of the ecological requirements of the species is necessary also in order to plan field work at the optimal time and weather conditions, otherwise the reported number may not reflect the actual abundance of the species due to low detection probability. On the other hand, the qualitative monitoring requires less efforts at a single sampling site (transect) of observation and could allow covering a bigger area of monitoring with increased sample size. At the same time, the change in frequency of occurrence is a good early indicator for changes in population trends. If a negative trend in frequency is registered, it is necessary to carry out a quantitative study. In order for the results of the national monitoring to be compatible with those of the reports under the Habitat Directive of EEC, we propose the following changes in the monitoring scheme for standardization and easier data management: 1. Field observations should be carried out at least 8 of the 10 × 10 km squares of the ETRS grid that contain medium and highly suitable habitats according to the presented ENM. In each of the 10 km squares, between 10–30 sample sites with a size of 1 km × 1 km should be selected, in which 1 sampling unit – a transect with a length of 1 km should be sampled randomly. 2. Parameters to count: presence of the species (walking along the transect until its registration or the transect ends); reporting the presence of threats to the species for the entire sample site. 3. Assessment parameters: the proportion of the 1 km^2 squares with registered presence of the *M. asper* from the total number of sampled 1 km^2 squares; the proportion of 1 km^2 squares with registrations of the species-specific threats (listed in the standard protocol) from the total number of sampled squares. In such manner the monitoring scheme could easily incorporate citizen science data to become much more effective, although these data alone are not sufficient for the status of the species to be assessed.

To fill the gap in the scientific capacity and to develop citizen science in Bulgaria, it is necessary for a broad educational campaign to be carried out in partnership between the Bulgarian Ministry of Environment and Waters, the Executive Environment Agency, and scientific and educational institutions. Only this can ensure reliable forest certification and the implementation of measures for the protection of forest habitats and forest specialist species, including *Morimus asper*.

Acknowledgements

We thank all colleagues, friends, and members of the Bulgarian Facebook group “The insects and the entomologists” for sharing *Morimus asper* occurrence data.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

The research was supported by the project “Cybertaxonomic approach to phylogenetic studies of model invertebrate genera (Invertebrata, Arachnida, Insecta) clarifying the problems of origin, formation and conservation of the Invertebrate Fauna of the Balkan Peninsula” (National Science Fund, Ministry of Education and Science of the Republic of Bulgaria, Grant KP-06H21/1-17.12.2018).

Author contributions

Conceptualization: RK, RB. Data curation: RB. Formal analysis: GP, RK. Investigation: RB. Methodology: GP, YVK, RK. Resources: RB. Software: RK, GP. Validation: YVK, GP. Visualization: GP, RK. Writing – original draft: YVK, RK, RB. Writing – review and editing: GP, RB, YVK, RK.

Author ORCIDs

Rumyana Kostova https://orcid.org/0000-0002-8119-3275
Rostislav Bekchiev https://orcid.org/0000-0001-6143-0184
Georgi Popgeorgiev https://orcid.org/0000-0002-7625-8898
Yurii V. Kornilev https://orcid.org/0000-0002-8596-8728

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

References

Angelov PA (1964) Coleoptera aus der Thrakischen Tiefebene und Einigen Angrenzenden Gebieten. Bulgarische Akademie der Wissenschaften, Sofia, 307–324.

Angelov PA (1967) Beitrag zur Kenntnis der bulgarische Cerambyciden-Arten. Travaux scientifiques de l'Ecole Normale Superieure Paisii Hilendarski – Plovdiv 5: 113–128.

Anguélov P (1960) Etudes sur l'entomofaune du parc Delassement et culture Ostrova (l'île au milieu de Maritza) près de Plovdiv, avec quelques notes faunologiques. Annuaire des Musées de Plovdiv 3: 7–40.

Bechev D, Stojanova A (2004) Geographical localities of invertebrates of conservation importance in the Rhodopes (Bulgaria). Travaux Scientifiques d'Universite de Plovdiv, Biologie. Animalia 40: 19–25.

Beck HE, Zimmermann NE, McVicar TR, Vergopolan N, Berg A, Wood EF (2018) Present and future Köppen-Geiger climate classification maps at 1-km resolution. Scientific Data 5(1): e180214. https://doi.org/10.1038/sdata.2018.214

Bełcik M, Goczał J, Ciach M (2019) Large-scale habitat model reveals a key role of large trees and protected areas in the metapopulation survival of the saproxylic specialist Cucujus cinnaberinus. Biodiversity and Conservation 28(14): 3851–3871. https://doi.org/10.1007/s10531-019-01854-0

Bringmann HD (1996) Die Morimus- und Acanthoderes Arten Bulgariens (Col., Cerambycidae). Entomologische Nachrichten und Berichte 40: 237–239.

Cálix M, Alexander KNA, Nieto A, Dodelin B, Soldati F, Telnov D, Vazquez-Albalate X, Aleksandrowicz O, Audisio P, Istrate P, Jansson N, Legakis A, Liberto A, Makris C, Merkl O, Mugerwa Pettersson R, Schlaghamersky J, Bologna MA, Brustel H, Buse J, Novák V, Purchart L (2018) European Red List of Saproxylic Beetles. IUCN, Brussels, Belgium, 12 pp.

Cateau E, Herrault PA, Sheeren D, Ladet S, Brustel H (2018) The influence of spatial and temporal discontinuities of forest habitats on the current presence of flightless saproxylic beetles. PLoS ONE 13(5): e0197847. https://doi.org/10.1371/journal.pone.0197847

Chehlarov E (2014) Monitoring methodology of *Lucanus cervus*, *Morimus asper funereus*, *Rosalia alpina*, *Osmoderma barnabita*, order Coleoptera. Bulgaria, 14 pp.

Chorbadzhiev P (1932) Pests on the culture plants in Bulgaria during 1928–1929. Svedenia po zemedelieto 13: 3–64.

Csiki E (1943) Coleopteren von Alibotusch-Gebirge in Süd-Bulgarien. Mitteilungen aus den Königlichen Naturwissenschaftlischen Instituten, Sofia 16: 214–218.

Danilevsky M (2023) Palaearctic Chrysomeloidea catologue (Vesperidae, Disteniidae, Cerambycidae). 235 pp.

Danilevsky M, Gradinarov D, Sivilov O (2016) A new subspecies of *Morimus verecundus* (Faldermann, 1836) from Bulgaria and a new subspecies of *Morimus asper* (Sulzer,

1776) from Greece (Coleoptera, Cerambycidae). Humanity space International almanac 5: 187–191.

Della Rocca F, Stefanelli S, Pasquaretta C, Campanaro A, Bogliani G (2014) Effect of deadwood management on saproxylic beetle richness in the floodplain forests of northern Italy: Some measures for deadwood sustainable use. Journal of Insect Conservation 18(1): 121–136. https://doi.org/10.1007/s10841-014-9620-1

Doychev D, Topalov P, Zaemdjikova G, Sakalian V, Georgiev G (2017) Host plants of xylophagous longhorn beetles (Coleoptera: Cerambycidae) in Bulgaria. Acta Zoologica Bulgarica 69: 511–528.

Doychev D, Topalov P, Zaemdzhikova G, Sakalian V, Georgiev G (2018) Addition to xylophagous longhorn beetles (Coleoptera: Cerambycidae) host plants in Bulgaria. Silva Balcanica 19: 47–54.

Fick SE, Hijmans RJ (2017) WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37: 4302–4315. https://doi.org/10.1002/joc.5086

EIONET Central Data Repository (2023) EIONET Central Data Repository.

Fick SE, Hijmans RJ (2017) WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37: 4302–4315. https://doi.org/10.1002/joc.5086

Georgiev G, Hubenov Z (2006) Vertical distribution and zoogeographical characteristics of Cerambycidae (Coleoptera) family in Bulgaria. Acta Zoologica Bulgarica 58: 315–343.

Georgiev G, Doychev D, Migliaccio E (2005a) Studies on Cerambycid fauna (Coleoptera: Cerambycidae) in Western Rhodopes in Bulgaria. Forest Science 2: 81–90.

Georgiev G, Simov N, Stojanova A, Doychev D (2005b) New and interesting records of longhorn beetles (Coleoptera: Cerambycidae) in some Bulgarian Mountains. Acta Zoologica Bulgarica 57: 131–138.

Georgiev G, Migliaccio E, Doychev D (2006) Longhorn beetles (Coleoptera: Cerambycidae) in Western Rhodopes (Bulgaria). In: Beron P (Ed.) Biodiversity of Bulgaria 2 Biodiversity of Western Rhodopes (Bulgaria and Greece). Pensoft, Sofia, 347–360.

Georgiev G, Doychev D, Simov N, Guéorguiev B, Bekchiev R (2013) Contribution to the knowledge to the of Cerambycid fauna (Coleoptera: Cerambycidae) of Belasitsa Mountain in Bulgaria. Silva Balcanica 14: 109–116.

Georgiev G, Gjonov I, Sakalian V (2015) New records of longhorn beetles (Coleoptera: Cerambycidae) in Strandzha Mountain. Journal of Entomological Research Society 17: 73–88.

Georgiev G, Gradinarov D, Gjonov I, Sakalian V (2018) A Check list and areography of longhorn beetles (Coleoptera:Cerambycidae) in Strandzha Mountain – Bulgaria and Turkey. Silva Balcanica 19: 89–116.

Georgiev G, Gradinarov D, Sivilov O, Gjonov I, Doychev D, Gashtarov V, Cvetkovska, Gjorgjievska A, Sakalian V (2019) A check list and areography of longhorn beetles (Coleoptera: Cerambycidae) in Belasitsa Mountain, Bulgaria and North Macedonia. ZooNotes (Supplement 8): 1–27. http://www.zoonotes.bio.uni-plovdiv.bg/Supplements/ZooNotes_Suppl%208_Cerambicidae.pdf

Gossner MM, Lachat T, Brunet J, Isacsson G, Bouget C, Brustel H, Brandl R, Weisser WW, Muller J (2013) Current near-to-nature forest management effects on functional trait composition of saproxylic beetles in beech forests. Conservation Biology 27(3): 605–614. https://doi.org/10.1111/cobi.12023

Guéorguiev B (2001) Beetles (Insecta: Coleoptera) in Kresna Gorge (SW Bulgaria). National Museum of Natural History-BAS, Sofia, 163–188.

Guéorguiev B (2018) Coleoptera (Insecta) collected or observed around Chelopech Village, Western Bulgaria. Historia Naturalis Bulgarica 34: 1–8. https://doi.org/10.48027/hnb.34.01001

Guéorguiev B, Ljubomirov T (2009) Coleoptera and Hymenoptera (Insecta) from Bulgarian section of Maleshevska Planina Mountain: Study of an until recently unknown biodiversity. Acta Zoologica Bulgarica 61: 235–276.

Guéorguiev B, Georgiev G, Mirchev P, Tsankov G (2003) Four new coleopteran species (Insecta: Coleoptera) for Bistrishko branishte biosphere reserve in Vitosha Mountain, Bulgaria. Acta Zoologica Bulgarica 55: 107–112.

Hardersen S, Bardiani M, Chiari S, Maura M, Maurizi E, Roversi PF, Mason F, Bologna MA (2017a) Guidelines for the monitoring of *Morimus asper funereus* and *Morimus asper asper*. Nature Conservation 20: 205–236. https://doi.org/10.3897/natureconservation.20.12676

Hardersen S, Cuccurullo A, Bardiani M, Bologna MA, Maura M, Maurizi E, Roversi PF, Sabbatini Peverieri G, Chiari S (2017b) Monitoring the saproxylic longhorn beetle *Morimus asper*: Investigating season, time of the day, dead wood characteristics and odour traps. Journal of Insect Conservation 21(2): 231–242. https://doi.org/10.1007/s10841-017-9970-6

Heyrovský L (1931) Beitrag zur Kenntnis der bulgarischen Cerambyciden. Mittelungen aus den Koniglichen naturwissenschaftlichen Instituten in Sofia – Bulgarien 4: 78–86.

Hubenov Z, Beshovski V, Iosifov M, Popov A, Kuntanski K, Sakalian V, Abadjiev S, Vidinova Y, Lyuhontirov T (2000) Entomofaunistic diversity of the Rila National Park. In: Sakalian M (Ed.) Biological Diversity of the Rila National Park. Pensoft, Sofia, 285–464.

Jugovic J, Pogorelec N, Lužnik M (2022) Habitat preferences, dispersal and demography of two saproxylic longhorn beetles (Cerambycidae: *Rosalia alpina*, *Morimus asper funereus*): A case study from the Julian Alps, Slovenia. Research Square, Preprint (Version 1), 1–14. https://doi.org/10.21203/rs.3.rs-1764780/v1

Jurc M, Ogris N, Pavlin R, Borkovic D (2008) Forest as a habitat of saproxylic beetles on Natura 2000 sites in Slovenia. Revue d'Écologie 63(1): 53–66. https://doi.org/10.3406/revec.2008.1462

Kantardjiewa-Minkova S (1934) Die Arten der Familie Cerambycidae (Col.). 11. (Lamiinae). Bulletin de la Societe Entomologique de Bulgarie 8: 132–144.

Kodzhabashev N, Penev L (1998) Order Coleoptera. Context & Pensoft, Sofia, 130 pp.

Kornilev Y, Popgeorgiev G, Naumov B, Stoyanov A, Tzankov N (2017) Updated distribution and ecological requirements of the native freshwater turtles in Bulgaria. Acta Zoologica Bulgarica 10: 65–76.

Kostova R, Bekchiev R, Beshkov S (2019) Coleoptera and Lepidoptera (Insecta) diversity in the central part of Sredna Gora Mountains (Bulgaria). Bulletin of the Entomological Society of Malta 10: 75–95.

Kovachev V (1905) Contribution pour l'etude de la faune entomologique de Bulgarie. Godishnik na rusenskata gimnazia: 3–12.

Leonarduzzi G, Onofrio N, Bardiani M, Maurizi E, Zandigiacomo P, Bologna MA, Hardersen S (2017) Attraction of different types of wood for adults of *Morimus asper* (Coleoptera, Cerambycidae). Nature Conservation 19: 135–148. https://doi.org/10.3897/natureconservation.19.12659

Liu C, Berry PM, Dawson TP, Pearson RG (2005) Selecting thresholds of occurrence in the prediction of species distributions. Ecography 28(3): 385–393. https://doi.org/10.1111/j.0906-7590.2005.03957.x

Liu C, Newell G, White M (2015) On the selection of thresholds for predicting species occurrence with presence-only data. Ecology and Evolution 6(1): 337–348. https://doi.org/10.1002/ece3.1878

Manu M, Lotrean N, Nicoară R, Bodescu F, Badiu DL, Onete M (2017) Mapping analysis of saproxylic Natura 2000 beetles (Coleoptera) from the Prigoria-Bengeşti Protected Area (ROSCI0359) in Gorj County (Romania). Travaux du Muséum National d'Histoire Naturelle. Grigore Antipa 60: 445–462. https://doi.org/10.1515/travmu-2017-0012

Manu M, Băncilă RI, Lotrean N, Badiu D, Nicoară R, Onete M, Bodescu F (2019) Monitoring of the saproxylic beetle *Morimus asper funereus* Mulsant, 1862 (Coleoptera: Cerambycidae) in Măcin Mountains National Park, Romania. Travaux du Muséum National d'Histoire Naturelle. Grigore Antipa 62: 61–79. https://doi.org/10.3897/travaux.62.e38591

Markovich A (1904) Contribution for insect fauna in the neighborhood of Razgrad Town. Traveaux de la société bulgare des sciences naturelles 2.

Markovich A (1909) Contribution for insect fauna in the neighborhood of Razgrad Town. Sbornik za umotvorenia, nauka i knizhnina, Sofia 25: 3–20.

Migliaccio E, Georgiev G, Mirchev P (2004) Studies on Cerambycid fauna (Coleoptera: Cerambycidae) of the Vitosha Mountain, Bulgaria. Acta Zoologica Bulgarica 56: 137–144.

Migliaccio E, Georgiev G, Gashtarov V (2007) An annotated list of Bulgarian Cerambycids with special view on the rarest species and endemics (Coleoptera: Cerambycidae). Lambillionea 107: 3–79.

Nedelkov N (1906) Contribution to the entomological fauna of Bulgaria. Periodichno spisanie na balgarskoto knizhovno druzhestvo 66: 404–439.

Nedelkov N (1909a) Fifth contribution to the entomological fauna of Bulgaria. Sbornik za umotvorenia, nauka i knizhnina, Sofia 25: 3–37.

Nedelkov N (1909b) Fourth contribution to the entomological fauna of Bulgaria. Sbornik za umotvorenia, nauka i knizhnina, Sofia 25: 3–32.

Netolitzky F (1912) Eine sammelreise nach Bulgarien. Coleopterologische Rundschau 1: e156163.

Nieto A, Alexander KNA (2010) European Red List of Saproxylic Beetles. Publications Office of the European Union, Luxembourg, 43 pp.

Palm T (1966) På koleopterologiska excursioner vid Bulgariens Svarta havskust. Entomologisk Tidskrift 87: 5–22.

Phillips SJ, Anderson RP, Dudík M, Schapire RE, Blair ME (2017) Opening the black box: An open-source release of MaxEnt. Ecography 40(7): 887–893. https://doi.org/10.1111/ecog.03049

Popgeorgiev G, Spasov S, Kornilev Y (2015) SmartBirds – Information system with biological information. http://www.smartbirds.org [Accessed on: 2019-9-20]

Rapuzzi P, Georgiev G (2007) Contribution to the knowledge of species composition and regional distribution of longhorn beetles (Coleoptera: Cerambycidae) in Bulgaria. Acta Zoologica Bulgarica 59: 253–266.

Redolfi De Zan L, Rossi de Gasperis S, Andriani V, Bardiani M, Campanaro A, Gisondi S, Hardersen S, Maurizi E, Mosconi F, Nardi G, Zapponi L, Rombolà P, Romiti F (2023) The big five: Species distribution models from citizen science data as tool for preserving the largest protected saproxylic beetles in Italy. Diversity 15(1): 96. https://doi.org/10.3390/d15010096

Rossi de Gasperis S, Passacantilli C, Redolfi De Zan L, Carpaneto GM (2016) Overwintering ability and habitat preference of *Morimus asper*: A two-year mark-recapture study with implications for conservation and forest management. Journal of Insect Conservation 20(5): 821–835. https://doi.org/10.1007/s10841-016-9913-7

Roubal J (1931) Fragmente zur Koleopterenfaunistik des balkanischen Festlandes. Entomologischer Anzeiger 11: 311–312. [362–363, 393–396, 437–438, 453–454.]

Samuelian S (1998) Species of family Cerambycidae, Coleoptera found at Jundola (near Velingrad). Acta Entomologica Bulgarica 1: 39–42.

Seibold S, Brandl R, Buse J, Hothorn T, Schmidl J, Thorn S, Müller J (2015) Association of extinction risk of saproxylic beetles with ecological degradation of forests in Europe. Conservation Biology 29(2): 382–390. https://doi.org/10.1111/cobi.12427

Siering G, Beier W (2005) Notizen zur Verbreitung von Bockkaefern (Col., Cerambycidae) im Suedosten Bulgariens. Teil I. Entomologische Nachrichten und Berichte 49: 223–225.

Simeonov Y, Totzev M (1997) Morphometrics. In: Jordanova M, Donchev D (Eds) Geography of Bulgaria Physical Geography Socio-economic Geography Prof. Marin Drinov Academic Publishing House, Sofia, 49–54.

Solano E, Mancini E, Ciucci P, Mason F, Audisio P, Antonini G (2013) The EU protected taxon *Morimus funereus* Mulsant, 1862 (Coleoptera: Cerambycidae) and its western Palaearctic allies: systematics and conservation outcomes. Conservation Genetics 14(3): 683–694. https://doi.org/10.1007/s10592-013-0461-3

Topalov P, Doychev D, Simov N, Sakalian V, Georgiev G (2014) New records of longhorn beetles (Coleoptera: Cerambycidae) in Vitosha Mountain. Forest Science 1/2: 95–102.

Trabucco A, Zomer R (2019) Global Aridity Index and Potential Evapotranspiration (ET0) Climate Database v2. figshare. Fileset. https://doi.org/10.6084/m9.figshare.7504448.v3

Tzonev R, Dimitrov M, Chytrý M, Roussakova V, Dimova D, Gussev C, Pavlov D, Vulchev V, Vitkova A, Gogoushev G, Nikolov I, Borisova D, Ganeva A (2006) Beech forest communities in Bulgaria. Phytocoenologia 36(2): 247–279. https://doi.org/10.1127/0340-269X/2006/0036-0247

Veen P, Fanta J, Raev I, Biriş I-A, de Smidt J, Maes B (2010) Virgin forests in Romania and Bulgaria: Results of two national inventory projects and their implications for protection. Biodiversity and Conservation 19(6): 1805–1819. https://doi.org/10.1007/s10531-010-9804-2

Vrezec A, de Groot M, Kobler A, Ambrožič Š, Kapla A (2014) Ecological characteristics of habitats and potential distribution of selected qualification species of beetles (Coleoptera) in the scope of Natura 2000 network in Slovenia: The first modelling approach. Gozdarski Vestnik 72: 452–471.

World Conservation Monitoring Centre (1996) *Morimus funereus*. The IUCN Red list of threatened species 1996: e.T13875A4359886. https://doi.org/10.2305/IUCN.UK.1996.RLTS.T13875A4359886.en [Accessed on 13 February 2023]

Yoakimoff D (1899) Prinos kam faunata ot nasekomi na Rila planina (Contribution to the insect fauna of Rila Mountain). Periodichesko spisanie na Balgarskoto knizhovno druzhestvo [Periodical Journal of the Bulgarian Literary Society] 60: 858–884.

Yoakimov D (1904) Contribution to the bulgarian insect fauna. Coleoptera. Sbornik za umotvorenia, nauka i knizhnina, Sofia 20: 1–43.

Zapponi L, Cini A, Bardiani M, Hardersen S, Maura M, Maurizi E, Redolfi De Zan L, Audisio P, Bologna MA, Carpaneto GM, Roversi PF, Sabbatini Peverieri G, Mason F, Campanaro A (2017) Citizen science data as an efficient tool for mapping protected saproxylic beetles. Biological Conservation 208: 139–145. https://doi.org/10.1016/j.biocon.2016.04.035

Supplementary material 1

Morimus asper occurrence in Bulgaria data set

Authors: Rostislav Bekchiev, Georgi Popgeorgiev, Rumyana Kostova, Yurii V. Kornilev

Data type: occurences

Explanation note: Data set with locations of *Morimus asper* in Bulgaria. Includes occurrence data from literature, field studies and citizen science incorporated into the SmartBirds database, as well as occurrence data from GBIF used to create a distribution map of the species, *M. asper* Ecological Niche Model and its verification.

Link: https://doi.org/10.3897/natureconservation.53.104243.suppl1

Nature Conservation

Nature Conservation 53: 61–83 (2023)
DOI: 10.3897/natureconservation.53.85421

Research Article

Coastal beaver, Chinook, coho, chum salmon and trout response to nearshore changes resulting from diking and large-scale dam removals: synergistic ecosystem engineering and restoration in the coastal zone

J. Anne Shaffer[1,2], Dave Parks[1], Katrina Campbell[1], Anna Moragne[1], Bligh Hueske[1,2], Pamela Adams[1], Jenise M. Bauman[2]

1 *Coastal Watershed Institute (CWI) PO Box 266, Washington 98362, Port Angeles, USA*
2 *Western Washington University, College of the Environment, 516 High St, WA 98225, Bellingham, USA*
Corresponding author: J. Anne Shaffer (anne.shaffer@coastalwatershedinstitute.org)

Abstract

In this paper we assess long-term trends and habitat changes to understand the relationships between coastal beaver (*Castor canadensis*), salmon, shoreline alterations, large-scale dam removals and nearshore ecological restoration. From this work we conclude that the removal of two large scale dams in the Elwha River has benefited beaver use of the coastal zone through water quality changes that allow beaver to re-establish high-quality zones and the expansion of riparian zones that provide extensive new food resources to beaver. However, the lower river hydrodynamic processes continue to be disrupted by a 200-meter earthen dike installed by local government and landowners for flood protection in the Elwha coastal zone in the 1960's. The dike acts as a driver of lower river geomorphology and has resulted in the formation of a large and persistent lateral bar along the lower river channel. Associated disrupted hydrodynamics are causing a critical coastal zone of the unimpounded lower river side channels to fill in. This channel habitat has decreased by 23%, with an annual average shrinkage rate of 13%, from pre-dam removal size, resulting in a decrease in both quality and quantity of nursery function for juvenile wild fish in a coastal zone that was historically documented to be the highest functioning for endangered juvenile salmon and trout. Inversely, physical changes including improved water quality in the adjacent impounded west side channel and continued expansion of riparian vegetation along the west delta lateral bar benefitted coastal beaver that recolonized the west delta after dam removals. The newly colonized coastal beaver may provide ecological engineering services to offset side channel loss as well as promote continued fish access. However recreational use was found to negatively impact beaver use of the area. We therefore recommend a series of additional ecosystem restoration actions that incorporate beaver as an ecosystem restoration component of the coastal zone. These actions include a public outreach program to encourage passive recreation measures to prevent negative impacts to beaver, and legacy, ecosystem scale restoration projects that reconnect the hydrodynamics of the west delta to complete Elwha ecosystem restoration. Together, these steps, if implemented, will result in a synergistic ecosystem restoration throughout the watershed to the benefit of the coastal ecosystem, including both beaver and salmon, as intended by the large-scale dam removal project.

Academic editor: Mathias Scholz
Received: 17 April 2022
Accepted: 24 June 2023
Published: 18 July 2023

ZooBank: https://zoobank.org/D5C91245-7DBC-4978-A465-E9248413DD7B

Citation: Shaffer JA, Parks D, Campbell K, Moragne A, Hueske B, Adams P, Bauman JM (2023) Coastal beaver, Chinook, coho, chum salmon and trout response to nearshore changes resulting from diking and large-scale dam removals: synergistic ecosystem engineering and restoration in the coastal zone. Nature Conservation 53: 61–83. https://doi.org/10.3897/natureconservation.53.85421

Key words: hydrodynamics, marine ecology, shoreline impediments

---

Introduction

Beavers are documented to be ecosystem engineers that are important for the restoration of watershed functions and provide invaluable ecosystem services including fish and wildlife habitat, flood mediation, water quality improvements, and fire management (Brazier et al. 2021). The role of beaver (*Castor canadensis*) in nearshore ecosystem conservation and restoration is an emerging topic. Hood (2012) and Hood and Larson (2014) documented that estuaries with beaver dams produced significantly more juvenile Chinook (*Oncorhynchus tshawytscha*) and coho (*Oncorhynchus kisutch*) salmon than coastal regions without beaver dams. Concomitantly, the removal of human-built large-scale in river dams is emerging as an important restoration action to restore watershed ecosystem functions, and juvenile fish use as an important and useful metric for understanding these ecosystem functions (Shaffer et al. 2009, 2017a). However, the relationships between coastal ecosystem restoration responses to large-scale dam removals and beaver, including in the nearshore, have not been defined.

Located in Washington State, USA, the Elwha River is the site of the largest dam removal restoration to date (Magirl et al. 2015). The Elwha River is 72 km long and falls 1372 m in elevation as it flows from the glaciated Olympic Mountains north to discharge into the Strait of Juan de Fuca. The majority of the river and watershed is located within the federally protected Olympic National Park, however the lower linear 12 km of river and coastal shoreline are not protected federally and instead have a myriad of private, Tribal, and public ownership (Fig. 1). The use of the delta coastline is primarily residential and recreational. The Elwha ecosystem, including beaver and salmon, was catastrophically impacted by the installation of two large-scale dams at the turn of the century (Winter and Crain 2008). Removal of the two large scale dams began in 2011 and ended in 2014. Dam removal resulted in extensive riverbed changes including evolution of braiding and bars in the lower river (Draut and Ritchie 2015). These include lateral bars, defined by Riverstyle (2022) as “a bank-attached unit bar that develops along low-sinuosity reaches of gravel- and mixed-bed channels with a bar surface that is generally inclined gently towards the channel”. The riverbed along the delta raised by over a meter and the river mouth extended upwards of 300 meters offshore (Ritchie et al. 2018).

Post-dam removal restoration goals were framed around restoring wildlife populations which included understanding baseline distribution and abundance of terrestrial fauna and describing the role of anadromous salmon on trophic riverine structures. Knapp (2009) surveyed beaver of the entire Elwha watershed and found numerous cuttings, but only one beaver dam and two bank lodges that were located along the lower reach of the eastern shore of the entire 72 km long watershed. After the two dams were removed McCaffery et al. (2018) documented beaver activity on both reservoir beds and developed a framework predicting wildlife response, including beaver, and their functional roles within large-scale restoration. However, a decade after dam removal, beaver response in the Elwha nearshore is largely undefined.

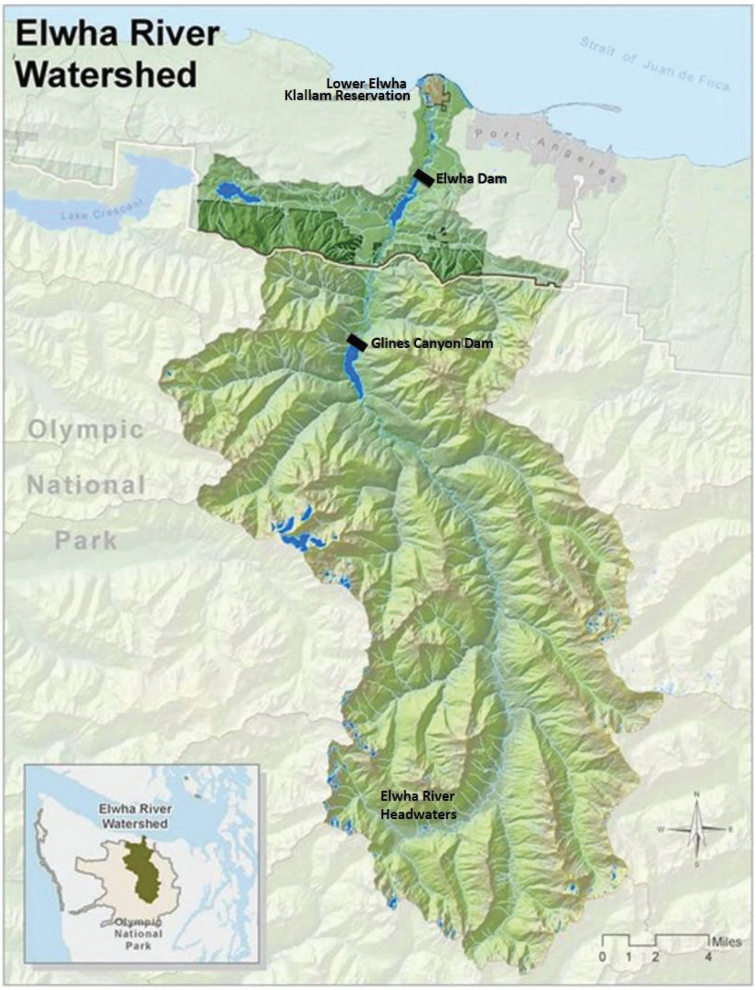


Figure 1. Elwha watershed in Washington State. The headwaters of the Elwha River reside 72 km within the Olympic Mountains where it flows south to north into the Strait of Juan de Fuca. Image maps the placement of two former dams, the formerly 32 m high Elwha dam located 8 km and the formerly 64 m high Glines Canyon dam located 22 km from the river mouth. Base map provided by Olympic National Park.

The nearshore of the Elwha is a critical component of the Elwha watershed. The Elwha delta consists of three zones: the east and west delta (which are separated by the Elwha River), and within the west delta, two zones, termed the impounded zone (also termed 'impounded Place Pond'), due to a 200-meter-long earthen dike, and the un-impounded west delta zone (Fig. 2). Shaffer et al. (2009) documented that proportionally, the Elwha west delta and side channels supported the majority of juvenile fish, more than 90% of all juvenile salmon across the entire Elwha delta. The west delta was therefore identified as one of the most important fish habitats in the Elwha delta nearshore, and thus is one of the highest priorities for additional monitoring and restoration actions in the Elwha watershed (Shaffer et al. 2017a). The adjacent impounded zone of the west Elwha delta was found to also be highly functioning for fish, but inaccessible to salmonids due to the dike. The impounded west delta was also defined by persistent green algae (*Ulva* spp.), from here forward called 'macroalgae blooms', that were a product of a combination of saline conditions, lack of circulation and nutrient loading from residential lawn treatment and septic systems (Nelson and Lucas 2011).

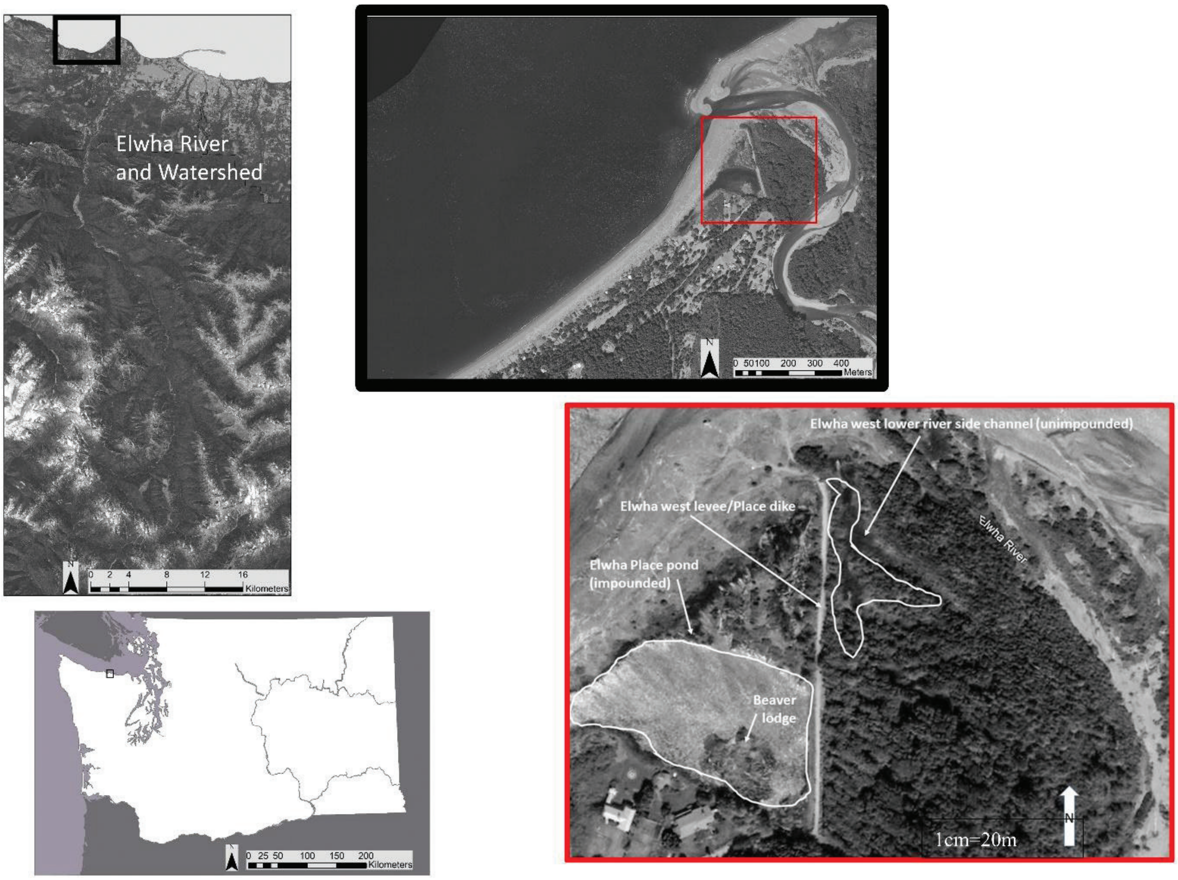


Figure 2. Figure of Elwha watershed, delta, and west delta including un-impounded west side channel and impounded west delta (Place Pond) study area.

The dramatic changes in the riverbed, delta extent, and water quality also resulted in changes in Elwha riparian corridors including the lower river and west delta (Fuentes et al. 2011; Foley et al. 2015; Warrick et al. 2015; Shaffer et al. 2017a, b; Prach et al. 2019). It is unclear how removing large dams will restore pre-dam communities important for beaver habitat when faced with the potential of nonnative plant species invading the newly deposited sediments (Shafroth et al. 2011). Brown et al. (2022) reported lower native plant species richness downstream from the two dams along the Elwha River; however, while dam removal caused species richness to increase in the middle reach, it did not increase within the lower river reaches within five years after dam removal. The authors speculate that the reconnection of the river channel through sediment transport and increased hydrochory may increase species richness over time in the lower river segments. Nonnative species richness or cover did not appear to be affected by the dams or dam removal but they increased 32% and 36% respectively from 2005 to 2017 (Brown et al. 2022). This suggests that nonnative species invasion could become a problem for the large, persistent lateral bar along the public, west lower river channel and delta that is the focus of our study. Early detection and rapid removal of exotic species will be required to ensure the recovery of habitat most conducive for beaver activity.

To date, no detailed study has been undertaken to understand the coastal beaver response to large-scale dam removals or the relationship between coastal beaver, shoreline impediments, dam removals, and salmon recovery.

In this study we provide an overview of the key post-dam removal physical and ecological changes to the Elwha west delta, including the delta extent and associated riparian vegetation; changes in water quality and macroalgae blooms in the impounded area of the west Elwha delta, changes in water quality, topography, and fish use of the un-impounded west side channel of the Elwha delta, recreational use of the Elwha west delta site, and how these are related to beaver use of the Elwha west delta coastal zone. We use a series of field studies and mapping to test our hypothesis that dam removals alone were not driving physical and ecosystem changes along the lower west delta of the Elwha.

We conclude that a series of factors, not just dam removals, are synergistically contributing to the Elwha west delta ecosystem. Specifically: 1. The west Place dike is playing a role in defining changes in lower river physical attributes including profiles, area, and water quality; 2. These changes are in turn affecting the ecological function of the west lower Elwha lateral bar and unimpounded west side channel extent, including riparian plant composition and cover, juvenile salmon and trout, and beaver use; 3. Riparian vegetation extent and composition changes post-dam removal are related to lateral bar extent, and may be contributing to beaver recolonization; 4. Beaver recolonization of the west delta is positively related to habitat changes driven by water quality changes documented to be associated with dam removals, but may be offset/ challenged by human use and; 5. Using juvenile fish abundance over time as an indicator of ecological function of the unimpounded west side channel, there has been a decline in habitat function of the un-impounded west delta due to the site shrinking and the concomitant decrease in the habitat quality for fish due to an increase in temperature and a decrease in dissolved oxygen. Finally, we discuss the future relationship the beaver may play in sustained ecosystem function of this important and evolving nearshore region given the persistent impaired nature of hydrodynamic function of the Elwha delta nearshore, and steps to support beaver in this role.

Methods

Physical changes to the Elwha delta

A. Elwha-west side channel topography: The topography and areal extent of the Elwha west side-channel between 2002 and 2018 was mapped using LiDAR bare earth digital elevation models (DEMs) in ArcGIS 10.6.1. A topographic cross-section from east to west across the side-channel was evaluated using the 3-D Analyst extension in ArcGIS 10.6.1 to map the extent of channel in-filling before and after dam removals on the Elwha River (Fig. 2).

B. Elwha-west side channel habitat mapping: The wetted area of un-impounded west side channel area was digitized from LiDAR Digital Terrain Models using Global Mapper Version 22.0. For each year LiDAR imagery was available the un-impounded westside channel areas were digitized three times, and the average used to estimate area.

We used the elevation NAVD88 2.13 meters to establish wetted area (ha) across all years using Crescent Bay Tidal Station 9443826 MHHW = 2.152 meters NAVD88.

C. Elwha-west side water quality: The un-impounded west side channel of the Elwha lower river was sampled once a month during beach seining for basic water quality parameters of temperature, salinity, and dissolved oxygen (DO) from 2008 to present using a handheld YSI model proDSS meter. Prior to beach seining the probe was positioned in 0.6 meters of water and values recorded. These point data were summarized by month and year and dam removal phase. Data were analyzed using single factor ANOVA analysis and a post hoc Tukey test between water quality parameters before, during, and after dam removal.

Ecological changes to the Elwha west delta

A. Elwha west delta riparian extent and riparian vegetation cover: The Elwha delta and the west delta riparian areas were mapped using ortho-rectified aerial photographs with ARC GIS 10.6.1 software (ESRI 2021). Historical aerial photographs were georeferenced to 1-meter digital ortho-photographs and the area (hectares) of habitats were digitized and recorded for the years 1939–2021 (Table 1).

B. Elwha west delta riparian composition: Vegetation plots were located along the un-impounded zone along the west side of the mouth of the Elwha River within the riparian and shrub-emergent marsh habitats. On May 21, 2020,

Table 1. Annual (from previous year) percent change of west delta and riparian vegetation extent. Single asterisk (*) indicates west Elwha delta/Place levee installation, double asterisks (**) designate when the Elwha dam removals began, and triple asterisks (***) denote when the Elwha dam removals were completed.

Year	Change in west Elwha delta area (m^2)	Change in Elwha west delta riparian vegetation area (m^2)
1939	–	–
1965*	5%	–
1974	180%	–
1976	10%	109%
1977	-1%	356%
1981	-22%	36%
1985	-9%	41%
1990	15%	25%
2005	63%	76%
2006	18%	-8%
2009	3%	23%
2011**	4%	12%
2012	-2%	1%
2013	-1%	2%
2014***	40%	-9%
2015	-29%	14%
2016	30%	15%
2017	-18%	-2%
2021	-5%	13%

transects were placed parallel to the river along the lower bank edge where the first vegetation patch was located, as described in the PacFish InFish Biological Opinion Monitoring Program (Archer et al. 2016). Eight, 50-meter transects were placed, starting at the river's mouth where riparian vegetation began, and continued south (total of 400 m of transect). Line-point intercept data was recorded along each meter of each transect wherein the species with the nearest stem, leaf, or plant base intercepted was recorded. Along each 50 m transect, three 1 m × 1 m quadrats were randomly placed (8 transects × 3 quadrats = 24 total 1 m^2 quadrats). Within each quadrat, vegetation cover was estimated using the following cover classes: 1 = 0–1%, 2 = >1–10%, 3 = 11–25%, 4 = 26–50%, 5 = 51–75%, 6 = 76–90% and 7 = >90%. Both transect and line intercept data were standardized to reflect relative abundances. All plants were identified to species using Hitchcock and Cronquist (1973) and Pojar and MacKinnon (1994). Brome species were difficult to identify to species; therefore, they were categorized as *Bromus* spp. This documentation provides a species list for adaptive management as well as a baseline for long-term comparisons.

C. Impounded Elwha west delta area and macroalgae cover: This was mapped from ortho-rectified aerial images using Google Earth Pro. All images were analyzed with north facing orientation at 475-meter altitude. Images were gathered from all years that had distinguishable images of the west delta Place Pond and macroalgae. The area of the main impounded Place Pond and the area of floating macroalgae were mapped from 2004 to 2021 for the main growing season (July-October) using Google Earth Pro built-in mapping tool. The area, in square meters, of the pond and visible macroalgae were calculated to determine the relative percent cover of macroalgae.

D. Fish use: The Elwha un-impounded west side channel was sampled monthly from 2008-current for juvenile salmon abundance using a standardized sampling technique known as beach seining (Miller et al. 1980; PSWQA 1996), as described in Shaffer et al. 2009; 2017b; 2018). Seining involves deploying and retrieving a standard 11.2 × 1.8 m net with 0.32 cm mesh size using a small rowboat, and then idling, counting, and measuring fish intercepted. The beach seine net mesh is designed specifically to entrain small, young fish. This site has been studied since 2007 and subsets of these data have been included in a number of earlier studies (Shaffer et al. 2009; 2017b; 2018). Shaffer et al. (2009) documented that the west delta had the highest abundance of fish and proportion of salmon of the entire delta. As a result, the un-impounded west side channel of this study was designated a long-term study site and continuously sampled before, during, and after dam removal. The impounded area was not expected to have changes in fish use due to the fish barrier of the dike and so was not sampled regularly. We analyzed water quality parameters of temperature, DO, salinity, and juvenile salmon and trout abundance to illustrate changes in water quality over the course of dam removals and the relationships between juvenile salmon and habitat associated water quality changes. Fish abundances' distributions were not normally distributed, therefore a Spearman's Rank correlation was used to model correlative relationships.

E. Beaver presence: We determined beaver behavior using three main indicators: 1) Cataloging the evidence of the beaver lodge, beaver channel, vegetation harvesting, and food caches of the west delta in May, September, and December of 2020, and again March, June, September, and October of

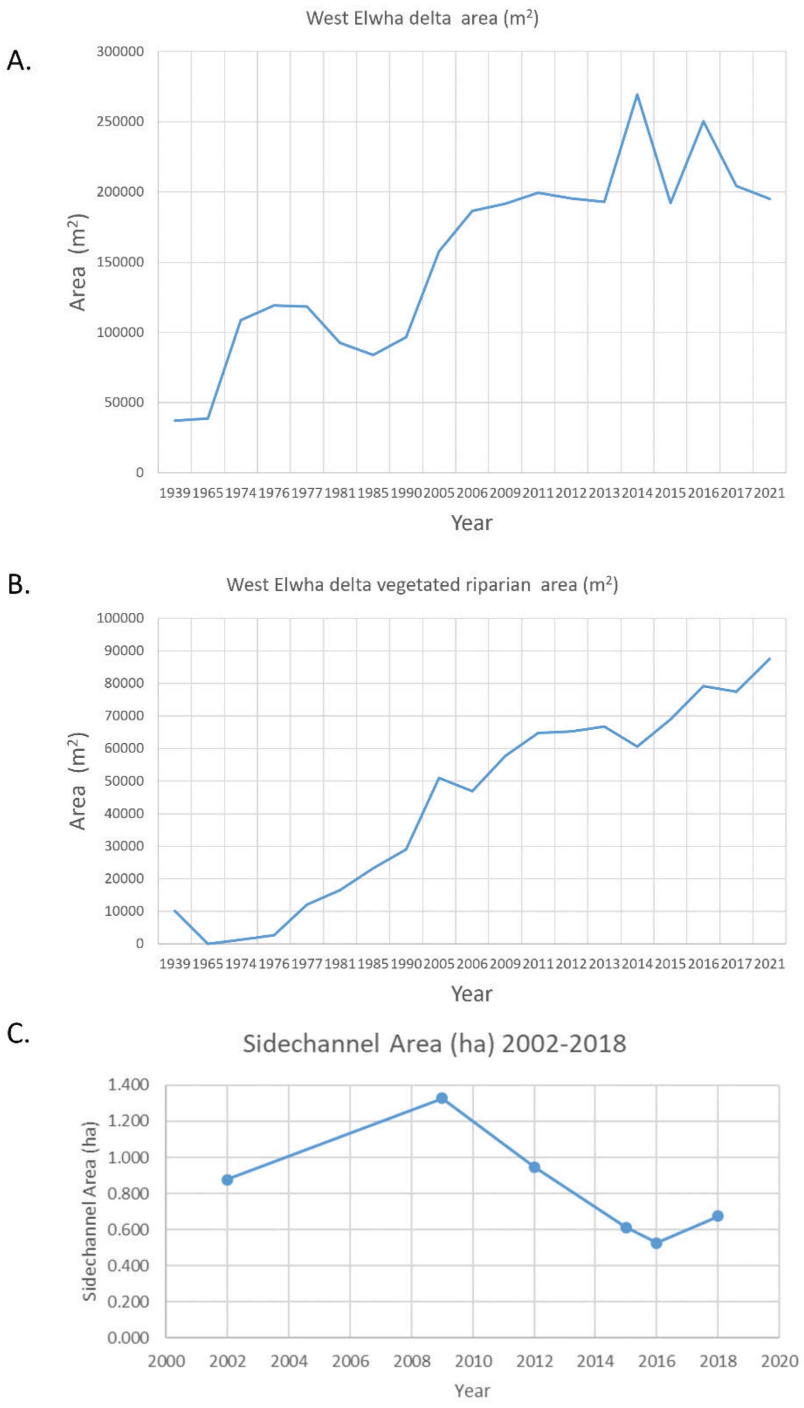


Figure 3. A–C West Elwha delta mapping graphs.

2021 using aerial imagery (Figs 2, 3), 2) an inventory of beaver observations reported by the community, and; 3) a two-year field survey of the west Elwha delta for beaver sign from 2020–2021 (Fig. 3). The west Elwha delta dike, side channel and river were split into three zones, and then walked quarterly for beaver sign which was recorded. Beaver sign included fresh drag marks, fresh chew marks, fresh scent mounts, and actual beaver sightings. For each survey, every definitive new beaver sign observed was recorded by GPS location and photographed.

Results

Physical changes to the Elwha delta

Elwha west delta extent, topography, and side channel area

The area of the west delta increased from 1965 to 1977, decreased until 1990, and then began a trend of growing again steeply over the next 15 years to and through 2005, with temporary dramatic increases in 2014, and 2016 that corresponded to delivery of large volumes of dam removal sediment, followed by decreases in 2015 to 2017. The lateral bar that borders the east of the un-impounded west delta increased by an average of 26% prior to dam removals but only 2% on average for the years during and after dam removals. In 2021, the Elwha west delta appeared to decrease to the 2011 extent of 200,000 square meters (Table 1, Fig. 4). The side channel area decreased from an average of 1.104 hectares before dam removal to an average of 0.641 hectare after dam removal, which is a decrease of 42% overall after dam removals at an average rate of -12.5% per year (Table 2, Fig. 3C).

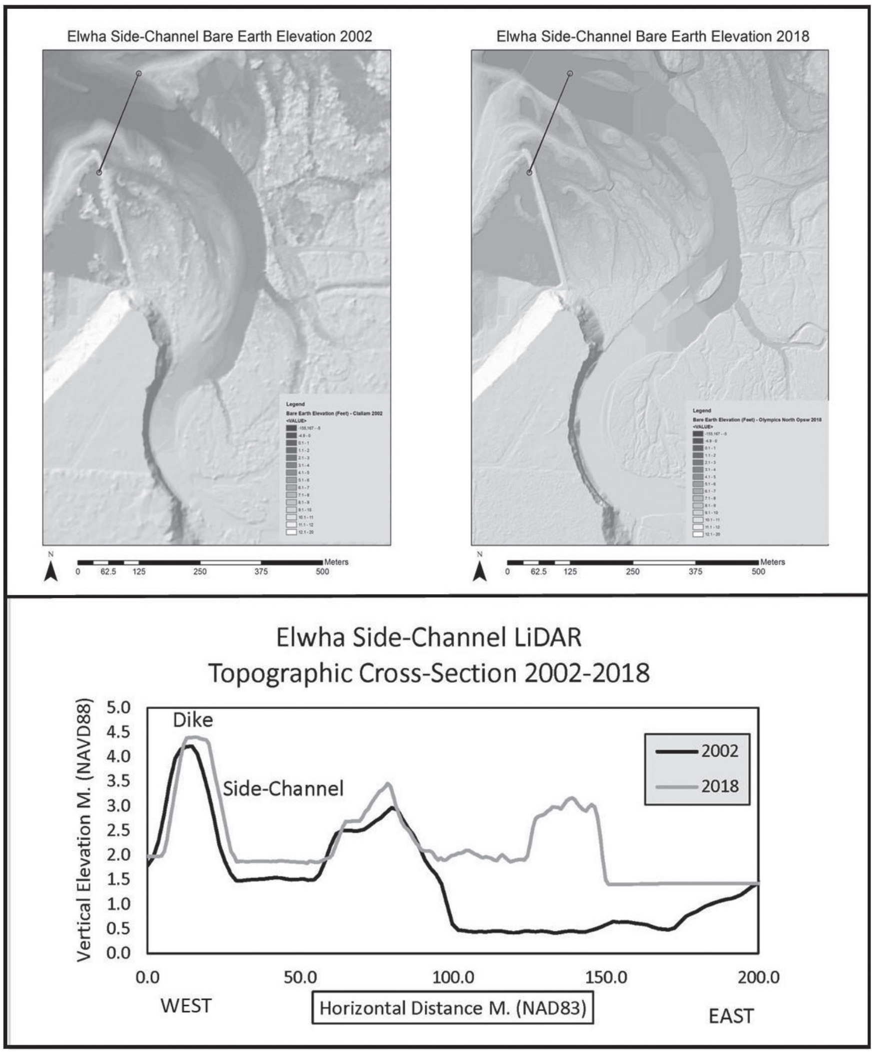


Figure 4. Elwha side-channel topography between 2002 and 2018 from bare earth LiDAR. LB = lateral bar.

Table 2. Summary of the side channel area after dam removal and percent change from previous year.

Year	West Elwha Delta Area (m2)	West delta Veg Area (m2)	West side channel habitat
2011	4%	12%	–
2012	-2%	1%	-29%
2013	-1%	2%	–
2014	40%	-9%	–
2015	-29%	14%	-35%
2016	30%	15%	-14%
2017	-18%	-2%	28%
Average % change since dam removals	3%	5%	-13%

The side channel between the Place dike and Elwha River lost approximately a half meter of depth between 2002 and 2018. The main river channel moved east and lost almost three meters of depth over the same time period (Fig. 4).

Water quality

Water quality parameters of the west Elwha delta have changed over the last fourteen years. Salinity decreased significantly following dam removal, and temperature changed seasonally, consistent with findings of Foley et al. (2015). Dissolved oxygen (DO) values of the un-impounded west side channel have significantly declined since dam removal to some of the seasonally lowest recorded observations since monitoring began (Table 3, Fig. 5 A–C).

Ecological changes to the Elwha west delta

Riparian vegetation extent

The riparian vegetation extent along this west delta steadily increased by an average of 82% from 1965 to 2011, and was positively correlated to delta extent (R^2=0.86; $P_{(2,18)}$,<0.001. However, the riparian vegetation cover does not correspond to west delta extent changes during and after dam removal, ($R^2_{(2,6)}$=-0.166; P>0.05) but instead continued to steadily increase by an average of 6% from 2011–2021 with a continued increase in vegetation cover in 2014 and 2016.

Riparian zone vegetation composition

Spring vegetation surveys documented a total of 52 plant species (Suppl. material 1, Table 4). Forbs had the greatest species richness (28) and were comprised of 21% native, 9% naturalized non-native, and 6% noxious weeds (Table 4). The most notable forbs by plant origin included coastal mugwort (*Artemisia suksdorfii*), nonnative common plantain (*Plantago major*), and nonnative herb-Robert (*Geranium robertianum*), respectively. Grasses comprised 25% of the vegetation and consisted mostly of native dunegrass (*Elymus mollis*) and naturalized,

Table 3. Water quality trends un-impounded west side channel 2008–2021. DR = During dam removal; Pre = pre dam removal, Post = post dam removal. NS = Not significant. DO = dissolved oxygen). Salinity values were natural log.

WQ Metric	DR Phases	Significant Change	p-value
DO (%)	Pre:DR	NS	0.43
DO (%)	DR:Post	decrease	<0.001
DO (%)	Pre:Post	decrease	0.001
Temperature (C)	Pre:DR	NS	0.49
Temperature (C)	DR:Post	NS	0.86
Temperature (C)	Pre:Post	NS	0.15
Salinity (g/kg)	Pre:DR	decrease	<0.001
Salinity (g/kg)	DR:Post	NS	0.5558
Salinity (g/kg)	Pre:Post	decrease	<0.001

non-native orchard grass (*Dactylis glomerata*), with one noxious invasive species present, reed canary (*Phalaris arundinacea*). Woody trees (29%) and shrubs (9%) collectively made up 38% of all plant species sampled. Most were native species with one exception, evergreen blackberry (*Rubus laciniatus*).

The native woody species that were reported to have been used by beaver included Sitka willow (*Salix sitchensis*), red alder (*Alnus rubra;* 8.3%), Hooker's willow (*Salix hookeriana;* 6.2%), big-leaf maple (*Acer macrophyllum;* 2%), and black cottonwood (*Populus trichocarpa;* <1%) (Suppl. material 1).

Impounded Elwha west delta area and macroalgae cover

Percent macroalgae cover in west delta (location of original beaver lodge) was approximately 25% of the total impounded Place Pond area until 2013, when the proportion of the pond covered with macroalgae dropped to ~7%. Macroalgae percent cover decreased to 0% by 2015, where it has remained since (Fig. 6, Table 5).

Beaver observations

Aerial imagery indicates the west delta lodge area was well vegetated with no sign of channels or beaver caches until 2015. By 2021 all the vegetation in the area of the west delta lodge had been harvested by beaver and a large cache area with a lodge had been created (Fig. 7).

Interviewing long-time landowners revealed that beavers were first observed and recorded along the west delta, and specifically the impounded Place Pond in October 2014-but not before. After 2014, observations of beaver were consistently and regularly reported along both sides of the dike, in the impounded Place Pond, and west side channel from 2014 until May 2018, when a beaver was killed on the dike via predation. No beavers were observed again until 2019 when they were spotted intermittently.

Standard surveys for signs of beaver including tree chewing, scent mounds, tail drag trails, and actual beaver sitings were conducted along the west delta quarterly from 2020 and 2021 and are summarized in Table 6. No fresh signs of beaver were seen in summer surveys. Fresh beaver sign was observed during all other seasons.

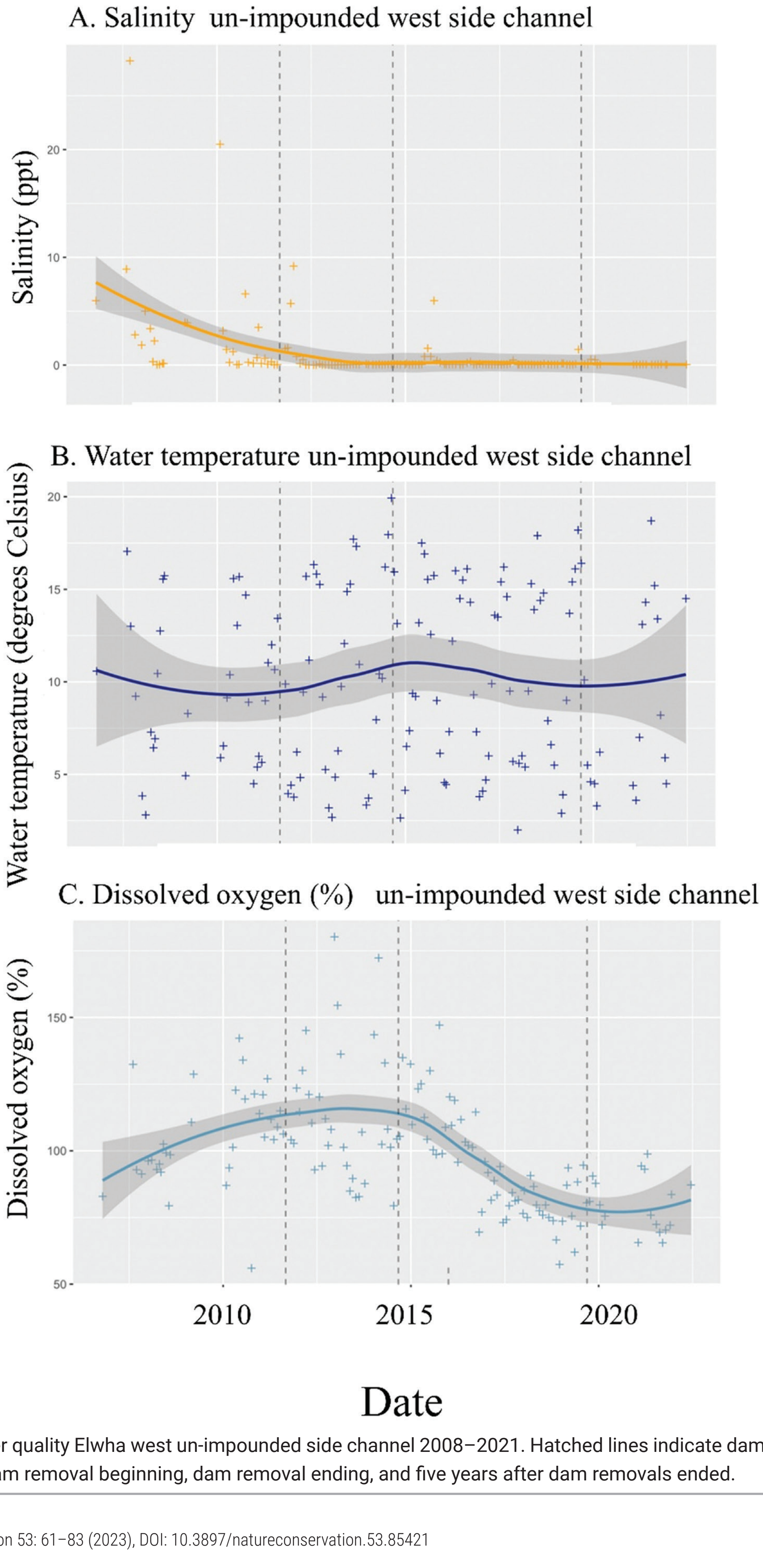


Figure 5. Water quality Elwha west un-impounded side channel 2008–2021. Hatched lines indicate dam removal phases left to right: dam removal beginning, dam removal ending, and five years after dam removals ended.

Table 4. Summary of mean plant abundance by life form and origin surveyed from the riparian and shrub-emergent marsh vegetation present along the west side of the mouth of the Elwha River in 2021. SR = Species Richness. A complete species list is reported in Suppl. material 1.

Life Form	SR	Origin	Abundance	Most Abundant per life form and origin
Forbs	28	Native	21%	Coastal Mugwort (*Artemisia suksdorfii*)
		Naturalized	9%	Common plantain (*Plantago major*)
		Noxious	6%	Herb Robert (*Geranium robertianum*)
Grass	9	Native	16%	Dune grass (*Elymus mollis*)
		Naturalized	8%	Orchard grass (*Dactylis glomerata*)
		Noxious	1%	Reed Canary (*Phalaris arundinacea*)
Shrubs	6	Native	9%	Twin-berry (*Lonicera involucrate*)
		Naturalized	0	NA
		Noxious	1%	Evergreen Blackberry (*Rubus laciniatus*)
Trees	9	Native	29%	Sitka Willow (*Salix sitchensis*)
		Naturalized	0	NA
		Noxious	0	NA

Table 5. Place pond area (m²), macroalgae area (m²) and percent cover, and change in percent macroalgae cover recorded between 2004–2021.

Year	Month	Pond Area (m^2)	Algae Area (m^2)	Percent of pond covered with macro algae	Change in Pond area	macro algae area
2004	8	16,539	5,355	32%	–	–
2006	10	15,922	6,432	40%	-4%	20%
2009	9	17,341	7,273	42%	9%	13%
2011	11	17,124	5,912	35%	-1%	-19%
2012	8	16,394	5,594	34%	-4%	-5%
2013	7	17,549	1,818	10%	7%	-68%
2015	9	16,768	0	0%	-4%	-100%
2016	7	17,888	0	0%	7%	0%
2017	7	18,701	0	0%	5%	0%
2021	7	18,568	0	0%	-1%	0%

Table 6. Summary of quarterly beaver surveys Elwha west delta 2020–2021.

Season	Month	Year	Beaver sign observed	West of dike	East of dike
Spring	5	2020	y	y	y
Summer	9	2020	n	n	n
Winter	12	2020	y	n	y
Spring	6	2021	y	y	n
Summer	9	2021	n	n	n
Fall	10	2021	y	y	y
Winter	12	2021	y	y	y

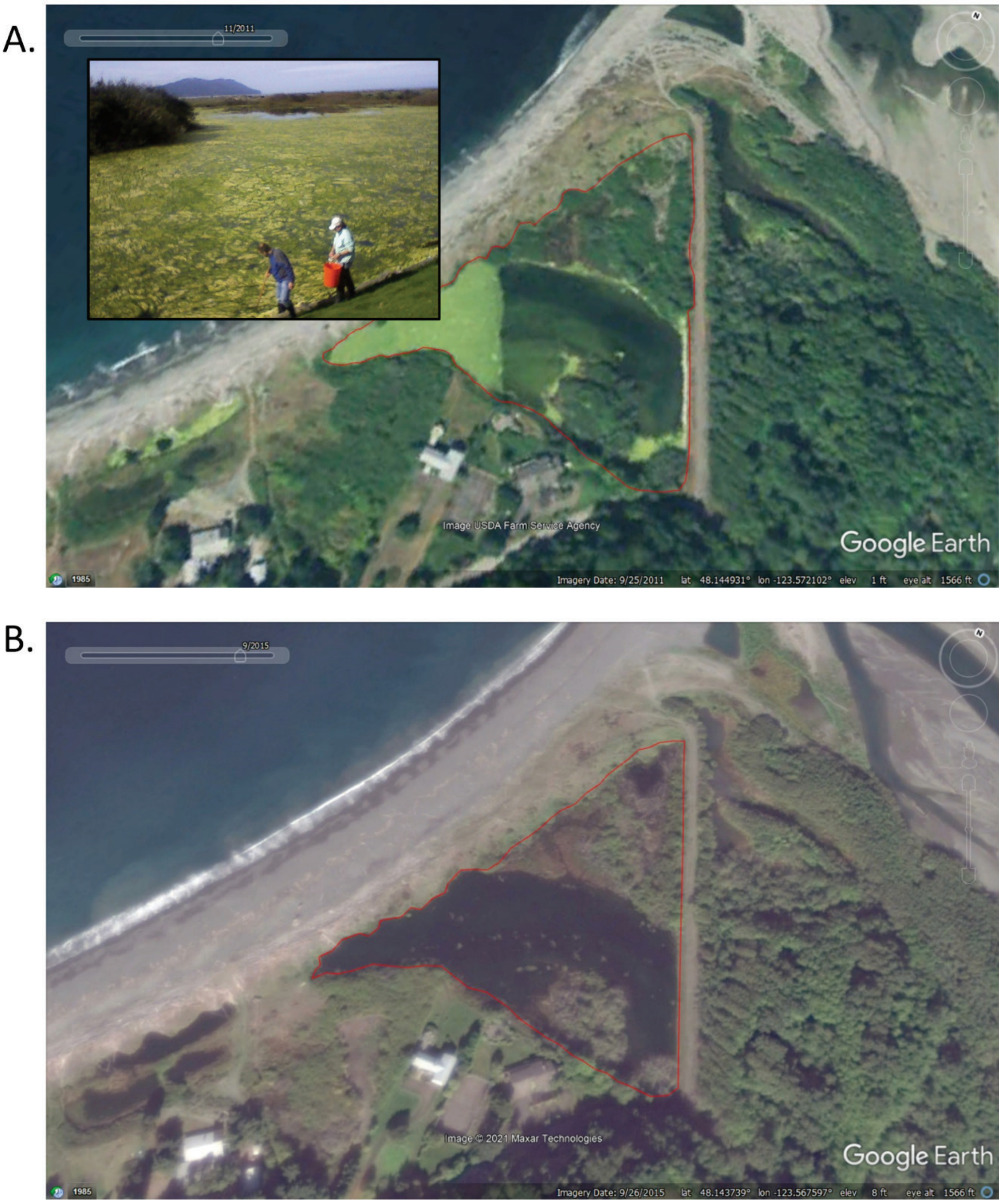


Figure 6. A, B Impounded west delta (Place Pond) illustrating-high macroalgae cover prior to dam removals (2011) and B post dam removal with no macroalgae cover (2014).

Table 7. Summary of beaver (this study), and humans, and dog numbers by month 2020–2021 for the Elwha west delta. Recreation data reprinted with permission from CWI 2022.

Month	Humans	Dogs	Beaver
1	45	9	2
3	24	3	2
5	11	2	4
6	41	5	1
9	46	9	0
10	16	0	4

Figure 7. Photograph of Elwha west delta impounded area beaver lodge in 2021.

We correlated beaver observations to dog and human use data collected for a different study but from the same area and over the same time period (CWI 2022, Table 7). Beaver observations were negatively correlated with dogs ($R^2 = -0.79, P_{1,4} < 0.02$), and humans ($R^2 = -0.88, P_{1,4} < 0.01$).

Fish use

Fish abundance and composition of the impounded Place pond have remained unchanged over the last 15 years and is comprised solely of high numbers of three spine stickleback (*Gasterosteus aculeatus*; Shaffer et al 2009; Michel 2021 unpublished data).

In the un-impounded Elwha River west side channel, the abundance of individual species of juvenile salmon and trout changed before and after dam removal. Overall, juvenile Chinook (*Oncorhynchus tshawytscha*) and coho salmon (*Oncorhynchus kisutch*) numbers increased, while juvenile chum salmon (*Oncorhynchus keta*), steelhead (*Oncorhynchus mykiss*), and cutthroat trout (*Oncorhynchus clarkia*) decreased (Fig. 8).

Spearman rank correlations between individual species abundance and water quality parameters revealed that coho and chum abundance were related to water quality parameters of dissolved oxygen, temperature, and salinity (Table 8). Specifically regarding coho, fish abundance was negatively correlated to DO ($r = -0.33, P < 0.001$) and salinity ($r = -0.32, < 0.001$), yet positively correlated to temperature ($r = 0.19, P = 0.05$). The opposite pattern

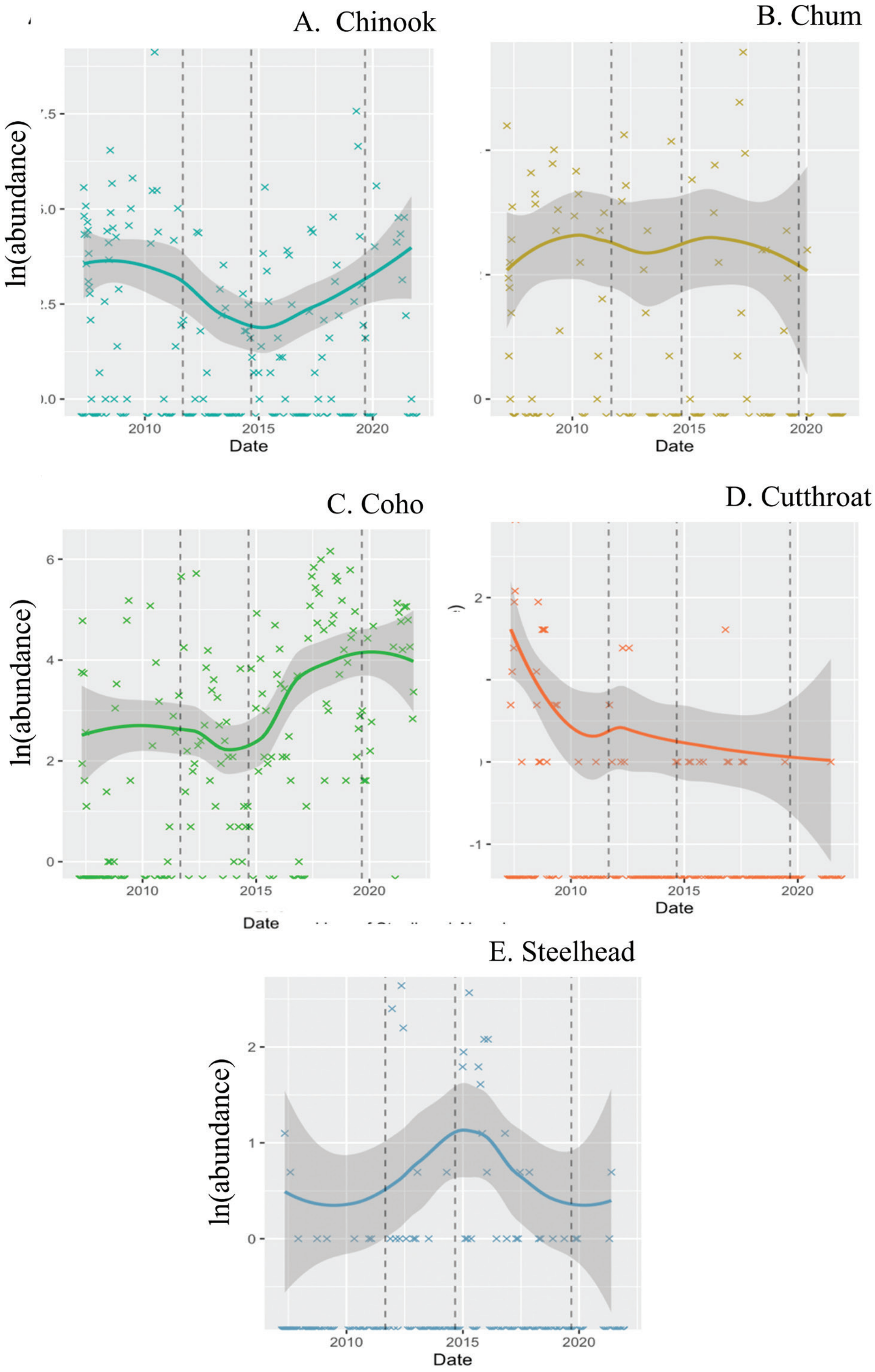


Figure 8. Monthly fish abundance Elwha un-impounded west side channel 2007–2021 (data are natural log; some data reprinted with permission from authors). Hatched lines indicate dam removal phases left to right: dam removal beginning, dam removal ending, and five years after dam removals ended.

for chum revealed a positive relationship between DO (r = 0.20, P = 0.04) and salinity (r = 0.20, P = 0.04) and a negative correlation with temperature (r = -0.51, P < 0.001). Chinook had a positive correlation to water temperature (r = 0.19, P = 0.05).

Table 8. Spearman correlation tests using an alpha level of 0.05. of natural log of species abundances (+1) and their monotonic correlation to different water quality metrics. Table indicates fish species, water quality variable, and the correlation coefficient (r) with sign indicating either positive or negative correlation. Significant p-values are emphasized in bold font.

Species	Water Quality Metric	r	P-value
Chinook	DO (%)	-0.08	0.43
Chinook	Temperature (C)	0.19	**0.05**
Chinook	Salinity (g/kg)	-0.14	0.17
Coho	DO (%)	-0.33	**<0.00**1
Coho	Temperature (C)	0.19	**0.05**
Coho	Salinity (g/kg)	-0.32	**<0.001**
Chum	DO (%)	0.20	**0.04**
Chum	Temperature (C)	-0.51	**<0.001**
Chum	Salinity (g/kg)	0.20	**0.04**
Cutthroat	DO (%)	0.11	0.11
Cutthroat	Temperature (C)	0.21	**0.03**
Cutthroat	Salinity (g/kg)	0.02	0.84
Steelhead	DO (%)	0.16	0.10
Steelhead	Temperature (C)	-0.06	0.52
Steelhead	Salinity (g/kg)	-0.15	0.14

Discussion

Both dam removal and beaver are important for river ecosystem function, and in coastal restoration their benefits may be synergistic. In the coastal Elwha nearshore, both dam removals and lower river alterations have contributed to an expanded beaver habitat and colonization through water quality improvements along the impounded west delta, and expansion of riparian vegetation growth along the un-impounded west delta (specifically the lateral bar driven by the dike along the west delta). Interestingly, the lateral bar persistence is the result of the west Place dike and not dam removal.

The vegetation of the lateral bar of the west Elwha has increased significantly since dam removal, likely to the benefit of beaver. Fuentes et al. (2011) showed that prior to dam removal the impounded Elwha west delta vegetation was primarily shrub-marsh transition. The un-impounded west delta, including the lateral bar, was dominantly riparian forest and willow-alder forest with dune grass ringing the northern portion of the delta. These vegetation groups and relative cover have stayed largely the same, expanding a bit (6% on average) after dam removal. Further, the vegetation collected in the newly expanded riparian zone and shrub-marsh transition reflected the developing complexity of the nearshore environment, which has been extended to connect with the expanded river delta and was comprised of abundant Sitka willow (*S. sitchensis*) red alder (*A. rubra*), and Hooker's Willow (*S. hookeriana*). Both genera were previously reported prior to dam removal and their proximity prior to restoration allowed for rapid recruitment along the newly deposited sediment (Shafroth et al.

2011; Prach et al. 2019). Both willow (*Salix spp.*) and alder (*A. rubra*) have been cited as important forage tree species for food, dam, and lodge construction for beaver (Stoffyn-Egli and Willison 2011; Ritter et al. 2020). Black cottonwood (*P. trichocarpa*), another preferred beaver forage source was present, as well as big-leaf maple (*A. macrophyllum*), but both had relatively low abundance when compared to mature riparian forest that previously dominated the nearshore environment prior to dam removal and beach expansion.

Habitat complexity is further represented by sub-canopies of small trees and shrubs that were there prior to restoration and include Indian plum (*O. cerasiformis*), bitter cherry (*P. emarginata*), Pacific dogwood (*Cornus nuttallii*), thimbleberry (*R. parviflorus*), Nootka rose (*R. nutkana*), and salmonberry (*R. spectabilis*). Vegetation followed the nearshore expansion and included species previously sampled within the shrub-marsh and riparian zones such as pacific silverweed and slough sedge (Shafroth et al. 2011). The significant extension of the beach post-dam removal has also expanded the dunegrass habitat (*E. mollis*), which prior to dam removal, were confined to narrow corridors of the sediment depleted beach. Some native species such as Coastal mugwort (*A. suksdorfii*) and riverbank lupine (*L. rivularis*) were new to the nearshore and represented native species that were used in the upstream restoration of riparian forests in the former lake reservoirs (Michel et al. 2011; Chenoweth et al. 2021).

Non-native species sampled post-dam removal included mostly naturalized species with some class B and C noxious weeds, which are widespread and recommended for control. Noxious weeds included species such as herb-Robert (*G. robertianum*), hairy cat's-ear (*H. radicata*), oxeye daisy (*L. vulgare*), wild teasel (*D. fullonum*), and reed canary grass (*P. arundinacea*). These weedy species are facilitated by recent disturbance and will need to be monitored due to the influence these species invasions can have on native species richness (Woodward et al. 2011). The establishment of a larger proportion of riparian vegetation will hopefully stabilize and promote the long-term re-establishment of the west delta beaver habitat (Bruner 1989; Bailey et al. 2019), however, management and monitoring should emphasis control of non-native plant species.

These vegetation changes combined with water quality improvements likely benefit beaver, allowing them to expand into additional areas not used before dam removals. Beaver use of the Elwha west delta appears complex and changing. Regular surveys of beaver presence along the impounded west estuary, dike, and un-impounded west estuary from 2020–2022 indicate that the most use (based on chewing and tail drag marks) was during winter months. Over the course of the study no beaver use of the Place dike area was observed during summer months. This could be a result of the beaver changing their food source during summer months. However, as beaver have to chew on hard wood/substrates regularly to maintain teeth we would expect to see some sign even if food resource use changed seasonally. Given the high volume of food resource on the east side of the dike along the lateral bar, we assume there are other reasons apart from low food availability that explain why the beaver do not transit across the dike to the heavily vegetated areas. There are a number of reasons for beaver seasonal use of this area including lodge access due to increased summer human/dog presence, which would discourage beaver use, and the calmer river conditions which would make larger reaches of the main river channel more suitable during the summer months. Beaver and human observations are significantly negative correlated, indicating that beaver avoid using the area when humans and dogs are present. The negative impact of dogs on wildlife is well documented, including for beaver (Hennings 2016; Schüttler et al. 2018). The Place dike, which sits between the lodge area and the west Elwha River riparian and side channel areas, is the sole recreation access to the west delta and used heavily used for recreation, including heavy domestic dog use.

Alternatively, beaver use of this region may be defined by other factors. For example, beaver may use the impounded Place Pond lodge zone in the winter when river flows are high and challenging for beaver. Beaver use of the area in summer may be more variable when water levels in the impounded pond are low, the pond is shallower, and possibly making accessing the lodge more complicated. A broader more comprehensive study on beaver use of this area would help explain these fluctuations.

Juvenile salmon and trout use of the unimpounded west side channel indicate that the physical changes in area, depth, and water quality are having ecological repercussions. Fish use of the nearshore has been documented to be a very sensitive metric defining ecosystem changes that are foundational components of coastal systems. In this study we used water quality as an indicator of habitat quality attributed to changes in the unimpounded west delta extent. Relative to water quality, we find relationships between fish abundance relative to dissolved oxygen and temperature. As the site shallows and becomes smaller it transitions to lower salinity, warmer off-channel habitat that is suitable for coho. The significant decrease in DO is likely due to both the change to freshwater dominated system, and the site becoming smaller and shallower with poorer connection to the river. This, in turn, results in the site becoming less accessible and/or less hospitable to fish, and significantly so for chum. The relationship between salmon, habitat, and water quality, however, which is extremely complex and varies by species and life history stage, can also be masked by hatchery releases, and which in the Elwha have increased log scale for Chinook and coho salmon since dam removals (RMPC 2022; Shaffer et al. 2017b). While our data indicate both trends and relationships in the physical habitat and fish response of the unimpounded west delta side channel, our data are only point data (monthly). A more detailed study will provide resolution needed to fully define causative relationships between water quality, salmon and trout abundance.

Conclusion

Beaver, as ecosystem engineers that increase channels and ponded water in their habitat zones, may be a positive coastal response to dam removals. As the Elwha system, including the lower river and riparian zones, continue to restore two decades after dam removals, we expect beaver to continue to expand their numbers and range throughout the Elwha coastal zone. We recommend recovery and conservation measures be developed to support the beaver of the Elwha west delta that include public outreach to educate about the impacts of off leash dogs on wildlife (Sterl et al. 2008; Schüttler et al. 2018).

An additional finding illustrated the problems associated with the Place dike that blocks fish passage between the Elwha River and the otherwise high quality historic west side channel zone of the impounded west delta. This is also causing a decrease in the size and quality of one of the most important and persistent areas of the Elwha delta for salmon rearing. Therefore, we also recommend continued pursuit of legacy, ecosystem scale restoration actions to hydrologically reconnect the two sides of the Place pond/west Elwha delta. Implementing education and ecosystem engineering restoration actions will result in a synergistic ecosystem benefit for the entire Elwha recovery, including salmon and coastal beaver.

Acknowledgements

Western Washington University and Peninsula College students, including the 2021 WWU Ecological Restoration Course, assisted with the project vegetation surveys. Jamie Michel, CWI, and Washington Conservation Corps staff led by Sam Miller Impresario also assisted with field work. John Gussman assisted with final graphics. Landowners and staff of the Malcolm Dudley estate, including Malcolm and Cozette Dudley and Chuck Janda, as well as the Lower Elwha Klallam Tribe, provided property access. Three anonymous reviewers provided a very helpful critical review. Funding for this work was provided by Patagonia Inc and CWI. Thank you to all.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

Funding for this work was provided by Patagonia Inc and CWI.

Author contributions

Anne Shaffer led all aspects of this project; Dave Parks provided physical habitat mapping science, Katrina Campbell conducted macroalgae mapping, Anna Morgan provided fish and water quality data synthesis, Pamela Adams provided beaver monitoring and expertise, Jenise Bauman provided riparian zone field data and analysis.

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

References

Archer EK, Van Wagenen ER, Coles-Ritchie M, Ojala JV, Roseen TP, Gavin A (2016) Effectiveness Monitoring Sampling Methods for Riparian Vegetation Parameters. Logan, UT. https://www.fs.usda.gov/Internet/FSE_DOCUMENTS/fseprd494542.pdf

Bailey DR, Dittbrenner BJ, Yocom KP (2019) Reintegrating the North American beaver (Castor canadensis) in the urban landscape. Wiley Interdisciplinary Reviews: Water 6(1): e1323. https://doi.org/10.1002/wat2.1323

Brazier RE, Puttock A, Graham HA, Auster RE, Davies KH, Brown CM (2021) Beaver: Nature's ecosystem engineers. Wiley Interdisciplinary Reviews: Water 8(1): e1494. https://doi.org/10.1002/wat2.1494

Brown R, Thomas CC, Cubley ES, Clausen AJ, Shafroth PB (2022) Does Large Dam Removal Restore Downstream Riparian Vegetation Diversity? Testing Predictions on the Elwha River, Washington, USA. Ecological Applications 32(6): e2591. https://doi.org/10.1002/eap.2591

Bruner KL (1989) Effects of beaver on streams, streamside habitat, and coho salmon fry populations in two coastal Oregon streams. Master's Thesis, Oregon State University, Corvallis, Oregon.

Chenoweth J, Bakker JD, Acker SA (2021) Planting, seeding, and sediment impact restoration success following dam removal. Restoration Ecology. https://doi.org/10.1111/rec.13506

CWI [Coastal Watershed Institute] (2022) Data reporting, Bird and human use field study of the Elwha delta 2020–2022. Port Angeles, CWI112022: 1.

Draut AE, Ritchie AC (2015) Sedimentology of new fluvial deposits on the Elwha River, Washington, USA, formed during large-scale dam removal. River Research and Applications 31(1): 42–61. https://doi.org/10.1002/rra.2724

ESRI (2021) Environmental Systems Research Institute (ESRI), ArcGis Software, version 10.6.1. Redlands, California.

Foley MM, Duda JJ, Beirne MM, Paradis R, Ritchie A, Warrick JA (2015) Rapid water quality change in the Elwha River estuary complex during dam removal. Limnology and Oceanography 60(5): 1719–1732. https://doi.org/10.1002/lno.10129

Fuentes TL, Shafroth PB, Pritekel C, Beirne MM, Beauchamp VB (2011) Vegetation of the Elwha River Estuary. USGS: 225–248. https://doi.org/10.3133/sir201151208

Hennings L (2016) Impacts of dogs on wildlife and water quality: A literature review.

Hitchcock CL, Cronquist A (1973) Flora of the Pacific Northwest: An Illustrated Manual. 5th edn. University of Washington Press.

Hood WG (2012) Beaver in tidal marshes: Dam effects on low-tide channel pools and fish use of estuarine habitat. Wetlands 32(3): 401–410. https://doi.org/10.1007/s13157-012-0294-8

Hood GA, Larson DG (2014) Beaver-created habitat heterogeneity influences aquatic invertebrate assemblages in boreal Canada. Wetlands 34(1): 19–29. https://doi.org/10.1007/s13157-013-0476-z

Knapp RL (2009) North American Beaver Castor Canadensis Habitat Use in the Olympic Peninsula's Elwha Valley. Thesis, Western Washington University.

Magirl CS, Hilldale RC, Curran CA, Duda JJ, Straub TD, Domanski M, Foreman JR (2015) Large-scale dam removal on the Elwha River, Washington, USA: Fluvial sediment load. Geomorphology 246: 669–686. https://doi.org/10.1016/j.geomorph.2014.12.032

McCaffery R, McLaughlin J, Sager-Fradkin K, Jenkins KJ (2018) Terrestrial fauna are agents and endpoints in ecosystem restoration following dam removal. Ecological Restoration 36(2): 97–107. https://doi.org/10.3368/er.36.2.97

Michel JT, Helfield JM, Hooper DU (2011) Seed Rain and Revegetation of Exposed Substrates Following Dam Removal on the Elwha River. Northwest Science 85(1): 15–29. https://doi.org/10.3955/046.085.0102

Miller B, Simenstad CA, Cross JN, Fresh KL, Steinfort SN (1980) Nearshore fish and macroinvertebrate assemblages along the Strait of Juan de Fuca including food habits of common nearshore fish. Final report of three years sampling 1976–1979. Fisheries Research Institute College of Fisheries University of Washington Seattle, Seattle.

Nelson T, Lucas R (2011) Pilot Study on Macroalgae Bloom in the Impounded West Elwha Estuary. Proceedings, Elwha Nearshore Workshop, Port Angeles, Washington 98362.

Pojar J, MacKinnon A (1994) Plants of the Pacific Northwest Coast: Washington, Oregon, British Columbia & Alaska. Edited by Paul Alaback, Joe Antos, Trevor Goward, Ken Lertzman, Rosamund Pojar, Andrew Reed, Nancy Turner, and Dale Vitt. Lone Pine Publishing, Vancouver, British Columbia.

Prach K, Chenoweth J, del Moral R (2019) Spontaneous and assisted restoration of vegetation on the bottom of a former water reservoir, the Elwha River, Olympic National Park, WA, U.S.A. Restoration Ecology 27(3): 592–599. https://doi.org/10.1111/rec.12915

PSWQA [Puget Sound Water Quality Authority Olympia] (1996) Recommended protocol for sampling soft bottom demersal fishes by beach seines and trawling in Puget Sound, Washington. Puget Sound Partnership, Olympia.

RMPC [Regional Management Process Center] (2022) Regional Management Process Center. https://www.rmpc.org/ [Accessed 14 December 2022]

Ritchie AC, Warrick JA, East AE, Magirl CS, Stevens AW, Bountry JA, Randle TJ, Curran CA, Hilldale RC, Duda JJ, Gelfenbaum GR (2018) Morphodynamic evolution following sediment release from the world's largest dam removal. Scientific reports 8(1): 1–3. https://doi.org/10.1038/s41598-018-30817-8

Ritter TD, Gower CN, McNew LB (2020) Habitat conditions at beaver settlement sites: Implications for beaver restoration projects. Restoration Ecology 28(1): 196–205. https://doi.org/10.1111/rec.13032

Riverstyle (2022) Riverstyle. https://riverstyles.com/?portfolio=lateral-bar-alternate-or-side-bar

Schüttler E, Saavedra-Aracena L, Jiménez JE (2018) Domestic carnivore interactions with wildlife in the Cape Horn Biosphere Reserve, Chile: Husbandry and perceptions of impact from a community perspective. PeerJ 6: e4124. https://doi.org/10.7717/peerj.4124

Shaffer JA, Beirne M, Ritchie T, Paradis R, Barry D, Crain P (2009) Fish habitat use response to anthropogenic induced changes of physical processes in the Elwha estuary, Washington, USA. Hydrobiologia 636(1): 179–190. https://doi.org/10.1007/s10750-009-9947-x

Shaffer JA, Higgs E, Walls C, Juanes F (2017a) Large-scale Dam Removals and Nearshore Ecological Restoration: Lessons Learned from the Elwha Dam Removals. Ecological Restoration 35(2): 87–101. https://doi.org/10.3368/er.35.2.87

Shaffer JA, Juanes F, Quinn TP, Parks D, McBride T, Michel J, Naumann C, Hocking M, Byrnes C (2017b) Nearshore fish community responses to large scale dam removal: Implications for watershed restoration and fish management. Aquatic Sciences 79(3): 1–18. https://doi.org/10.1007/s00027-017-0526-3

Shaffer JA, Munsch S, Juanes F (2018) Functional diversity responses of a nearshore fish community to restoration driven by large-scale dam removal. Estuarine, Coastal and Shelf Science 213: 245–252. https://doi.org/10.1016/j.ecss.2018.08.030

Shafroth PB, Fuentes TL, Pritekel C, Beirne MM, Beauchamp VB (2011) Vegetation of the Elwha River Estuary. In: Duda JJ, Magirl C, Warrick JA (Eds) Coastal Habitats of the Elwha River: Biological and Physical Patterns and Processes Prior to Dam Removal: U.S. Geological Survey Scientific Investigations Report: 2011–5120. https://doi.org/10.3133/sir201151208

Sterl P, Brandenburg C, Arnberger A (2008) Visitors' awareness and assessment of recreational disturbance of wildlife in the Donau-Auen National Park. Journal for Nature Conservation 16(3): 135–145. https://doi.org/10.1016/j.jnc.2008.06.001

Stoffyn-Egli P, Willison JH (2011) Including wildlife habitat in the definition of riparian areas: The beaver (*Castor canadensis*) as an umbrella species for riparian obligate animals. Environmental Reviews 19: 479–493. https://doi.org/10.1139/a11-019

Warrick JA, Bountry JA, East AE, Magirl CS, Randle TJ, Gelfenbaum G, Ritchie AC, Pess GR, Leung V, Duda JJ (2015) Large-scale dam removal on the Elwha River, Washington, USA: Source-to-sink sediment budget and synthesis. Geomorphology 246: 729–750. https://doi.org/10.1016/j.geomorph.2015.01.010

Winter BD, Crain P (2008) Making the Case for Ecosystem Restoration by Dam Removal in the Elwha River, Washington. Northwest Science 82(sp1): 13–28. https://doi.org/10.3955/0029-344X-82.S.I.13

Woodward A, Torgersen C, Chenoweth J, Beirne K, Acker S (2011) Predicting spread of invasive exotic plants into de-watered reservoirs following dam removal on the Elwha River, Olympic National Park, Washington: U.S. Geological Survey Open-File Report 2011–1048, 64 pp. https://doi.org/10.3133/ofr20111048

Supplementary material 1

Mean woody plant abundance surveyed from the riparian and shrub-emergent marsh vegetation

Authors: J. Anne Shaffer, Dave Parks, Katrina Campbell, Anna Moragne, Bligh Hueske, Pamela Adams, Jenise M. Bauman

Data type: occurrences

Explanation note: Mean woody plant abundance surveyed from the riparian and shrub-emergent marsh vegetation present along the west side of the mouth of the Elwha River in 2021. Species-level data are relative abundances (percentages of the total counts). Plant species and Family names are divided by life form (tree, shrub, forb, and grass) then sorted by abundance (from high to low). Plant origin: N, native plant and I, introduced plant. If introduced, plant is classified as naturalized (n) or class of noxious weed (B, C).

Link: https://doi.org/10.3897/natureconservation.53.85421.suppl1

Nature Conservation 53: 85–103 (2023)
DOI: 10.3897/natureconservation.53.104000

Research Article

Hope is the last thing lost: Colombian captive-bred population of the critically endangered Orinoco crocodile (*Crocodylus intermedius*) is a genetic reservoir that could help to save the species from extinction

Ana M. Saldarriaga-Gómez[1,2,3], María Cristina Ardila-Robayo[2†], Federico Medem[2†], Mario Vargas-Ramírez[1,2]

1 *Grupo Biodiversidad y Conservación Genética, Instituto de Genética, Universidad Nacional de Colombia, Calle 53 # 35–83, Edificio 426, Bogotá D.C., Colombia*
2 *Estación de Biología Tropical Roberto Franco (EBTRF), Carrera 33 #33–76, Villavicencio, Meta, Colombia*
3 *Department of Biological Sciences, Fordham University, Bronx, New York 10458, USA*
Corresponding authors: Ana M. Saldarriaga-Gómez (asaldarriaga@fordham.edu); Mario Vargas-Ramírez (maavargasra@unal.edu.co)

Academic editor: William Magnusson
Received: 24 March 2023
Accepted: 24 May 2023
Published: 24 July 2023

ZooBank: https://zoobank.org/F35A0EA7-6E10-497D-A933-259AA09A77DC

Citation: Saldarriaga-Gómez AM, Ardila-Robayo MC, Medem F, Vargas-Ramírez M (2023) Hope is the last thing lost: Colombian captive-bred population of the critically endangered Orinoco crocodile (*Crocodylus intermedius*) is a genetic reservoir that could help to save the species from extinction. Nature Conservation 53: 85–103. https://doi.org/10.3897/natureconservation.53.104000

Abstract

A purpose of *ex-situ* populations is the preservation of genetic variation, but this is a challenging task since genetic diversity is commonly lost through each generation, and so the establishment of management guidelines should be a high priority. Fifty years ago, the National University of Colombia began a breeding program in the Roberto Franco Tropical Station (in Villavicencio, Meta) to conserve the critically endangered Orinoco crocodile *Crocodylus intermedius*. Despite the large number of individuals raised and kept in captivity, the Station has not been able to release individuals due to a lack of a complete genetic characterization that could determine whether the population is genetically viable. In this study we used a panel of 17 microsatellite loci to overcome this problem. We estimated from the founder animals and the live crocodiles the inbreeding, heterozygosities, the number of alleles, and their richness, and frequencies to understand the effects of managing a captive breeding program without considering genetic profiles. Our results revealed that the living population maintains much of its founder diversity with high levels of heterozygosity and low overall inbreeding, making it suitable for maintaining captive breeding and for implementing wild releases. We estimated the individual genetic diversity of the living crocodiles, as well as their relationships. This information, combined with the size, sex, and location, allowed us to propose combinations and to restructure the breeding groups. We demonstrated that molecular data could be used to improve the management of *ex-situ* conservation programs well beyond what could be achieved with pedigree information alone.

Key words: Critically endangered species, *ex-situ* conservation, genetic diversity, microsatellites, population genetics

Introduction

Despite *in-situ* conservation representing the most effective way to protect endangered species, *ex-situ* conservation programs and reintroduction of captive-bred animals have become an important tool for managing the same spe-

---

† Deceased.

cies (Witzenberger and Hochkirch 2011). And in many cases these programs might be the only way to save them from extinction (Bertorelle et al. 2009). Nowadays, the aim of the *ex-situ* conservation centers goes beyond the survival of individuals targeting the conservation of genetic diversity (Ramírez et al. 2006). The probability of species long-term survival will be increased by efforts to restore as much genetic variation as possible through the production and reintroduction of offspring with high genetic diversity, capable of resisting and adapting to the environmental pressures of natural habitats (Araki et al. 2007; Goncalves da Silva et al. 2010). Nevertheless, this is not always an easy task, and several management considerations must be contemplated.

Despite detailed studbooks being the simplest means for the proper management of captive populations, the correct parental allocation of individuals is not always possible without the use of molecular data, since pedigree information is often insufficient to select the best breeding pairs (Tzika et al. 2008). Additionally, founders are assumed to be unrelated (i.e., founder assumption), although this is not always true (e.g., individuals born of the same brood) and this may lead to an underestimation of relatedness, resulting in incorrect management decisions (Russello and Amato 2004). Genetic information can guide the choice of individuals with the lowest mean kinship and highest diversity as parents of subsequent generations, reducing the overall level of relatedness, maximizing founder representation, and minimizing the expression of deleterious alleles in inbred animals (Montgomery et al. 1997).

The Orinoco crocodile (*Crocodylus intermedius*. Fig. 1) is endemic to the Orinoco Basin in Colombia and Venezuela, being considered as the most endangered and least-studied of the New World crocodilians (Ross 1998; Antelo et al. 2010; Seijas et al. 2010; Moreno-Arias and Ardila-Robayo 2020; Parra-Torres et al. 2020). It is one of the largest species of crocodiles, with males reaching lengths of up to seven meters. Sexual maturity is typically attained by females between seven and ten years of age, while males reach sexual maturity between nine and twelve years (Thorbjarnarson 1987; Garcés-Restrepo et al. 2014). During the 20th century, commercial hunting of the Orinoco crocodile, motivated by the high demand for its skin, brought the species to the brink of extinction and consequently, the species is categorized as Critically Endangered (CR) in the IUCN Red Book List (IUCN 2020). Currently the population status of the species is unknown, and the last censuses reported a general trend of poor recovery or population decline (Medem 1981; Lugo 1996; Seijas et al. 2010; Espinosa-Blanco and Seijas 2012; Babarro 2014; Parra-Torres et al. 2020).

To tackle this situation, two direct conservation strategies have been suggested and followed in Colombia. First, its protection has been legally regulated by prohibition decrees and through practices of improvement and protection of its habitats (Castro Casal et al. 2013). Second, as in other crocodilian species (e.g., *Alligator sinensis*; Xu et al. 2005), a captive breeding program was established in 1971 by Federico Medem at the Roberto Franco Tropical Biological Station (EBTRF by its acronym in Spanish) of the National University of Colombia located in Villavicencio, Meta department. Since 1998 the EBTRF has been part of the National Program for the Conservation of the Orinoco Crocodile (PROCAIMAN; see MAM 2002), and it represents the largest and only stock of individuals of the species in Colombia, allegedly containing more crocodiles than those found in the wild in the country (Posso-Peláez et al. 2018). Currently it contains more than

Figure 1. Adult female of *Crocodylus intermedius* at the Roberto Franco Tropical Biological Station. Photograph MVR.

600 individuals distributed in five different locations known as *ex-situ* subpopulations: Piscilago, Wisirare, Merecure, Ocarros, and EBTRF, being the last hope for the recovery of the species in the country. Nevertheless, despite the variety of ages and the large number of individuals raised and kept in captivity, the EBTRF has been unable to reintroduce animals due to the lack of a robust and conclusive genetic characterization that determines whether the population is genetically viable and has no signs of inbreeding (see MAM 2002). Therefore, it is unknown if the individuals have enough genetic diversity and are genetically adequate for release and if the individuals can be used to maintain and increase the *ex-situ* population´s genetic variability. In addition, due to the extended time that the program has been in operation, it is unknown whether there has been a loss of diversity.

In this study we used a panel of 17 microsatellite loci to genetically characterize the *ex-situ* population of the EBTRF and to tackle the previously described issues. We estimated allelic richness, frequencies, and heterozygosities in living and founder crocodiles to understand at the genetic level the effects of managing a captive breeding program without considering the genetic profile of the individuals and the population. Based on this data, we also estimated relationships of living individuals and developed recommendations for the combination of breeding groups.

Materials and methods

Sampling

Since 2004 tissue samples have been taken from most of the individuals comprising the *ex-situ* population in charge of the EBTRF. Scales and muscle samples were preserved in pure ethanol and kept at -20 °C until processing. We searched EBTRF records to clarify the geographic origins, status, and current location of each crocodile. All the animals were microchipped for individual identification.

In total, we included 551 individuals in the study. The complete dataset includes 40 crocodiles that were wild in origin (Suppl. material 1: appendix S1). They were either acquired through direct captures as wildlife, from the breeding center or as confiscations. These animals included 24 seized individuals whose provenance was unknown (13 were dead in 2021), nine young individuals from Cravo Norte River (by 2021 one was dead and eight were 11 years old), and seven dead wild founders from the Cuisiana River, the Metica River, the Humea River, the Meta River, the Guachiría River, and the Vichada River. The remaining samples corresponded to 458 individuals of the captive offspring (F1 and F2) and 53 released crocodiles.

From the living individuals we evaluated, 82% belonged to the captive breeding program from five subpopulations: 316 were from the main headquarters of the program at the EBTRF in Villavicencio, Meta department; 19 were from the Parque Agroecológico Merecure in Puerto López, Meta department; five were from the Bioparque los Ocarros in Villavicencio, Meta department; four were from the Aquatic and Conservation Park Piscilago in Nilo, Cundinamarca department; and 127 were from the Parque Ecotemático Wisirare in Orocué, Casanare department. The remaining samples were from the two largest subpopulations: 44 from the EBTRF and 56 from Wisirare.

Laboratory procedures and genotyping

Genomic DNA was extracted from preserved tissue using the Invisorb Spin Tissue Mini Kit (Stratec) following manufacturer protocols. Seventeen microsatellite primers developed for other species of the genus and already evaluated for cross amplification by Lafferriere et al. (2016) were used. We implemented four PCRs multiplex using the Multiplex PCR kit MyTaq HS Mix (Bioline, USA) M1: CpP302, CpP305, CpP314, CpP1409, and CpP3216; M2: Cj16, Cj122, and Cu5123; M3: Cj18, Cj109, C391, CUJ131 and M4: Cj101, Cj127, Cp801, and CpDi13. Reactions were prepared in a final volume of 10 µL including: 5 µL of MyTaq HS Mix, 0.2 µL of 10X each primer (except for Cj122 and Cj109 with 0.4 µL added), a final concentration of 4 ng/ µL of DNA and the excess of ultra-pure water. Thermocycling conditions were as follows: a preliminary denaturation stage at 95 °C for 4 minutes, followed by 30 denaturation cycles at 95 °C for 30 seconds, two different annealing temperatures (57 °C for M1, M2 and M4 and 60 °C for M3) for 45 seconds and extension at 72 °C for 30 seconds, ending with a temperature of 72 °C for 5 minutes. Fragment lengths were determined using an ABI 3500 genetic analyzer. For this purpose, 1 µl of the PCR product was diluted in 99 µl water; 1 µl of this dilution was mixed with 8.5 µl Hi-Di Formamide (Applied Biosystems), 0.25 µl water and 0.25 µl GeneScan-600 LIZ Size Standard (Applied Biosystems). The Gene-Mapper 3.7 (Applied Biosystems Foster City, CA) and Osiris 2.13.1 (NCBI) software were used for scoring fragment lengths. A single person carried out the visualization and determination of allele sizes. Genetic laboratory work was conducted at the Molecular Ecology Laboratory of the Genetics Institute, National University of Colombia in Bogotá.

Data analysis

Estimation of loss of genetic diversity

To evaluate any loss of genetic diversity, the EBTRF crocodilian population was subdivided into two groups. The first group was composed of 40 F0 dead and alive crocodiles representing the genetic potential that the station has had since it was founded. The second group contained 468 live individuals, including F0, F1 and F2 distributed in the different *ex-situ* subpopulations, representing the current potential diversity of the population. Null allele frequencies at each locus on the whole dataset were estimated using the software FreeNA (Chapuis and Estoup 2007) and CERVUS 3.0.7 (Kalinowski et al. 2007), and null alleles were considered when the frequency was higher than 0,05. Allele dropout was estimated using MICRO-CHECKER 2.2.3 (van Oosterhout et al. 2004).

For the F0 population and the whole living population the number of alleles per locus (nA), allelic richness (AR), allelic frequencies and inbreeding coefficient (F_{IS}) were calculated using the software FSTAT 2.9.3.2 (Goudet 2001). F_{IS} significance for excess and defect of heterozygous was evaluated in Genepop 4.7.5 ($p\text{-value} < 0.005$; Raymond and Rousset 1995). Statistically significant differences for AR, Ho and F_{IS} between population subdivisions were tested with 15,000 permutations in FSTAT 2.9.3.2 (Goudet 2001). We removed F0 individuals from the living crocodilians to maintain independence between both groups. Expected heterozygosities (He) and observed heterozygosities (Ho) were estimated using ARLEQUIN 3.5.1.2 (Excoffier et al. 2005). The same software was used to test for Hardy Weinberg equilibrium (HWE) and linkage equilibrium and Bonferroni corrections were applied to both calculations.

Assessment of parental veracity

To assess the veracity of the provenance of the captive bred individuals registered in the records, we ran a parental pairs analysis with known sexes using the likelihood-based approach implemented in the software CERVUS 3.0.7 (Kalinowski et al. 2007). Two levels of confidence were set at 80% (relaxed) and 95% (strict). Positive LOD scores (the logarithms of the likelihood ratios) and Delta scores were compared to identify the most likely parents for each offspring. We evaluated separately five groups: 1) seven individuals registered as coming from the founding couple Pancho/Dabeiba; 2) two individuals registered as coming from Ocarros; 3) 54 individuals registered as coming from Merecure; 4) 90 individuals registered as coming from Piscilago; and 5) 246 individuals registered as coming from Wisirare. To determine samples' distributions and using the potential fathers by location for each of the groups, we ran simulations of 10,000 offspring genotypes, each at a sampling rate of 100% and with a proportion of mistyped loci set at 0.01. Determinations were made conforming to the established sets with the location and origin of the individuals.

Assessment of founder assumption

Relationships among the founder crocodiles were inferred using ML-RELATE (Kalinowski et al. 2006), a maximum likelihood-based software that estimates relatedness coefficients (r) for each pair of individuals and provides the relationships that have the highest likelihood for each pair of individuals (half-sibling, full-sibling, and unrelated). The coefficient goes from zero (i.e., individuals not related) to one (i.e., identical genotypes). We evaluated relatedness in two groups: nine individuals from Cravo Norte River and 12 individuals seized from the Rango Rudd hatchery.

Management formulations

To facilitate the development of management guidelines, the living crocodile population was subdivided into five groups according to the location of individuals in the subpopulations (i.e., EBTRF, Ocarros, Piscilago, Wisirare, and Merecure). The number of alleles per locus (nA) and allelic frequencies were calculated for each group using FSTAT 2.9.3.2 (Goudet 2001). Using this information, we genetically identified key individuals with rare alleles.

We estimated inbreeding coefficients at the individual level for each of the living and dead crocodiles using the GENHET 2.3 R script (Coulon 2010). We calculated the homozygosity by loci (HL), a homozygosity index that correlates with the inbreeding coefficient and weighs the contribution of each locus depending on their allelic variability (Aparicio et al. 2006). This allowed us to identify the most genetically diverse individuals. Homozygosity by loci indices for founders and live crocodiles (disregarding founders that may be alive to maintain sample independence) were compared using a U Mann Whitney test.

Relationships among all the living crocodiles were inferred using ML-RELATE (Kalinowski et al. 2006). The relatedness index allowed us to evaluate the relationships of the crocodiles that are alive and have reproduced. To guide the choice of reproductive pairs that will generate neonates with high genetic diversity, the *r* and the HL indexes, and the presence of alleles detected at low frequencies (see below), were combined with additional information from each single crocodile (i.e., size, age, sex, origin, current location), to propose options of viable crosses with reproductive, non-related, and highly diverse individuals from the five subpopulations already established and from two newly formed reproductive nuclei integrated at the Universidad de los Llanos in Villavicencio, Meta.

Results

The 17 microsatellite loci were successfully amplified for 548 of the 551 individuals. Between one and six loci failed to amplify for the other three samples. Locus CpP1610 resulted as monomorphic and therefore it was removed from the analyses. There was no evidence for null alleles or for allele dropout.

Estimation of loss of genetic diversity

Our data set represents 82% of the living crocodiles of the Station and 91% of the F0 population. Of the missing founders, three corresponded to juveniles from Cravo Norte that had not reproduced to date and only one founder breeder from which no tissue sample was taken. A total of 72 alleles were revealed: 69 in F0 crocodiles and 65 in live crocodiles (89.9% of the F0 alleles, Table 1). The F0 population had eight private alleles while the live population had three, suggesting that in the living population we found wild individuals or non-genotyped parents. The average number of alleles per locus, the average allelic richness, the expected heterozygosity, and the inbreeding coefficient F_{IS} were higher in the F0 population than in the live population (Table 1). Fourteen loci in the live population showed significant deviation from the Hardy-Weinberg equilibrium. No significant linkage disequilibrium was found between pairs of loci. In the F0 population two loci had deviations in the F_{IS} coefficient for heterozygous defect and one locus for heterozygous excess. The live population showed deviations in the F_{IS} coefficient in seven loci for heterozygous excess (Table 1).

Even though the live population showed a higher Ho than the F0 population, differences between each group were not significant (H0 p = 1). Likewise, although the F0 crocodiles showed generally higher AR and F_{IS}, differences were statistically not significant (AR p = 0.332, FIS p = 0.332). Although there were loci where allele frequencies did not change considerably between F0 and the live populations (e.g., CpP3216, Cj127; Table 2), there were other loci that showed strong changes, and even a loss of alleles (e.g., Cj109, Cj18, Cj391, Cpp801; Table 2).

Assessment of parental veracity and the founder assumption test

Of the 399 individuals evaluated, 325 (81.5%) had potential fathers in the subpopulation of origin registered; and for 74 (18.5%) individuals the physical registry does not correctly indicate the origin of these crocodiles (Table 3). When testing the relationships between the founding crocodiles that came from the same sites, we found that several were related (Table 4). Although the nine individuals from Cravo Norte showed unrelatedness in some cases, most were related as half and full-siblings. We found a similar result for the seized crocodiles from the Rango Rudd hatchery.

Table 1. Genetic diversity of the F0 and live populations of *Crocodylus intermedius* in the Roberto Franco Tropical Biological Station. N – sample size; nA – alleles per locus; AR – allelic richness; Ho – observed heterozygosity; He – expected heterozygosity; HWE – Hardy-Weinberg equilibrium; F_{IS} – inbreeding coefficient; * Significance for heterozygous defect; ** Significance for heterozygous excess.

Locus	Null alleles	F0 population (total alleles = 69)								Live population (total alleles = 65)							
		N	nA	Private alleles	AR	Ho	He	HWE	F_{IS}	N	nA	Private alleles	AR	Ho	He	HWE	F_{IS}
CpP3216	No	40	2	–	1.984	0.475	0.481	Yes	0.012	468	2	–	1.971	0.561	0.460	**No**	-0.217**
CpP305	No	40	3	–	2.877	0.600	0.664	Yes	0.097	468	3	–	2.566	0.530	0.588	**No**	0.100
CpP1409	No	40	3	–	2.198	0.375	0.445	Yes	0.159	468	3	–	2.622	0.650	0.565	**No**	-0.152**
CpP302	No	40	5	–	3.646	0.750	0.693	Yes	-0.084	468	5	–	3.481	0.750	0.707	**No**	-0.062**
CpP314	No	40	3	–	2.795	0.550	0.619	Yes	0.113	468	3	–	2.874	0.639	0.663	**No**	0.034
Cj16	No	40	5	1	3.168	0.600	0.596	Yes	-0.007	468	4	–	2.721	0.620	0.561	**No**	-0.102
CU5123	No	40	4	–	3.058	0.800	0.682	Yes	-0.175**	468	4	–	3.292	0.741	0.689	**No**	-0.079**
Cj122	No	40	5	–	4.064	0.700	0.781	Yes	0.105	468	5	–	3.942	0.816	0.771	**No**	-0.057
Cj18	No	40	5	1	3.404	0.775	0.702	Yes	-0.106	468	5	1	3.071	0.635	0.612	**No**	-0.040
CUJ131	No	40	4	1	2.325	0.400	0.492	Yes	0.189*	468	3	–	2.009	0.560	0.502	Yes	-0.114**
Cj109	No	40	6	2	3.543	0.675	0.716	Yes	0.059	468	4	–	3.266	0.786	0.699	**No**	-0.123**
Cj391	No	40	10	2	4.546	0.675	0.806	Yes	0.164*	468	8	–	2.859	0.583	0.537	**No**	-0.089
CCj101	No	40	3	–	2.184	0.575	0.529	Yes	-0.087	468	4	1	2.151	0.596	0.485	**No**	-0.230
CpDi13	No	40	2	–	1.969	0.475	0.453	Yes	-0.050	468	3	1	2.057	0.506	0.479	Yes	-0.053
Cj127	No	40	3	–	1.291	0.075	0.074	Yes	-0.017	468	3	–	1.828	0.344	0.299	**No**	-0.152**
CpP801	No	40	6	–	3.500	0.725	0.703	Yes	-0.032	468	6	–	2.974	0.637	0.582	**No**	-0.094
Mean			4.313		2.910	0.577	0.590		0.019		4.063		2.730	0.622	0.575		-0.013
SD			1.991		0.871	0.187	0.179		0.086		1.482		0.610	0.117	0.118		0.059

Table 2. Allelic frequencies of 16 polymorphic microsatellite loci in F0 and live populations of *Crocodylus intermedius* in the Roberto Franco Tropical Biological Station. [a] Private allele in that population; [b] Private allele in that subpopulation; [c] Alleles with low frequencies.

Locus	Allele	F0 population (N = 40; nA = 69)	Live population					
			Total (N = 465, nA = 65)	EBTRF (N = 314, nA = 63)	Ocarros (N = 5, nA = 52)	Piscilago (N = 4, nA = 43)	Wisirare (N = 127, nA = 53)	Merecure (N = 18, nA = 49)
CpP3216	137	0.613	0.643	0.642	0.900	0.625	0.638	0.667
	141	0.388	0.357	0.358	0.100	0.375	0.362	0.333
CpP305	176	0.325	0.103	0.080	0.100	0.375	0.169	0.000
	192	0.413	0.435	0.482	0.800	0.500	0.303	0.444
	196	0.263	0.461	0.438	0.100	0.125	0.528	0.556
CpP1409	245	0.263	0.286	0.299	0.200	0.750	0.248	0.250
	249	0.700	0.578	0.605	0.700	0.250	0.512	0.611
	253	0.038	0.135	0.096	0.100	0.000	0.240	0.139
CpP302	194	0.500	0.431	0.422	0.700	0.750	0.429	0.472
	196	0.138	0.173	0.164	0.100	0.000	0.197	0.194
	200	0.150	0.133	0.140	0.100	0.125	0.134	0.056
	202	0.138	0.017 [c]	0.022	0.100	0.000	0.000	0.028
	208	0.075	0.245	0.252	0.000	0.125	0.240	0.250
CpP314	254	0.525	0.367	0.433	0.400	0.375	0.217	0.278
	258	0.238	0.351	0.330	0.300	0.250	0.382	0.556
	262	0.238	0.283	0.237	0.300	0.375	0.402	0.167
Cj16	141	0.125	0.053	0.064	0.200	0.250	0.000	0.194
	151	0.038 [a]	0.000	0.000	0.000	0.000	0.000	0.000
	167	0.600	0.592	0.596	0.700	0.625	0.587	0.528
	171	0.175	0.286	0.291	0.100	0.125	0.283	0.278
	173	0.063	0.069	0.049	0.000	0.000	0.130	0.000
CU5123	204	0.250	0.246	0.260	0.200	0.375	0.201	0.333
	214	0.025	0.094	0.111	0.100	0.000	0.039	0.222
	216	0.375	0.216	0.221	0.600	0.375	0.181	0.222
	220	0.350	0.444	0.408	0.100	0.250	0.579	0.222
Cj122	378	0.175	0.156	0.169	0.200	0.375	0.126	0.056
	380	0.175	0.310	0.275	0.200	0.125	0.406	0.278
	386	0.350	0.178	0.215	0.400	0.250	0.051	0.417
	390	0.163	0.092	0.080	0.100	0.125	0.138	0.000
	392	0.138	0.263	0.261	0.100	0.125	0.280	0.250
Cj18	203	0.000	0.005 [a,c]	0.008 [b,c]	0.000	0.000	0.000	0.000
	207	0.300	0.209	0.231	0.400	0.250	0.122	0.361
	209	0.163	0.157	0.140	0.000	0.000	0.228	0.028
	211	0.425	0.560	0.572	0.600	0.750	0.520	0.611
	213	0.100	0.069	0.049	0.000	0.000	0.130	0.000
	215	0.013 [a]	0.000	0.000	0.000	0.000	0.000	0.000
CUJ131	185	0.650	0.517	0.463	0.400	0.500	0.685	0.222
	189	0.013 [a]	0.000	0.000	0.000	0.000	0.000	0.000
	191	0.300	0.481	0.537	0.400	0.500	0.311	0.750
	193	0.038	0.002 [c]	0.000	0.200	0.000	0.004	0.028
Cj109	372	0.213	0.338	0.347	0.400	0.375	0.307	0.361
	374	0.238	0.072	0.045	0.100	0.000	0.130	0.139
	376	0.013 [a]	0.000	0.000	0.000	0.000	0.000	0.000
	382	0.100	0.231	0.240	0.100	0.125	0.213	0.222
	384	0.425	0.359	0.368	0.400	0.500	0.350	0.278
	388	0.013 [a]	0.000	0.000	0.000	0.000	0.000	0.000
Cj391	153	0.350	0.646	0.611	0.300	0.500	0.728	0.722
	157	0.075	0.017 [c]	0.025	0.000	0.000	0.000	0.028
	159	0.013 [a]	0.000	0.000	0.000	0.000	0.000	0.000
	161	0.013	0.001 [c]	0.000	0.100	0.000	0.004	0.000
	169	0.050	0.098	0.126	0.100	0.000	0.016	0.250
	171	0.013	0.003 [c]	0.005 [b]	0.000	0.000	0.000	0.000

Locus	Allele	F0 population (N = 40; nA = 69)	Live population					
			Total (N = 465, nA = 65)	EBTRF (N = 314, nA = 63)	Ocarros (N = 5, nA = 52)	Piscilago (N = 4, nA = 43)	Wisirare (N = 127, nA = 53)	Merecure (N = 18, nA = 49)
Cj391	173	0.163	0.033 [c]	0.041	0.100	0.500	0.000	0.000
	175	0.175	0.191	0.178	0.200	0.000	0.252	0.000
	179	0.125	0.01 [c]	0.013	0.200	0.000	0.000	0.000
	183	0.025 [a]	0.000	0.000	0.000	0.000	0.000	0.000
CCj101	354	0.000	0.012 [a,c]	0.003	0.000	0.000	0.035	0.000
	356	0.513	0.625	0.634	0.600	0.375	0.587	0.833
	358	0.025	0.012 [c]	0.016	0.000	0.000	0.004	0.000
	360	0.463	0.352	0.347	0.400	0.625	0.374	0.167
CpDi13	358	0.000	0.011 [a]	0.013	0.000	0.125	0.000	0.028
	360	0.663	0.624	0.613	0.600	0.625	0.650	0.556
	362	0.338	0.366	0.374	0.400	0.250	0.350	0.417
Cj127	337	0.963	0.817	0.820	1.000	0.875	0.815	0.750
	341	0.013	0.004 [c]	0.003	0.000	0.000	0.000	0.056
	343	0.025	0.178	0.177	0.000	0.125	0.185	0.194
CpP801	166	0.050	0.002 [c]	0.002	0.100	0.000	0.000	0.000
	170	0.063	0.168	0.177	0.100	0.000	0.146	0.222
	174	0.013	0.001 [c]	0.002 [b]	0.000	0.000	0.000	0.000
	178	0.338	0.167	0.170	0.300	0.125	0.177	0.000
	182	0.413	0.599	0.557	0.400	0.750	0.677	0.778
	186	0.125	0.063	0.092	0.100	0.125	0.000	0.000

Table 3. Number of individuals with registered provenance with and without potential parents in each assigned subpopulation. N – sample size.

	Dabeiba-Pancho (N = 7)	Ocarros (N = 2)	Merecure (N = 54)	Piscilago (N = 90)	Wisirare (N = 246)
Individuals with potential parents	7	1	53	62	202
Individuals without potential parents	0	1	1	28	44

Table 4. Coefficient of relationship and possible relationships within the founder crocodiles from Cravo Norte River (a) and Rango Rudd hatchery (b). Relationships: U = unrelated; HS = half sibling; FS = full sibling.

a. Cravo Norte

	575	579	581	584	592	593	1021	1072	1266			
575	–											
579	0.125 HS	–										
581	0.000	0.650 FS	–									
584	0.406 HS	0.000	0.000	–								
592	0.178 HS	0.614 FS	0.747 FS	0.000	–							
593	0.116 HS	0.445 FS	0.508 FS	0.000	0.544 FS	–						
1021	0.000	0.282 HS	0.401 HS	0.050 U	0.398 FS	0.483 FS	–					
1072	0.042 U	0.310 FS	0.395 HS	0.000	0.500 FS	0.417 HS	0.601 FS	–				
1266	0.000	0.269 U	0.369 FS	0.011 U	0.349 HS	0.285 HS	0.464 FS	0.592 FS	–			

b. Rango Rudd hatchery

	105	106	122	127	128	156	162	163	213	214	215	385
105	–											
106	0.575 FS	–										
122	0.500 FS	0.200 HS	–									
127	0.000	0.085 U	0.500 FS	–								
128	0.576 FS	0.243 HS	0.294 HS	0.302 FS	–							
156	0.142 HS	0.151 HS	0.353 HS	0.500 FS	0.000	–						
162	0.304 HS	0.275 HS	0.787 FS	0.366 HS	0.220 HS	0.272 HS	–					
163	0.000	0.000	0.402 HS	0.451 FS	0.102 U	0.264 HS	0.261 HS	–				
213	0.492 FS	0.306 FS	0.180 HS	0.172 U	0.006 U	0.283 HS	0.205 HS	0.000	–			
214	0.173 HS	0.132 HS	0.642 FS	0.321 HS	0.000	0.547 FS	0.615 FS	0.170 HS	0.248 HS	–		
215	0.000	0.000	0.434 FS	0.392 HS	0.000	0.714 FS	0.317 FS	0.213 HS	0.034 U	0.694 FS	–	
385	0.028 U	0.000	0.5 FS	0.303 HS	0.500 FS	0.000	0.330 HS	0.627 FS	0.027 U	0.188 HS	0.000	–

Management formulations

The number of alleles differed among the five subpopulations (Table 2). As expected, the largest subpopulations (EBTRF and Wisirare) showed unique alleles and the highest number of alleles (63 and 53 alleles, respectively), while the population with the lowest number of alleles was Piscilago (43 alleles). Although the other subpopulations did not show unique alleles, they did have alleles at very low frequencies (e.g., allele 203 locus Cj18 was present in five individuals or allele 193 locus Cj131 in only three individuals; Suppl. material 1: appendix S2). We identified and prioritized the management guidelines of 76 individuals that contained those rare alleles with low frequencies. The individual diversity (HL) of the living individuals that make up the entire *ex-situ* population varied between 0.075 and 0.947. However, 95.5% of the individuals that had an index lower than 0.6 and 74.8% were grouped between 0.2 and 0.5 (Suppl. material 1: appendix S3). No differences were found between the HL of founding crocodiles and living crocodiles (p-value = 0.292; $\alpha = 0.05$).

We found that in some cases current combinations of individuals are not the most appropriate when considering their genetic profiles. For example, the EBTRF represents the largest of the *C. intermedius* subpopulations and contains 97% of the alleles from the entire captivity program including 55 priority crocodiles and three unique alleles; but the subpopulation has no active reproductive nucleus. Piscilago has an F0 priority male in an isolated tank only for exhibition. The three males found in Ocarros are priority crocodiles since they have scarce alleles, but two of them are related to the females located there and they have not contributed to the growth of the captive population. Since genetic parameters for the selection of reproductive individuals must be urgently considered, we proposed changes and reorganized crocodiles in the subpopulations with combinations that guarantee the recovery of rare alleles and minimize the mean kinship. All the parental combinations were assembled by the combination of the *r* and HL indexes with important complementary information regarding every single crocodile (i.e., size, age, sex, origin, current location, capacity of the tanks). Using this information, we considered the priority crocodiles identified with the allele frequencies, combining them with unrelated crocodiles of reproductive age that showed the lower HL. We also considered whether the selected individuals had the appropriate size and health status, as well as if they had normal growth according to the growth model estimated for the EBTRF.

We reorganized the individuals that make up the reproductive nucleus of Ocarros and the two nuclei of Piscilago, and we selected the individuals of the two new nuclei from the Universidad de los Llanos. In Wisirare we proposed not to make changes considering that transport to Wisirare is complex, and since we found an unrelated kinship level and a low HL in the individuals that made up the breeding stock. For now, we recommend considering only the six reproductive nuclei mentioned above (Table 5). Once the individuals are reorganized, we provided program officials with the necessary information to establish new pairs in EBTRF and Merecure that will depend on the capacity of the tanks. We selected six males not suitable for reproduction (with no priority and high HL) to be taken to Piscilago (one individual) and to the Hacienda Nápoles Park in Puerto Triunfo, Antioquia (five individuals). These individuals are to take part in environmental education and awareness-raising, but not for reproduction.

Table 5. Past and present reproductive combinations for four *ex-situ* subpopulations of *Crocodylus intermedius* in Colombia. The values in parentheses represent the homozygosity by loci for each individual. The values in the table represent the relatedness (relationship) coefficient between both individuals compared. Females are in the rows, males in the columns. Individuals in bold represent priority crocodiles. Relationships: U Unrelated; HS Half sibling; FS Full sibling.

<table>
<tr><th></th><th colspan="4">Ocarros</th><th colspan="5">Piscilago</th></tr>
<tr><td rowspan="4">Past Situation</td><td colspan="4">Unique tank</td><td colspan="2">Tank 1</td><td colspan="3">Tank 2 (isolated)</td></tr>
<tr><td>F/M</td><td>**154 (0.195)**</td><td>**156 (0.478)**</td><td>**157 (0.610)**</td><td>F/M</td><td>**214 (0.456)**</td><td colspan="3">**213 (0.351)**</td></tr>
<tr><td>155 (0.453)</td><td>0</td><td>0</td><td>0</td><td>115 (0.233)</td><td>0</td><td colspan="3">0</td></tr>
<tr><td>158 (0.226)</td><td>0.144 HS</td><td>0</td><td>0.14 HS</td><td>**118 (0.351)**</td><td>0.248 HS</td><td colspan="3">1 FS</td></tr>
<tr><td rowspan="5">Present combinations</td><td>F/M</td><td colspan="3">**156 (0.478)**</td><td colspan="2">Tank 1</td><td>Tank 2</td><td colspan="2">Tank 3</td></tr>
<tr><td>155 (0.453)</td><td colspan="3">0</td><td>F/M</td><td>**214 (0.459)**</td><td rowspan="4">193 (0.599)
Isolated</td><td>F/M</td><td>**213 (0.351)**</td></tr>
<tr><td>158 (0.226)</td><td colspan="3">0</td><td>115 (0.233)</td><td>0</td><td>**238 (0.323)**</td><td>0</td></tr>
<tr><td>**172 (0.245)**</td><td colspan="3">0</td><td>258 (0.203)</td><td>0</td><td>239 (0.319)</td><td>0</td></tr>
<tr><td>**272 (0.239)**</td><td colspan="3">0</td><td>345 (0.306)</td><td>0</td><td>268 (0.289)</td><td>0</td></tr>
<tr><th></th><th colspan="4">Universidad de los Llanos</th><th colspan="5">Wisirare</th></tr>
<tr><td rowspan="11">Present combinations</td><td colspan="2">Tank 1</td><td colspan="2">Tank 2</td><td colspan="5">Unique tank</td></tr>
<tr><td>F/M</td><td>**579 (0.303)**</td><td>F/M</td><td>**157 (0.610)**</td><td colspan="3">F/M</td><td>385 (0.405)</td><td>389 (0.189)</td></tr>
<tr><td>174 (0.384)</td><td>0</td><td>194</td><td>0</td><td colspan="3">384 (0.441)</td><td>0</td><td>0</td></tr>
<tr><td>203 (0.429)</td><td>0</td><td>240</td><td>0</td><td colspan="3">387 (0.310)</td><td>0</td><td>0</td></tr>
<tr><td>255 (0.347)</td><td>0</td><td>256</td><td>0</td><td colspan="3">388 (0.292)</td><td>0</td><td>0</td></tr>
<tr><td>262 (0.387)</td><td>0</td><td>257</td><td>0</td><td colspan="3">391 (0.171)</td><td>0</td><td>0.173 U</td></tr>
<tr><td>274 (0.274)</td><td>0</td><td>270</td><td>0</td><td colspan="3">392 (0.265)</td><td>0</td><td>0</td></tr>
<tr><td>276 (0.417)</td><td>0</td><td>275</td><td>0</td><td colspan="5"></td></tr>
<tr><td>286 (0.305)</td><td>0</td><td>332</td><td>0</td><td colspan="5"></td></tr>
<tr><td>290 (0.339)</td><td>0</td><td>450</td><td>0</td><td colspan="5"></td></tr>
<tr><td>576 0.309)</td><td>0</td><td>577</td><td>0.01 U</td><td colspan="5"></td></tr>
</table>

Finally, we selected 84 juvenile individuals that have the genetic, size, health, and age requirements for release (sizes less than 2.5 m and HL < 0.6), and we moved them to a tank under semi-natural conditions in Merecure park.

Discussion

This study represents one of the few examples of the application of genetic tools for the management of captive-bred populations of endangered reptiles (Witzenberger and Hochkirch 2011), and these results are pivotal for the feasibility of the breeding program of the Orinoco crocodile in Colombia and as a strategy for its conservation. Our results revealed that the EBTRF living population maintains much of its founder diversity, high levels of heterozygosity, and a low overall inbreeding, and make it suitable for maintaining captive breeding and allowing wild releases.

Genetic diversity of the captive population of EBTRF

The expected heterozygosity obtained in the currently living crocodiles of the EBTRF is similar and even higher than that reported for wild populations of other species of the genus *Crocodylus*, evaluated with the same loci (e.g., Isberg et al. 2004; McVay et al. 2008; Mauger et al. 2017). This shows that, although the captive population of the Orinoco crocodile experienced an allele loss compared with the founder population, it maintains an important part of the variability in terms of heterozygosity, with genetically viable individuals to be reproduced and used for conservation and management. This is because few breeding pairs comprised unrelated and genetically diverse individuals and there has not been a generational turnover that might result in reproduction between relatives.

Our results showed no statistical difference between observed heterozygosity and the allelic richness between the live and the founder populations. However, a decrease in variability was detected by the loss of alleles (Table 2). This phenomenon has already been reported in *ex-situ* population of other reptiles: in a captive population of the northern river terrapin *Batagur baska*, the first generation maintained only 73% of the founder alleles, while there were no significant differences in the heterozygosities between founders and the first generation (Spitzweg et al. 2018). Furthermore, in captive populations of the Jamaican yellow boa *Chilabothrus subflavus*, a loss of genetic diversity due to the differential reproduction of individuals was detected in the first generations by the allelic richness and not by the heterozygosities (Tzika et al. 2008). This reflects the limited efficiency of tests based on heterozygosity variations to detect recent inbreeding (Luikart et al. 1998; Tzika et al. 2008). A similar situation was detected in the EBTRF, where variations in allele frequencies showed that only a few reproducing founders segregated alleles to the next generation. Consequently, knowing the genetic profile of individuals is crucial for developing strategies that prevent genetic loss.

One of the objectives of captive breeding programs is to guarantee the survival of the offspring, which can be compromised by phenomena such as inbreeding and captive adaptation (Farquharson et al. 2021). We found that the inbreeding coefficient is higher in F0 than in the live population and in the living population we have deviations in seven loci due to excess heterozygotes while in the F0 population there are no loci with this deviation. This may be because, initially, many confiscated individuals came from the same breeding farm and thus may be closely related. However, most of these crocodiles did not reproduce; and, if they did, they were combined with wild or seized crocodiles, decreasing the F_{IS} of the living ones. For the future management of the program, generational change must be considered since species' response to captivity adaptation may differ in the first generation in relation to subsequent generations (Farquharson et al. 2021). Captive adaptation can improve fitness at the population level in the captive environment, but when animals are returned to the wild, captive adaptations can be maladaptive and contribute to the poor success of reintroduction programs (Frankham 2008; Jule et al. 2008).

The captive breeding program of the EBTRF plays a key role in Orinoco crocodile conservation. Nonetheless, management of these captive populations was not guided by the standards necessary to conserve and maximize genetic diversity, despite the previous recommendation for genetic monitoring (see Williams and Osentoski 2007). Furthermore, genetic management has been recommended within the actions framed in PROCAIMAN (MAM 2002). The only genetic characterization of the EBTRF *ex-situ* population was carried out by Cuervo-Alarcón and Burbano-Montenegro (2012). In this study, the sampling coverage of the captive population was limited; of the seven wild founders, only one was used in the analysis. We genotyped the same crocodiles and obtained different numbers of alleles for the same loci (e.g., 16 alleles at locus Cj16 versus five in our study). Furthermore, their division of the samples in two groups based on age is inadequate, returning inconsistent results with an admixture of

F0, F1, and F2 generations in the established groups, as well as an erroneous determination of crocodiles with wild origin.

The only other study of *C. intermedius* population genetics considering wild individuals was carried out in Hato El Frío in Venezuela by Lafferriere et al. (2016) and aimed at reporting multiple paternity in the species. When comparing the EBTRF population with El Frío Biological Station population, the Venezuelan individuals have a greater diversity in terms of allele composition with 90 alleles in the 17 loci. It is remarkable that the locus CpP1610 was monomorphic in our study while in Venezuela it was polymorphic with two alleles, but with one allele more frequent than the other (Lafferriere et al. 2016). However, the overall He / Ho level in the EBTRF was a little higher than in Venezuela (0.622 / 0.575 vs 0.524 / 0.544). This difference was probably due to different ways in which individuals reproduced since even though the Venezuelan individuals were born from reintroduced individuals, they follow the principles of a natural population; while, in the EBTRF they have been dependent on arbitrary human management that has reproduced the same individuals without a generational change. Finally, unlike the Venezuelan population that did not show significant deviations from Hardy-Weinberg expectations at any locus (Lafferriere et al. 2016), in the EBTRF we found deviations in 14 loci for live crocodiles. These deviations were expected since the animals originated from a few breeding pairs crossed without scientific basis or management.

Management guidelines

The breeding program for *Crocodylus intermedius* in Colombia aims to preserve and increase as much as possible the current genetic diversity and to produce neonates with the highest genetic diversity possible to support management actions (MAM 2002). Based on our data, we suggest that selective breeding should be implemented, and some mating combinations should be avoided. To achieve this goal, we proposed a robust system of 16 polymorphic microsatellite loci for estimating the relationship, the individual diversity and rarity of the living crocodiles. This, combined with information of age, size, sex, and location, allowed us to design combinations for planning breeding groups in each subpopulation. The tool enables a simultaneous maximization of genetic diversity, combining non-related diverse individuals and individuals containing rare alleles to achieve a genetic gain by minimizing the relationships between the individuals combined and guaranteeing no loss of alleles in the following generations.

Our work is necessary and complements the previous data, since most captive breeding projects are not monitored genetically, and only recently attention has been paid to the pedigree or relatedness of breeders using conservation genetic approaches (e.g. Spitzweg et al. 2018). Furthermore, this is one of the few studies combining relatedness information with the homozygosity by loci, which can be very useful when the number of individuals involved is large, allowing discrimination only when the *r* index may not be enough.

Despite the living crocodiles of our sample retaining approximately 90% of the genetic diversity of the wild-caught founder individuals with the presence of three unique and 13 rare alleles, the difference in the number of alleles and the allele frequencies among the five subpopulations revealed that the diversity is unevenly distributed between groups. If no action is taken to balance this, the loss of rare genetic diversity in the next few generations could be drastic, jeopardizing the viability of the program (Groombridge et al. 2012). To solve this, we explicitly recommend using the combination of genetic data with the information from the archive records so as not to rely solely on the latter; since, as we found when performing the parent pairs analysis, a large part of the archive files is wrong in determining the origin of the individuals. Finally, we explicitly recommend implementing conservation genetic assessments for other captive breeding projects to preserve maximum genetic diversity and to avoid inbreeding depression (recommended by Xu et al. 2005; Tzika et al. 2008; Spitzweg et al. 2018).

The EBTRF conservation program covers a very restricted range of the historical natural distribution of the species in Colombia, and key individuals (e.g., from Vichada department) had rare alleles, suggesting that the genetic diversity of the Station does not cover the unknown threatened possible diversity available in the wild. It is necessary and urgent to evaluate wild populations, as well as to enrich the diversity of the Station´s population by including wild individuals from unsampled sites (e.g., Guayabero / Duda / Lozada Rivers). These individuals must be genotyped to determine the presence of rare alleles, individual genetic diversity, and degree of relationship. As we demonstrated here, the basic assumption of unrelated founders may be incorrect, particularly given the often-imprecise nature of information on their origin (Gautschi et al. 2003). We recommended completing the dataset with the missing crocodile samples and including them in the management guidelines. In turn, it is necessary to genotype the crocodiles that are going to be born to have a complete genetic profile of the program, to evaluate future trends in allele frequencies and to restructure combinations if necessary.

The EBTRF contains the largest subpopulation (about 370 individuals), the largest number of tanks available, and a high genetic diversity involving three unique alleles. More than 150 crocodiles have passed through the EBTRF and have died from recent hatchlings to the first clutches of 1991 and the F0. After 2005, fewer eggs from the EBTRF were incubated since eggs from Wisirare, Piscilago and later Occarros began to be carried to the Station for incubation. Considering that the EBTRF subpopulation has the highest number of adult crocodiles with unique diversity, it is necessary to re-implement the breeding stock with these individuals. It is urgent to maintain a balance in the proportion of eggs incubated according to their origin and the number of parents that produce them. In the EBTRF we found juvenile individuals that we considered as priority because they contained alleles at low frequencies (Suppl. material 1: appendix S2). However, these individuals have not attained reproductive age, so we recommend keeping them until they can be included in the reproductive nuclei.

Future perspective

Through the implementation of the crosses proposed here, the program will ensure highly genetically variable offspring that preserve the available genetic diversity. By combining the offspring produced by different reproductive pairs, we will be able to form groups of unrelated and highly diverse individuals that, according to the requirements of natural populations, could be released into the wild.

This research supports the actions defined in PROCAIMAN to advance the recovery of populations of the Orinoco crocodile in Colombia. This is of urgent application since, even though management actions were established 20 years ago, the natural situation of the species has apparently not changed or even has deteriorated (see Medem 1981; Lugo 1996; Seijas et al. 2010; Espinosa-Blanco and Seijas 2012; Babarro 2014; Parra-Torres et al. 2020). By using the genetic system developed here, it is urgent to genetically characterize wild populations to define whether they need genetic management. The evaluation of the captive populations together with natural populations, as well as demographic and ecological studies, should guide the recovery of eggs and hatchlings both *ex-situ* and *in-situ* and define a reintroduction protocol, including the monitoring of introduced and wild populations.

However, more support and research are needed to comply with what has been established in PROCAIMAN (MAM 2002). Currently, neither the population sizes nor important ecological characteristics are known, such as whether there is reproduction in wild populations, the magnitude of reproduction, physiological parameters associated with reproductive events, nesting times, demographic structure of populations, etc. As a crucial component for the success in the recovery of the species, focused environmental education and awareness initiatives must be developed. These efforts should aim to foster actions and establish an extensive dialogue with human communities regarding the coexistence of this Colombian crocodile and its recovery.

Acknowledgments

This publication is dedicated to María Cristina Ardila-Robayo and Federico Medem, who devoted their lives to increasing the knowledge and conservation of the Orinoco crocodile. Thanks to Humberto Arboleda-Bustos and Luis Fernando Cadavid-Gutiérrez of the Instituto de Genética (IGUN), Universidad Nacional de Colombia (UNAL) and Uwe Fritz of Senckenberg Museum in Dresden, Germany, for their academic support. Thanks to Rita Mercedes Baldrich-Ferrer and Magda Yaneth López-López from the Servicio de Secuenciación y Análisis Molecular (SSIGMOL) of the IGUN. Thanks to Helena María Ahumada Cadena of Corporación para el Desarrollo Sostenible del Área de Manejo Especial La Macarena (CORMACARENA). Many thanks to the directors and workers of the Estación de Biología Tropical Roberto Franco (EBTRF), who have dedicated themselves to the hard task of maintaining and handling in captivity individuals of *Crocodylus intermedius*, and for whom the love for the Orinoco crocodile has been the engine that encourages them to keep going day by day, despite the adversities. Thanks to the members of the group Biodiversity and Conservation Genetics-IGUN-UNAL, for their permanent support. Samples were processed in accordance with the “Permiso Marco de Recolección de especímenes de especies silvestres de la Diversidad Biológica con fines de investigación científica no comercial Resolución 0255-2014” granted by the Autoridad Ambiental de Licencias Ambientales (ANLA) to Universidad Nacional de Colombia, subscribed by the Grupo Biodiversidad y Conservación Genética de la Universidad Nacional de Colombia.

Additional information

Conflict of interest

The author has declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

The development of this project was possible thanks to the financial support of the agreement N°PE.GDE. 1.4.8.1.20.009 between the Corporación para el Desarrollo Sostenible del Área de Manejo Especial La Macarena (CORMACARENA) and the Science Faculty of the Universidad Nacional de Colombia (UNAL): "Aunar esfuerzos técnicos, científicos y financieros para realizar la caracterización genética por medio de marcadores moleculares nucleares variables (microsatélites) de la mayor población cautiva del críticamente amenazado caimán del Orinoco (*Crocodylus intermedius*), presente en la Estación de Biología Tropical Roberto Franco (EBTRF)" and the Convocatoria para el Fortalecimiento de Alianzas Interdisciplinarias de Investigación y Creación Artística de la Sede Bogotá de la Universidad Nacional de Colombia (UNAL)-2018: "Genética de la conservación de la mayor población cautiva del críticamente amenazado caimán del Orinoco (*Crocodylus intermedius*): una contribución para su supervivencia".

Author contributions

Conceptualization: MVR, AMSG. Data curation: AMSG, MVR. Formal analysis: AMSG, MVR. Funding acquisition: AMSG, MVR. Investigation: MVR, AMSG. Methodology: AMSG, MVR. Project administration: AMSG, MVR. Resources: AMSG, MVR. Software: AMSG, MVR. Supervision: MVR, AMSG. Validation: AMSG, MVR. Visualization: AMSG, MVR. Writing – original draft: MVR, AMSG. Writing – review and editing: AMSG, MVR.

Author ORCIDs

Ana M. Saldarriaga-Gómez https://orcid.org/0000-0003-3466-6313
Mario Vargas-Ramírez https://orcid.org/0000-0001-8974-3430

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

References

Antelo R, Ayarzagüena J, Castroviejo J (2010) Reproductive ecology of Orinoco crocodiles (*Crocodylus intermedius*) in a newly established population at El Frío Biological Station, Venezuela. The Herpetological Journal 20: 51–58.

Aparicio JM, Ortego J, Cordero J (2006) What should we weigh to estimate heterozygosity, alleles or loci? Molecular Ecology 15(14): 4659–4665. https://doi.org/10.1111/j.1365-294X.2006.03111.x

Araki H, Cooper B, Blouin MS (2007) Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. Science 318(5847): 100–103. https://doi.org/10.1126/science.1145621

Babarro R (2014) Balance de las liberaciones de caimán del Orinoco (*Crocodylus intermedius*) en Venezuela: 25 Años De Esfuerzo. Boletín de la Academia de Ciencias Físicas. Matemáticas y Naturales 74: 75–87.

Bertorelle G, Bruford M, Hauffe H, Rizzoli A, Vernesi C (2009) Population Genetics for Animal Conservation (Conservation Biology). Cambridge University Press, Cambridge.

Castro Casal A, Merchán Fornelino M, Garcés Restrepo MF, Cárdenas Torres MA, Gómez Velasco F (2013) Uso histórico y actual del caimán llanero (*Crocodylus intermedius*) en la Orinoquia (Colombia-Venezuela). Biota Colombiana 14(1): 65–82.

Chapuis M, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Molecular Biology and Evolution 24(3): 621–631. https://doi.org/10.1093/molbev/msl191

Coulon A (2010) GENHET: An easy-to-use R function to estimate individual heterozygosity. Molecular Ecology Resources 10(1): 167–169. https://doi.org/10.1111/j.1755-0998.2009.02731.x

Cuervo-Alarcón LC, Burbano-Montenegro C (2012) Caracterización genética de la población *ex situ* de *Crocodylus intermedius* presente en Colombia. Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales 36: 373–383.

Espinosa-Blanco AS, Seijas AE (2012) Declinación poblacional del Caimán del Orinoco (*Crocodylus intermedius*) en dos sectores del sistema del río Cojedes, Venezuela. Ecotrópicos 25: 22–35.

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online: 47–50. https://doi.org/10.1177/117693430500100003

Farquharson KA, Hogg CJ, Grueber CE (2021) Offspring survival changes over generations of captive breeding. Nature Communications 12(1): e3045. https://doi.org/10.1038/s41467-021-22631-0

Frankham R (2008) Genetic adaptation to captivity in species conservation programs. Molecular Ecology 17(1): 325–333. https://doi.org/10.1111/j.1365-294X.2007.03399.x

Garcés-Restrepo MF, Castro A, Merchán M, Cárdenas-Torres MA, Gómez-Velasco F (2014) *Crocodylus intermedius* (Graves 1819) in Catálogo de Anfibios y Reptiles de Colombia 2(1): 5–10.

Gautschi B, Jacob G, Negro JJ, Godoy JA, Muller JP, Schmid B (2003) Analysis of relatedness and determination of the source of founders in the captive bearded vulture, *Gypaetus barbatus* population. Conservation Genetics 4(4): 479–490. https://doi.org/10.1023/A:1024730128819

Goncalves da Silva A, Lalonde DR, Quse V, Shoemaker A, Russello MA (2010) Genetic approaches refine *ex situ* lowland tapir (*Tapirus terrestris*) Conservation. The Journal of Heredity 101(5): 581–590. https://doi.org/10.1093/jhered/esq055

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (Version 2.9.3.2). https://www2.unil.ch/popgen/softwares/fstat.htm [Last accessed March 2023]

Groombridge J, Raisin C, Bristol R, Richardson D (2012) Genetic Consequences of Reintroductions and Insights from Population History. In: Ewen JG, Armstrong DP, Parker KA, Seddon PJ (Eds) Reintroduction Biology. Wiley-Blackwell, Oxford, 528 pp. https://doi.org/10.1002/9781444355833.ch12

Isberg SR, Chen Y, Barker G, Moran C (2004) Analysis of Microsatellites and Parentage Testing in Saltwater Crocodiles. The Journal of Heredity 95(5): 445–449. https://doi.org/10.1093/jhered/esh067

IUCN (2020) The IUCN Red List of Threatened Species. Version 2020-3.

Jule KR, Leaver LA, Lea SEG (2008) The effects of captive experience on reintroduction survival in carnivores: A review and analysis. Biological Conservation 141(2): 355–363. https://doi.org/10.1016/j.biocon.2007.11.007

Kalinowski ST, Wagner AP, Taper ML (2006) ML- RELATE: A computer program for Maximum Likelihood estimation of relatedness and relationship. Molecular Ecology Notes 6(2): 576–579. https://doi.org/10.1111/j.1471-8286.2006.01256.x

Kalinowski ST, Taper ML, Marshall TC (2007) Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16(5): 1099–1006. https://doi.org/10.1111/j.1365-294X.2007.03089.x

Lafferriere R, Antelo R, Alda F, Mårtensson D, Hailer F, Castroviejo-Fisher S, Amato G (2016) Multiple Paternity in a Reintroduced Population of the Orinoco Crocodile (*Crocodylus intermedius*) at the El Frío Biological Station, Venezuela. PLoS ONE 11(3): e0150245. https://doi.org/10.1371/journal.pone.0150245

Lugo LM (1996) Advances in the studies of the status of the Orinoco crocodile in Colombia. Newsletter Crocodile Specialist Group 15: 21–22.

Luikart G, Sherwin WB, Steele BM, Allendorf FW (1998) Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. Molecular Ecology 7(8): 963–974. https://doi.org/10.1046/j.1365-294x.1998.00414.x

Mauger LA, Velez E, Cherkiss MS, Brien ML, Mazzotti FJ, Spotila JR (2017) Conservation genetics of American crocodile, *Crocodylus acutus*, populations in Pacific Costa Rica. Nature Conservation 17: 1–17. https://doi.org/10.3897/natureconservation.17.9714

McVay JD, Rodriguez D, Rainwater TR, Dever JA, Platt SG, McMurry MRJ, Forstner S, Densmore LD III (2008) Evidence of multiple paternity in Morelet's Crocodile (*Crocodylus moreletii*) in Belize, CA, inferred from microsatellite markers. The Journal of Experimental Zoology 309A(10): 643–648. https://doi.org/10.1002/jez.500

Medem F (1981) Los Crocodylia de Sur América. Los Crocodylia de Colombia (Vol. I). Ministerio de Educación Nacional. Colciencias. Bogotá, 354 pp.

MAM (2002) MINISTERIO DE MEDIO AMBIENTE Programa Nacional para la Conservación del Caimán Llanero (PRICAIMÁN). Ministerio del Ambiente Dirección general de ecosistemas subdirección de fauna. Santafé de Bogotá, Colombia.

Montgomery ME, Ballou JD, Nurthen RK, England PR, Briscoe DA, Frankham R (1997) Minimizing kinship in captive breeding programs. Zoological Biology 16(5): 377–389. https://doi.org/10.1002/(SICI)1098-2361(1997)16:5<377::AID-ZOO1>3.0.CO;2-7

Moreno-Arias RA, Ardila-Robayo MC (2020) Journeying to freedom: The spatial ecology of a reintroduced population of Orinoco crocodiles (*Crocodylus intermedius*) in Colombia. Animal Biotelemetry 8: 1–15. https://doi.org/10.1186/s40317-020-00202-2

Parra-Torres F, Moreno-Arias R, Montenegro O (2020) Evaluation of crocodilian populations along the Bita River (Vichada, Colombia). Herpetological Conservation and Biology 15(2): 416–426.

Posso-Peláez C, Ibáñez C, Bloor P (2018) Low Mitochondrial DNA Variability in the Captive Breeding Population of the Critically Endangered Orinoco Crocodile (*Crocodylus intermedius*) from Colombia. Herpetological Conservation and Biology 13(2): 347–354.

Ramírez O, Altet L, Enseñat C, Vilà C, Sanchez A, Ruiz A (2006) Genetic assessment of the Iberian wolf *Canis lupus signatus* captive breeding program. Conservation Genetics 7(6): 861–878. https://doi.org/10.1007/s10592-006-9123-z

Raymond M, Rousset F (1995) GENEPOP (version-1.2)— Population-genetics software for exact tests and ecumenicism. The Journal of Heredity 86(3): 248–249. https://doi.org/10.1093/oxfordjournals.jhered.a111573

Ross JP [Ed.] (1998) Crocodiles. Status Survey and Conservation Action Plan (2nd edn.). IUCN/SSC Crocodile Specialist Group, Gland.

Russello A, Amato G (2004) *Ex situ* population management in the absence of pedigree information. Molecular Ecology 13(9): 2829–2840. https://doi.org/10.1111/j.1365-294X.2004.02266.x

Seijas AE, Antelo R, Thorbjarnarson JB, Ardila Robayo MC (2010) Orinoco crocodile *Crocodylus intermedius*. In: Manolis SC, Stevenson C (Eds) Crocodiles. Status Survey and Conservation Action Plan (3rd edn.). Crocodile Specialist Group, 59–65.

Spitzweg C, Praschag P, DiRuzzo S, Fritz U (2018) Conservation genetics of the northern river terrapin (*Batagur baska*) breeding project using a microsatellite marker system. Salamandra 54(1): 63–70.

Thorbjarnarson JB (1987) Status, Ecology and Conservation of the Orinoco Crocodile. Preliminary Report. FUDENA-WWF, Caracas, 74 pp.

Tzika AC, Remy C, Ginbson R, Milinkovitch MC (2008) Molecular genetic analysis of a captive-breeding program: The vulnerable endemic Jamaican yellow boa. Conservation Genetics 10(1): 69–77. https://doi.org/10.1007/s10592-008-9519-z

van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4(3): 535–538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Williams DA, Osentoski MF (2007) Genetic considerations for the captive breeding of tortoises and freshwater turtles. Chelonian Conservation and Biology 6(2): 302–313. https://doi.org/10.2744/1071-8443(2007)6[302:GCFTCB]2.0.CO;2

Witzenberger KA, Hochkirch A (2011) *Ex situ* conservation genetics: A review of molecular studies on the genetic consequences of captive breeding programs for endangered animal species. Biodiversity and Conservation 20(9): 1843–1861. https://doi.org/10.1007/s10531-011-0074-4

Xu Q, Fang S, Wang Z, Wang Z (2005) Microsatellite analysis of genetic diversity in the Chinese alligator (*Alligator sinensis*) Changxing captive population. Conservation Genetics 6(6): 941–951. https://doi.org/10.1007/s10592-005-9081-x

Supplementary material 1

Supplementary information

Authors: Ana M. Saldarriaga-Gómez, María Cristina Ardila-Robayo, Federico Medem, Mario Vargas-Ramírez

Data type: tables and figure (word document)

Explanation note: **appendix S1.** Provenance of the wild-born crocodiles. **appendix S2.** Information of priority crocodiles presenting alleles at low frequencies. **appendix S3.** Distribution of individual diversity (homozygosity by loci, HL) of the living crocodiles that make up the *ex-situ* population managed by the Roberto Franco Biological Tropical Station.

Link: https://doi.org/10.3897/natureconservation.53.104000.suppl1

Nature Conservation 53: 105–123 (2023)
DOI: 10.3897/natureconservation.53.86739

Research Article

Performance of SNP markers for parentage analysis in the Italian Alpine brown bear using non-invasive samples

Patrizia Giangregorio[1], Nadia Mucci[1], Anita J. Norman[2], Luca Pedrotti[3], Stefano Filacorda[4], Paolo Molinari[5], Göran Spong[2], Francesca Davoli[6]

1 *Unit for Conservation Genetics (BIO-CGE), Department for the Monitoring and Protection of the Environment and for Biodiversity Conservation, Italian Institute for Environmental Protection and Research (ISPRA), Ozzano dell'Emilia (BO), Italy*
2 *Molecular Ecology Group, Department of Wildlife, Fish and Environmental Studies, SLU, Umeå, Sweden*
3 *Autonomous Province of Trento, Large Carnivore Sector (PAT), Trento, Italy*
4 *Department of agricultural, food, environmental and animal science, University of Udine, Udine, Italy*
5 *Progetto Lince Italia, Tarvisio, Italy*
6 *Conservation, management and sustainable use of national marine resources (BIO-CIT), Department for the Monitoring and Protection of the Environment and for Biodiversity Conservation, Italian Institute for Environmental Protection and Research (ISPRA), Ozzano dell'Emilia (BO), Italy*

Corresponding author: Patrizia Giangregorio (patrizia.giangregorio@isprambiente.it)

Abstract

Determination of parentage provides valuable information for the conservation of wild populations, for instance, by allowing the monitoring of breeding success and inbreeding. Between 1999 and 2002, nine brown bears (*Ursus arctos*) were translocated to augment the remnant population of a few surviving individuals in the Italian Alps, but only part of them reproduced, with a higher inbreeding risk occurrence in the long-time. Currently, in the Alpine population, parentage tests are assessed through the analysis of 15 microsatellite loci (STRs), but the reduction of genetic variability in future generations will need the use of additional informative markers. Single nucleotide polymorphisms (SNPs) have been proven to be useful and reliable in individual identification and family reconstruction; moreover, they can perform well on low-quality samples. In this study, we analysed 51 SNPs to generate a SNP multilocus genotype dataset of 54 Alpine brown bears (*Ursus arctos*) and compared its performance in parentage analysis with the validated STR dataset. We found that SNPs alone are not sufficient to determine parentage relationships, but the combination of SNPs and STRs provided unambiguous parentage assignments. The combined panel also performed better than STRs when true parents were not present in the dataset and, consequently, showed higher values of assignment probabilities.

Key words: Colony, FRANz, markers combination, microsatellites, monitoring, *Ursus arctos arctos*

Academic editor: Klaus Henle
Received: 20 May 2022
Accepted: 3 July 2023
Published: 10 August 2023

ZooBank: https://zoobank.org/0A1DEBA4-B4B4-4416-9E40-69B8E0AA4391

Citation: Giangregorio P, Mucci N, Norman AJ, Pedrotti L, Filacorda S, Molinari P, Spong G, Davoli F (2023) Performance of SNP markers for parentage analysis in the Italian Alpine brown bear using non-invasive samples. Nature Conservation 53: 105–123. https://doi.org/10.3897/natureconservation.53.86739

Introduction

Parentage determination may greatly aid the management of wild populations of conservation concern (Jones and Ardren 2003; Hauser et al. 2011) since it provides information about reproductive success and inbreeding (Wilson et al. 2002; Vonholdt et al. 2008; De Barba et al. 2010a; Stenglein et al. 2011). Such information can also contribute to answering ecological questions related to the mating system, social organisation and dispersal behaviour (Webster and Reichart 2005; Moore et al. 2014) and can be used in reintroduction programmes to optimise translocation strategies for endangered species (Labuschagne et al. 2015; Wright et al. 2015). Molecular markers are useful for inferring parentage in wild populations when it is difficult to collect information from field observations (Blouin 2003; Pemberton 2008). Moreover, non-invasive genetic methods offer a great opportunity to infer parentage in wild and elusive populations without disturbing animals in their habitats (Taberlet et al. 1999), although DNA from biological traces is often degraded and scarce (Navidi et al. 1992; Broquet and Petit 2004).

Microsatellite loci, also known as short tandem repeats (STRs), are multi-allelic and highly polymorphic markers that have been routinely used in the last decade for parentage analysis in wild populations, also by starting from non-invasive samples (Constable et al. 2001; Nielsen et al. 2001; De Barba et al. 2010a; Caniglia et al. 2014). However, STRs are prone to genotyping errors with low or degraded DNA (Dewoody et al. 2006) and can produce false genotypes or incorrect parentage assignments (Pompanon et al. 2005). Moreover, recent simulation studies indicate that STRs provide less precision for relatedness, particularly in small populations where allelic diversity may be low (< 4 alleles per locus: Robinson et al. (2013); Taylor (2015)).

Single nucleotide polymorphisms (SNPs) are another type of marker of increasing popularity for many conservation genetic studies. They are polymorphic sites dispersed into the genome; differently from STRs, they can be easily scored, record high genotyping success and low error rates (Morin et al. 2004; Anderson and Garza 2006). Moreover, SNP data do not require allele calibration amongst different laboratories, thus genotypes can be easily compared when transboundary individuals move across different areas monitored by different research groups (Vignal et al. 2002; Pompanon et al. 2005). SNPs differ from STRs as they are bi-allelic and, therefore, offer less information on a marker-by-marker basis (Marth et al. 2001; Glaubitz et al. 2003); nevertheless, a high number of SNPs is easily obtained, thereby compensating for the low per-SNP information content.

Furthermore, the recently-emerged microfluidic genotyping platforms have shown very low copy number detection thresholds and are, thus, particularly suitable for the amplification of poor-quality DNA (von Thaden et al. 2020). SNPs have proven to be effective in several parentage studies of cattle and wild populations of fish, mammals and birds (see Flanagan and Jones 2019 for a recent compendium of 58 SNP-based parentage analyses published), even for populations that face low genetic diversity (Tokarska et al. 2009; Wright et al. 2015; Campbell et al. 2019; Galla et al. 2020). Several studies compared the efficiency of STRs and SNPs in parentage testing (Labuschagne et al. 2015; Weinman et al. 2015; Kaiser et al. 2017). SNP analysis is relatively easy to carry out, but finding and validating new SNP markers is still costly. Thus, it may be simpler to test pre-existing SNPs than to isolate new ones; sharing markers would be also a chance to allow a comparison of the genetic variability between populations. Recently, an increased number of empirical studies have demonstrated the utility of SNP markers in kinship analysis also by starting from non-invasive samples (Kleinman-Ruiz et al. 2017; Kraus et al. 2015; see also Carroll et al. (2018) and Ekblom et al. (2021)), thus enabling their use in the study of wild populations with no disturbance to individuals.

The Italian Alpine bear population (*Ursus arctos*) lives in two separate areas: the first subpopulation inhabits the central Italian Alps, while the second, in eastern Italy, constitutes the expansion front of the Dinaric Mountain population (Fig. 1). A geographic and numeric contraction occurred between the 18th and 20th centuries in the Alps, because of human persecution, habitat loss and fragmentation. By the 1900s, only a few individuals survived (≈ 3) in the Trentino Region (Kohn et al. 1995) and the population was considered biologically extinct (Mustoni et al. 2003). The translocation of nine bears from Slovenia, which took place during the 2000s, was the origin of the central Alpine bear population that is presently increasing both in numbers and distribution (De Barba et al. 2010a; Tosi et al. 2015). All released bears were VHF - radio collared and the reconstituted population has been intensively monitored since the first release till now. For management reasons, additional trapping was carried out from 2000 to 2014; 12 more individuals born in the area were fitted with GPS-collars and monitored through telemetry. VHF-/GPS-tracking and the possibility to combine fingerprinting analysis and camera trap data to determine litter size of reproductive females enabled us to keep good track of population development, individual fates and cub production, especially for founders and the first generation of new-borns, which had a narrow distribution area. The latest estimates, based on genetic Capture–Mark–Recapture from opportunistic and systematic sampling, approximate the population size to 73–92 bears in 2021 (Groff et al. 2019). Additionally, although males from the Dinaric population are regularly observed in the eastern Italian Alps, there are no cases of proven reproduction, thus gene flow to the reintroduced population is not known to occur (Krofel et al. 2010; Skrbinsek et al. 2012).

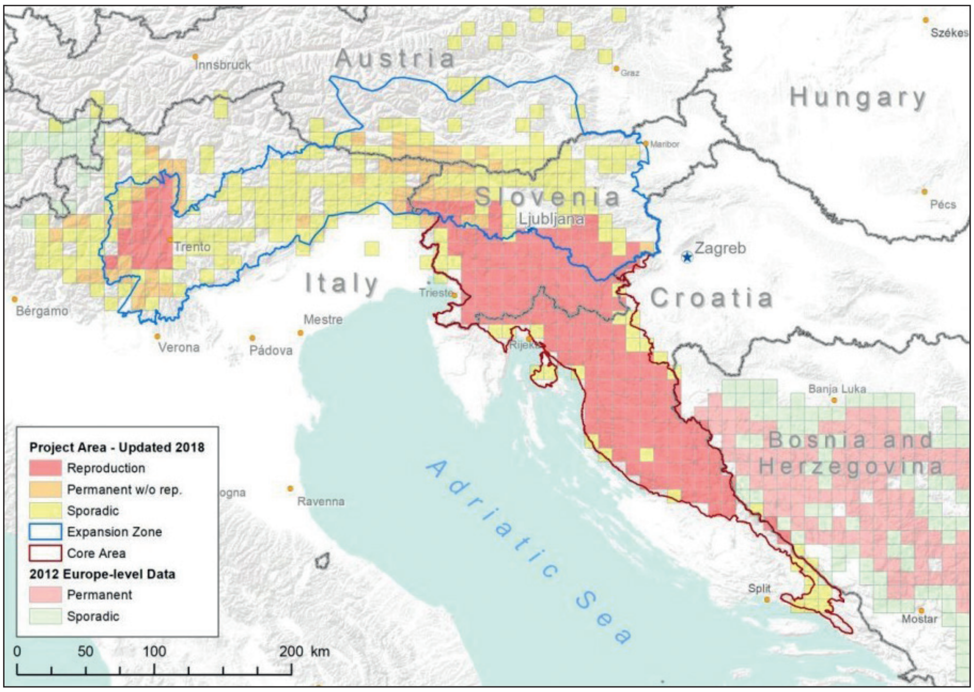


Figure 1. Brown bear distribution in the Italian Alps and neighbouring countries (from Skrbinšek et al. (2018)). Permanent presence, reproduction (red squares) – areas where cubs were confirmed within the last three years; permanent presence without reproduction (orange squares) – areas where bears have been present for at least three years over the last five years; sporadic presence (yellow squares) – areas where bear presence has been documented for fewer than three seasons in the last five years' period.

Although the status of the brown bear is categorised as “least concern” in its worldwide distribution area (McLellan et al. 2016), it is critically endangered in the Italian Alps (Stoch and Genovesi 2016) due to the limited number of individuals and geographic isolation. Therefore, it is essential to be able to study reproductive success and inbreeding levels to ensure the persistence of this population over time. Between 2000 and 2014, approximately 6000 non-invasive samples have been collected in the Alpine area to monitor the demographic trend of the reintroduced population. So far, 15 STR loci and two different regions for molecular sexing have been used for individual identification and parentage analysis (Probability of Identity PID = 3.7×10^{-13} and Probability of Identity between siblings PIDsibs = 6.6×10^{-06}; Allelic Drop-Out rates ADO = 0.041–0.168; De Barba et al. (2010a); Davoli et al. (2018); Giangregorio (2018)). Details on the genetic monitoring programme of the bear population in the Italian Alps during the reintroduction time frame are available in Suppl. material 1: tables S1, S2.

Despite the fact that the robustness of the STR protocol has been providing helpful information in the kinship analysis (Suppl. material 1: table S2), there are still critical issues in the peak scoring and the genotyping success due to fragment length, both conditions that increase the probability of genotyping error. Moreover, fluctuation in capillary electrophoresis makes difficult the recognition of the allele scoring when a reference genotype, previously analysed, is lacking.

Recently, a 96 × 96 SNP-chip comprising 85 autosomal SNPs, seven sex chromosome markers and four mtDNA markers was developed by Norman et al. (2013) and Norman and Spong (2015) and is currently used for bear monitoring in Scandinavia (J. Kindberg, pers. comm.). Amongst those, 51 autosomal and six sex chromosome SNP markers resulted in being variable also in the Alpine populations (Giangregorio et al. 2018) and were selected to be tested for individual and sex identification.

Here, we evaluate: i) the performance of the 51 SNPs in parentage analysis using non-invasive hair samples and ii) their reliability as compared to 15 STRs utilised in the monitoring of Alpine brown bear (Giangregorio 2018, Suppl. material 1: table S2) and a case study (Davoli et al. 2018). Given the intense monitoring programme, this population offers a great opportunity to test the suitability of SNP-based parentage analysis through non-invasive samples because the independent STR-based results and field data can be compared to SNP-based parental assignments.

Methods

Sampling methods

Hairs were collected in the Italian area of bear presence (Fig. 1) between 2000 and 2014, with both systematic and opportunistic methods through barbed-wire traps, transects and rub trees, during normal activities of various agency personnel and by volunteers or following notification by third parties, for example, after a damage event. The sampling activities were carried out for the annual monitoring of the species, coordinated by regional authorities and the Italian Institute for Environmental Protection and Research (ISPRA). Annual reports on bear presence are regularly published online (https://grandicarnivori.provincia.tn.it/Large-Carnivores-Report).

Sampling procedures followed the guidelines provided by the interregional action plan for the conservation of brown bears in the Italian Alps (PACOBACE) (AA.VV. 2010). Samples were collected using sterilised forceps or latex gloves and placed in envelopes, then stored in silica desiccant or alternatively in 95% ethanol (De Barba et al. 2010b).

STR genotypes, databank and sample selection

STR genotyping using methods developed by De Barba et al. (2010a) and implemented by Davoli et al. (2018), was carried out during the entire non-invasive monitoring project on the Alpine brown bear and made it possible to detect the asynchronous presence of 85 bears between 2000 and 2014 (De Barba et al. 2010a; Groff et al. 2016; Giangregorio 2018). For evaluating the SNPs panel, a total of 71 hair samples belonging to 71 different bears, genotyped and stored at -20 °C at ISPRA, were extracted using the Qiagen DNeasy Blood & Tissue Kit (Qiagen inc., Hilden, Germany) following the manufacturer's instructions. DNA or biological material of the remaining 14 bears was no longer available and they were not included in this comparative study. The samples of three bears from the Dinaric population were also added to test parentage analysis; thus a total of 74 genotypes, corresponding to the same number of individuals, was utilised in this study.

SNP genotyping

DNA aliquots (n = 74) were sent to the Swedish University of Agricultural Sciences (SLU), to be amplified on the Biomark platform (Fluidigm Corporation, San Francisco, USA) with the 96 × 96 SNP panel developed by Norman et al. (2013) and colleagues. Clusters obtained from SNP genotyping were visualised in the Fluidigm SNP Genotyping Analysis software v.3.1.2. We applied the same filtering procedures for SNP validation and sex identification identified in Giangregorio et al. (2018), who found 51 informative SNPs in the Alpine brown bear population: i) we removed SNPs which gave no amplification signal in any sample and those which were monomorphic, excluding Y-chromosome and mitochondrial SNPs, which are always haplotypic; ii) to prevent possible errors in genotypes, we removed all loci that showed unclear cluster affiliation or unusual clustering patterns and iii) those which showed a departure from Hardy Weinberg equilibrium. Only SNPs which passed the screening were analysed. A flowchart showing the SNP genotyping steps and the filtering process is shown in Suppl. material 1: table S3.

We replicated a proportion of the samples (16 samples were replicated once, while 13 were replicated three times – see Suppl. material 1: table S4 for details) to calculate the percentage of positive amplifications and error rates with GIMLET v. 1.3.3 (Valière 2002), following the procedures described in Giangregorio et al. (2018). Afterwards, consensus genotypes amongst the replicates were created using a conservative approach where allele inconsistencies amongst replicates were not called and were marked as missing alleles. Consensus genotypes with a call rate ≤ 70% were discarded.

SNP and STR marker statistics

Deviations from the Hardy-Weinberg equilibrium (HWE) were computed using the exact test in Genepop (Raymond and Rousset 1995). SNPs that showed a departure from the Hardy-Weinberg equilibrium were removed from the analyses. GeneAlEx 6.5 (Peakall and Smouse 2012) was used to describe allele frequencies, number of alleles (Na), effective number of alleles (Ne), observed (Ho) and expected (He) heterozygosity, Shannon's information index (I) and the ability in distinguishing individuals through the probability of identity test (PID). To overcome the bias caused by the presence of closely-related individuals in the population, which are more likely to share identical genotypes by chance (Waits et al. 2001), the equivalent probability for pairs of siblings (PIDsibs) was also calculated. These statistical tests were computed on SNP and STR-reference data to compare the genetic variability described by different marker datasets.

Parentage analyses

The reliability of parentage analysis is maximised when all individuals of the family trio (dam, sire and offspring) are sampled and fully genotyped. However, poor and degraded DNA can lead to incomplete or missing genotypes. The development of a protocol preserving the maximum number of individuals while minimising the effect of incomplete genotypes is advantageous. To test for the incidence of these limiting factors, two sample datasets were created, the first including samples with a SNP call rate ≥ 70% and a second with a reduced number of individuals, only those with a SNP call rate ≥ 90%. Parentage relationships were evaluated using FRANz v.2 (Riester et al. 2009), a commonly used likelihood-based parentage assignment software that incorporates multi-generation analyses using polymorphic co-dominant markers. Values of mistyping error rate and the maximum number of mismatches were set at 0.02 and 1, respectively.

Moreover, kinship analysis was also tested in Colony v. 2.0.6.4 (Jones and Wang 2010) to verify the robustness of results obtained from FRANz. A subset of genotypes was created for each monitoring year (n = 13), avoiding multi-generation analyses. Each annual parentage analysis includes bears born in the year of reference and putative parents. Year of birth and reproductive age (3 years for females and 5 years for males) were evaluated, based on the long-term monitoring programme (i.e. direct observations of females with cubs monitored through radio- and GPS-telemetry, at hair-trap sites equipped with camera traps and through *a posteriori* genetic parental assignments). We anticipate that reproductive traits (i.e. age of primiparity, interbirth interval, mean litter size) obtained from genetic data are always concordant with the reproductive biology of the species (Giangregorio 2018). Accepting these assumptions, we removed all bears who died within the last year, as they could not have taken part in the reproductive event in the year of reference.

Parameters used in FRANz v.2 and Colony v. 2.0.6.4 software, have been set following the procedure described in Davoli et al. (2018) and reported in Suppl. material 1: table S5.

To test the reliability of parental assignments, family trios obtained with SNPs were compared with those obtained formerly with STR. This was done by calculating the number of congruent, missing and incongruent parentage assignments in addition to significance values in the detected family trios. Missing assignments caused by the lack of the true parents in the SNP dataset were also deemed “congruent”. Finally, we combined SNP and STR genotypes in a single dataset and compared the results obtained through SNPs and STRs alone.

Results

Genotypes and marker datasets

Out of the 51 autosomal SNPs analysed, eight did not amplify, 15 were monomorphic, 11 showed unclear cluster affiliation or unusual clustering patterns, 5 showed call rates ≤ 70% and one showed a departure from the Hardy Weinberg equilibrium and were subsequently removed from the analyses. A total of 45 SNPs was thus retained for further analysis. Out of the 74 genotyped individuals, 54 were retained (including five founders and three bears from the remnant Dinaric population) as they had SNP genotyping call rates ≥ 70% (mean call rate was 85%), while 20 were rejected. Out of these 54 bears, 41 (including three founders and two Dinaric bears) showed a percentage of call rate ≥ 90% (mean call rate 97%). Details on genotyping success are shown in Suppl. material 1: table S4. A flowchart showing the filtering process with results is shown in Suppl. material 1: table S3.

The genotyping of 45 SNPs correctly identified 54 individual bears and sex determination, based on six SNPs on the sex chromosomes, confirmed STR-based results in 42 out of 54 cases (77%). The remaining 12 cases did not show incongruent results, but partially missing data at SNPs on the sex chromosomes prevented the sex determination. Amongst the 54 samples, 12 were replicated four times and showed 92% positive PCR amplifications amongst loci and 92% amongst samples. Allelic dropout interested only 1.6% of loci (mean value = 0.04) and 1.4% of samples (mean value = 0.02). Amongst 45 SNPs, 27 showed the three genotypic representatives (e.g. AT/TT/AA). The following statistics on variability and parentage analyses were performed using the reference dataset of 15 STRs, the total amount of 45 SNPs and the reduced dataset of 27 most variable SNPs. In addition, datasets formed by the combination of 15 STRs with 27 SNPs and 15 STRs with 45 SNPs were also processed.

STR and SNP marker statistics

Summary statistics for single markers are shown in detail in Suppl. material 1: table S6, while average statistics data of all marker subsets are summarised in Table 1. The mean number of alleles per locus (Na) was 4.53 (SD = 0.291) using the reference STRs panel, while 2.90 (SD = 0.215) and 2.63 (SD = 0.159) Na were recorded using the two STR and SNP combined panels. PID values ranged from 1.01×10^{-05} using 27 SNPs to 8.52×10^{-13} using the combined panel of 45 SNPs and 15 STRs, while PIDsibs values ranged from 2.17×10^{-10} to 1.95×10^{-26}. Values are plotted in Fig. 2.

Table 1. Marker summary statistics. The mean number of loci typed in 51 brown bear samples from the Central Italian Alps. (N), the mean number of alleles per locus (Na), the mean effective number of alleles (Ne), Shannon's information index (I), observed (Ho) and expected (He) heterozygosity. Standard error values (SE) are in brackets.

MARKER SETS	N (SE)	Na (SE)	Ne (SE)	I (SE)	Ho (SE)	He (SE)
15 STRs	51.00 (0.000)	4.53 (0.291)	3.25 (0.223)	1.25 (0.084)	0.73 (0.045)	0.66 (0.040)
27 SNPs	47.30 (0.443)	2.00 (0.000)	1.74 (0.049)	0.59 (0.023)	0.43 (0.026)	0.41 (0.020)
45 SNPs	47.18 (0.405)	2.00 (0.000)	1.58 (0.044)	0.52 (0.022)	0.38 (0.022)	0.34 (0.019)
27 SNPs & 15 STRs	48.26 (0.348)	2.90 (0.215)	2.28 (0.141)	0.83 (0.059)	0.54 (0.032)	0.50 (0.026)
45 SNPs & 15 STRs	47.88 (0.342)	2.63 (0.159)	2.00 (0.114)	0.70 (0.049)	0.47 (0.028)	0.42 (0.025)

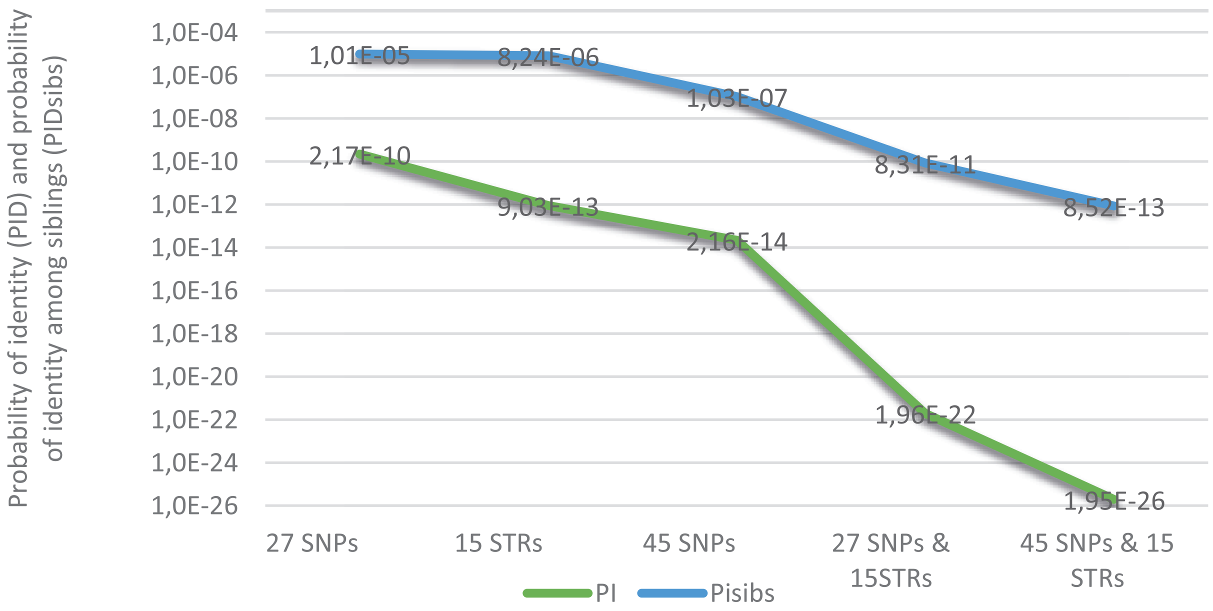


Figure 2. PID and PIDsibs values for increasing locus combination in the brown bear Alpine population. The values are calculated for the five marker sets: 27 most variable SNPs (showed the three genotypic representatives), the reference dataset of 15 STRs, the total amount of 45 SNPs, the combination of 15 STRs with 27 SNPs and the combination of 15 STRs with 45 SNPs.

Parentage analyses

Parentage tests were performed in two independent analyses excluding the five founder individuals in a total of individuals with call rates ≥ 70% (n = 49, 100%) and individuals with call rates ≥ 90% (n = 38, 77.5%). Amongst bears with call rates ≥ 70% and ≥ 90%, 2 out of 21 (9.5%) and 7 out of 17 (41.1%) of true parents (detected using the STR-based reference data) were removed from the dataset because of the filtering process, respectively. As a result, three (3.2%) and 30 (27.7%) assignments with the true parent were impossible to detect in the two analyses.

Parental assignments using bears with a call rate ≥ 70% and the complete set of 45 SNPs resulted in 8.16% of missing assignments and 7.14% inconsistencies with the STR reference data. Similar results were obtained using the reduced panel of 27 SNPs resulting in 11.22% of missing assignments and 6.12% of inconsistencies. This slight difference is probably due to the greater reliability of the 27 SNPs compared to the 45 SNPs as the former are characterised by the presence of all the three allelic forms. Variation was detected in samples with call rates ≥ 90%: the percentage of inconsistencies is similar (7.89% using 45 SNPs and 9.21% using 27 SNPs), but no missing assignments were found.

Using the two combinations of 15 STRs and 45/27 SNPs, amongst bears with call rates ≥ 70%, 8.16% and 6.12% had missing assignments and no inconsistencies were found. All parental assignments identified using bears with a call rate ≥ 90% were concordant with the STR reference data, despite the absence of a high proportion of parents in the dataset. On bears with SNP call rate ≥ 70%, the 15 STRs showed congruent results on all family trios, while bears with SNP call rate ≥ 90% led to one missing assignment (1.30%) and one incongruent assignment (1.30%). Proportions of congruent, incongruent and missing sire/dam assignments are summarised in (Table 2).

As expected, no parents were found for the three bears of Dinaric origin and all parental assignments were confirmed using Colony 2.0.6.4. Amongst congruent assignments, no mismatches were found and the mean number of common loci typed in the family trios was 58.7 out of 60 (min = 56, max = 60). Individual details about the combination of SNP and STR markers individuals' assignment are reported in Suppl. material 1: table S7.

Parental assignment probabilities were calculated for bears with the 36 most reliable genotypes (the Dinaric bears being excluded) using the five marker subsets. Results are displayed in Fig. 3: 27 SNPs showed the lowest mean values (mean = 0.75; min = 0.26), followed by 45 SNPs (mean = 0.84; min = 0.12). In both cases, a high number of bears with a parental assignment probability low-

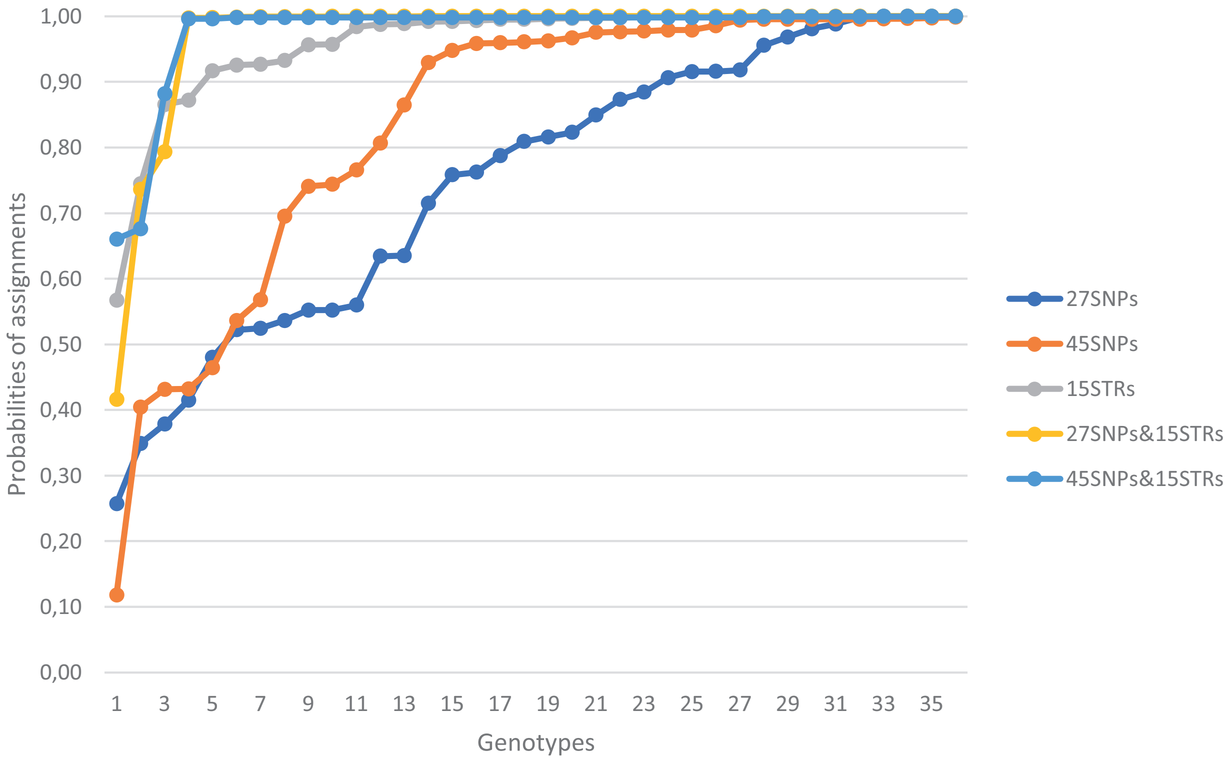


Figure 3. Parental assignment probabilities using FRANz v.2. The probabilities are calculated for the 36 most reliable bear genotypes born in the Central Italian Alps (the three bears of Dinaric origin are excluded) using the five marker sets described in Table 1. Probabilities are shown in ascending order for each genotype. Each marker set is indicated with a different colour (see the legend).

Table 2. Results of parental assignments using FRANz v.2. Values and percentages of correct, incongruent and missing parental assignments of 49 bear genotypes with call rate ≥ 70% (a) and 38 bear genotypes with call rate ≥ 90% (b) in the Italian Alpine brown bear population. Results are reported for each subset of SNP/STR marker. The total number of assignments to be determined for 49 (n = 98) and 38 (n = 76) bears genotypes are shown on the bottom row.

Call rates ≥ 70%	27SNPs	45SNPs	15STRs	27SNPs&15STRs	45SNPs&15STRs
Congruent	81 (82.65%)	83 (84.69%)	98 (100%)	92 (93.87%)	90 (91.8%)
Not assigned	11 (11.22%)	8 (8.16%)	0	6 (6.12%)	8 (8.16%)
Incongruent	6 (6.12%)	7 (7.14%)	0	0	0
TOT n assignments	**98**	**98**	**98**	**98**	**98**
Call rates ≥ 90%	**27SNPs**	**45SNPs**	**15STRs**	**27SNPs&15STRs**	**45SNPs&15STRs**
Congruent	69 (90.78%)	70 (92.10%)	74 (97.36%)	76 (100%)	76 (100%)
Not assigned	0	0	1 (1.31%)	0	0
Incongruent	7 (9.21%)	6 (7.89%)	1 (1.31%)	0	0
TOT n assignments	**76**	**76**	**76**	**76**	**76**

er than one was detected (31 and 27 out of 36 using 27 or 45 SNPs, respectively). For both STR/SNP marker combinations, mean and minimum probabilities of parental assignments were much higher (0.97/0.98; min = 0.42/0.66) and the number of bears with a probability of parental assignments lower than one was retrieved only in three cases. The probabilities of parental assignment using 15 STR markers showed intermediate mean values (0.96; min = 0.57).

Discussion

Our study demonstrated that a combination of SNPs and STRs provided robust assessments of parentage in the Italian Alpine brown bear population and performed better than STRs when a high proportion of true parents was not present in the dataset. The absence of parents in the dataset simulates a common situation in long-term monitoring projects of expanding populations, in which not all individuals are usually sampled. Interestingly, the numbers of assignments congruent with the reference STR-based data and probability values do not differ significantly when using the reduced set of the most variable 27 SNPs (He = 0.43) or the complete set of 45 SNPs (He = 0.38).

This result indicates that 27 SNPs, in combination with 15 STRs, are sufficient to considerably enhance data reliability compared to the use of 15 STRs alone. Combinations of SNPs and STRs were found to be more efficient than a higher number of SNPs alone and also in other species as in the African penguin (*Spheniscus demersus*; Labuschagne et al. (2017)). These authors obtained a > 99% correct cumulative parentage assignment probability, comparable to that obtained in this study. A similar study showed that a combined dataset of 33 SNPs and 6 STRs was most informative, with the highest confidence level, in the white rhino (*Ceratotherium simum*; Labuschagne et al. (2017)).

Conversely, 45 SNPs were not sufficient to determine parentage relationships in the Alpine population, although a similar number of SNPs were found to be adequate for assigning parents by Kaiser et al. (2017). They found 40 SNPs with a Ho value (0.37), similar to that obtained with 45 SNPs in our study (0.38), to be effective in the socially monogamous black-throated blue warbler (*Setophaga caerulescens*) and claimed that this small number of SNPs were just as powerful as six multi-allelic STRs. However, the mother was known, thus only a paternity test had to be performed. Additionally, Tokarska et al. (2009) and Labuschagne et al. (2015) found that SNPs performed slightly better than STRs in an ex-situ African penguin and in the European bison (*Bison bonasus*) population, respectively. However, differently from this study, invasive samples were used in all these former investigations.

Due to the wide-roaming of young male bears, international cooperation amongst labs involved in the monitoring of the species is pivotal for the conservation of the species in the Alps.

The long-term and intense monitoring of the brown bear population in the Italian Alps allowed us to empirically evaluate the performance of SNP markers in a wild population using non-invasive samples to assess family relationships. This information is usually difficult to achieve because field data (such as telemetry and direct observations of females with cubs), multiple sampling of individuals and multiple amplification of STR loci over years, are rarely available to confirm parentage assignments. In this study, reproductive data obtained from genetic and field data were available from more than a decade of research and management efforts and were always concordant with the reproductive biology of the species (see Giangregorio et al. (2018) for further information about the demographic history of the population). By considering the results obtained from this study, we can state that the 15 reference STRs and 27 SNPs can assess parental pairs, also with reduced genetic diversity. In the future, these markers could also be applied to other populations to make data comparable and useful for species conservation and management.

Genetic markers may also be less variable and informative when applied to a different population than the one for which they were developed; these SNPs were selected for being informative in the Scandinavian population and, as expected, only a portion was highly variable (51/96 = 53%). Ascertainment bias, due to the SNPs being selected for the Scandinavian bear population, likely contributed to the lower power of parentage assignment. Some of the 45 SNPs used in this study had low minor allele frequencies for the Alpine population, lowering their discretionary power.

In addition, a substantial number of studies concluded that SNP markers are entirely appropriate for parentage analyses, but the empirical data, thus far, indicate that a suite of 100–200 SNPs is generally needed to provide resolving power equal to or better than that provided by the available STR markers for the species under consideration (Flanagan and Jones 2019).

The integration of an additional set of SNPs specifically developed for the Alpine population would likely improve the effectiveness of parentage analyses, solving the problem of the low number of variable SNPs identified in this study. Benazzo et al. (2017) recently sequenced the brown bear genome and found several SNPs in the Apennine brown bear population that might prove variable in the Alpine population. SNP discovery within these variable regions could be conducted through advanced methodologies, such as genotyping-by-sequencing or RAD-tag sequencing (Gutierrez et al. 2017; Andrews et al. 2018; Zhao et al. 2018).

In this study, when using a lower call rate threshold (≥ 70%), a few incongruent parental assignments were found and a few assignments were missed. These errors are probably due to a combination of two factors: the lower number of common loci typed in the correct family trio and a few genotyping errors amongst SNPs. Our SNP results highlighted the importance of using special precautions when working with non-invasive samples, such as pre-selecting samples with high call rates (≥ 90%). Additionally, Kaiser et al. (2017) demonstrated that missing data in the SNP genotypes decrease the rate of congruent parental assignments. As in our study, increasing the filtering threshold of sample call rate by 70% and 90% reduced the number of offspring typed, but increased concordance in parentage assignments between STRs and SNPs. Missing data resulted in incomplete genotypes, which affected the ability of SNPs to resolve parentage in a few cases. The importance of using samples with a high call rate and including replicates when working with SNPs and non-invasive samples was also discussed by von Thaden et al. (2017). Our results thus emphasise the necessity of developing strategies and protocols for the use of SNPs when working with poor-quality samples, such as non-invasively collected faeces or hair.

We also underline that SNPs have some intrinsic disadvantages, especially when using non-invasive samples: since SNPs are bi-allelic, it is not straightforward how to recognise samples containing DNA from multiple individuals and rules concerning the number of replicates and call rates needed to obtain reliable genotypes are lacking.

More SNPs are needed to perform parentage analysis with an information content comparable or superior to that obtained through STRs (Morin et al. 2004) but, even with low error rates, parentage analysis may become problematic as the number of loci screened becomes very large (Hauser et al. 2011; Christie et al. 2013), thus making it necessary to find a compromise between the number and type of markers.

Despite the difficulties that may manifest when working with non-invasive samples and given the mentioned ascertainment bias, our results showed that a combination of 27 SNPs and 15 STRs was an effective panel in identifying parentage relationships in an isolated brown bear population, although several half- or full siblings amongst putative parents are present. The use of SNPs in parentage analysis is thus promising even if it should be evidenced that multiple factors could contribute to jeopardise the reliability of the results.

Considering the data obtained in this study, we provided simple guidelines to perform efficient parentage analysis in wild populations using non-invasive samples with STRs and SNPs: a) amplifying a congruent number of STRs and determining sex through the amplification of sex-specific regions for all collected samples should be the first step. The STR amplification can be used to discard bad-quality samples and identify single individuals; b) amongst multiple samples of the same individual, the one with the lower genotyping error rate and higher positive amplifications with STRs may be chosen for SNP genotyping; c) a SNP genetic data bank, including all putative parents, can be developed. 96 × 96, 48 × 48 or 192 × 24 plates can be used on the Fluidigm Biomark Platform, depending on the number of individuals and SNP availability; d) parentage analysis can be performed using FRANz, combining an appropriate number of SNP and STR markers, to allow for multi-generational analysis.

However, our data highlighted the need of using good-quality samples (e.g. call rates ≥ 90%, given our results) with a low likelihood of allelic drop-out. Indeed, parental assignments are particularly vulnerable to genotyping problems, as parent-offspring pairs must share at least one identical allele at each locus (Wang 2019). Eriksson et al. (2020) outlined a detailed protocol for cost-effective and accurate non-invasive SNP genotyping optimised for degraded DNA, while Ekblom et al. (2021) were the first to successfully reconstruct the pedigree in a wild population of wolverine (*Gulo gulo*) using SNP-genotyping of non-invasive samples, with individuals known to have high levels of relatedness (e.g. full siblings from inbred mating). In addition, choosing the most informative SNPs whether pursuing a SNP-PCR, SNP-chip or targeted capture method, is imperative, as concluded also by Flanagan and Jones (2019). Lastly, due to the risk of ascertainment bias, the SNP panel should be developed and/or validated for the population of interest, since it cannot always be easily transferable to other parts of the distribution range (Morin et al. 2004; Ekblom et al. 2021). Moreover, the use of STRs or SNPs of both of them can be considered depending on the situation for sample quality and quantity.

Acknowledgements

We acknowledge all the management authorities and people who gave major contributions to the collection of samples analysed in this study: the Autonomous Province of Bolzano, the Autonomous Province of Trento, the Autonomous Region of Friuli-Venezia Giulia, the general management of agricultural, forest, and fishing resources of the Region Friuli Venezia Giulia, the Lombardia Region, the staff of the provincial police force of Bergamo, Lecco, Brescia and Sondrio, the Veneto Region, the staff of Belluno and Verona Province. We also thank Helena Königsson (SLU) for having professionally analysed SNPs.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

No funding was reported.

Author contributions

Conceptualization: FD, GS, PG, NM. Data curation: GS, LP, PM, SF, PG, FD. Formal analysis: PG, GS. Investigation: NM, AJN, FD. Methodology: AJN, NM, GS. Project administration: FD, NM. Resources: LP, GS, SF, PM, AJN. Supervision: FD, GS, NM. Validation: NM, AJN, FD, GS. Writing - original draft: PG. Writing - review and editing: PM, LP, NM, SF, AJN, GS, FD.

Author ORCIDs

Patrizia Giangregorio https://orcid.org/0000-0003-1064-4494
Nadia Mucci https://orcid.org/0000-0002-7522-7213
Anita J. Norman https://orcid.org/0000-0002-9499-758X
Stefano Filacorda https://orcid.org/0000-0002-0984-2373
Paolo Molinari https://orcid.org/0009-0009-8556-246X
Göran Spong https://orcid.org/0000-0002-1246-5046
Francesca Davoli https://orcid.org/0000-0003-3231-2415

Data availability

The data that supports the findings of this study are available in the supplementary material of this article.

References

AA.VV. (2010) Piano d'Azione interregionale per la Conservazione dell'Orso bruno nelle Alpi centro-orientali – PACOBACE. Quad. Cons. Natura, 33, Min. Ambiente – ISPRA. https://www.isprambiente.gov.it/it/pubblicazioni/quaderni/conservazione-della-natura/piano-d2019azione-interregionale-per-la-conservazione-dell2019orso-bruno-nelle-alpi-centro-orientali-pacobace

Anderson EC, Garza JC (2006) The power of single-nucleotide polymorphisms for large-scale parentage inference. Genetics 172(4): 2567–2582. https://doi.org/10.1534/genetics.105.048074

Andrews KR, Adams JR, Cassirer EF, Plowright RK, Gardner C, Dwire M, Hohenlohe PA, Waits LP (2018) A bioinformatic pipeline for identifying informative SNP panels for parentage assignment from RADseq data. Molecular Ecology Resources 18(6): 1263–1281. https://doi.org/10.1111/1755-0998.12910

Benazzo A, Trucchi E, Cahill JA, Maisano Delser P, Mona S, Fumagalli M, Bunnefeld L, Cornetti L, Ghirotto S, Girardi M, Ometto L, Panziera A, Rota-Stabelli O, Zanetti E, Karamanlidis A, Groff C, Paule L, Gentile L, Vila C, Vicario S, Boitani L, Orlando L, Fuselli S, Vernesi C, Shapiro B, Ciucci P, Bertorelle G (2017) Survival and divergence in a small group: The extraordinary genomic history of the endangered Apennine brown bear stragglers. Proceedings of the National Academy of Sciences of the United States of America 114(45): E9589–E9597. https://doi.org/10.1073/pnas.1707279114

Blouin MS (2003) DNA-based methods for pedigree reconstruction and kinship analysis in natural populations. Trends in Ecology & Evolution 18(10): 503–511. https://doi.org/10.1016/S0169-5347(03)00225-8

Broquet T, Petit E (2004) Quantifying genotyping errors in noninvasive population genetics. Molecular Ecology 13(11): 3601–3608. https://doi.org/10.1111/j.1365-294X.2004.02352.x

Campbell MR, Vu NV, LaGrange AP, Hardy RS, Ross TJ, Narum SR (2019) Development and application of single-nucleotide polymorphism (SNP) genetic markers for conservation monitoring of burbot populations. Transactions of the American Fisheries Society 148(3): 661–670. https://doi.org/10.1002/tafs.10157

Caniglia R, Fabbri E, Galaverni M, Milanesi P, Randi E (2014) Noninvasive sampling and genetic variability, pack structure, and dynamics in an expanding wolf population. Journal of Mammalogy 95(1): 41–59. https://doi.org/10.1644/13-MAMM-A-039

Carroll EL, Bruford MW, DeWoody JA, Leroy G, Strand A, Waits L, Wang J (2018) Genetic and genomic monitoring with minimally invasive sampling methods. Evolutionary Applications 11(7): 1094–1119. https://doi.org/10.1111/eva.12600

Christie MR, Tennessen JA, Blouin MS (2013) Bayesian parentage analysis with systematic accountability of genotyping error, missing data and false matching. Bioinformatics 29(6): 725–732. https://doi.org/10.1093/bioinformatics/btt039

Constable J, Ashley MV, Goodall J, Pusey AE (2001) Noninvasive paternity assignment in Gombe chimpanzees. Molecular Ecology 10(5): 1279–1300. https://doi.org/10.1046/j.1365-294X.2001.01262.x

Davoli F, Cozzo M, Angeli F, Groff C, Randi E (2018) Infanticide in brown bear: A case-study in the Italian Alps – Genetic identification of perpetrator and implications in small populations. Nature Conservation 25: 55–75. https://doi.org/10.3897/natureconservation.25.23776

De Barba M, Waits L, Garton E, Genovesi P, Randi E, Mustoni A, Groff C (2010a) The power of genetic monitoring for studying demography, ecology and genetics of a reintroduced brown bear population. Molecular Ecology 19(18): 3938–3951. https://doi.org/10.1111/j.1365-294X.2010.04791.x

De Barba M, Waits LP, Genovesi P, Randi E, Chirichella R, Cetto E (2010b) Comparing opportunistic and systematic sampling methods for non-invasive genetic monitoring of a small translocated brown bear population. Journal of Applied Ecology 47(1): 172–181. https://doi.org/10.1111/j.1365-2664.2009.01752.x

Dewoody J, Nason JD, Hipkins VD (2006) Mitigating scoring errors in microsatellite data from wild populations. Molecular Ecology Notes 6(4): 951–957. https://doi.org/10.1111/j.1471-8286.2006.01449.x

Ekblom R, Aronsson M, Elsner-Gearing F, Johansson M, Fountain T, Persson J (2021) Sample identification and pedigree reconstruction in wolverine (*Gulo gulo*) using SNP genotyping of non-invasive samples. Conservation Genetics Resources 13(3): 261–274. https://doi.org/10.1007/s12686-021-01208-5

Eriksson CE, Ruprecht J, Levi T (2020) More affordable and effective noninvasive single nucleotide polymorphism genotyping using high-throughput amplicon sequencing. Molecular Ecology Resources 20(6): 1505–1516. https://doi.org/10.1111/1755-0998.13208

Flanagan SP, Jones AG (2019) The future of parentage analysis: From microsatellites to SNPs and beyond. Molecular Ecology 28(3): 544–567. https://doi.org/10.1111/mec.14988

Galla SJ, Moraga R, Brown L, Cleland S, Hoeppner MP, Maloney RF, Richardson A, Slater L, Santure AW, Steeves TE (2020) A comparison of pedigree, genetic and genomic estimates of relatedness for informing pairing decisions in two critically endangered birds: Implications for conservation breeding programmes worldwide. Evolutionary Applications 13(5): 991–1008. https://doi.org/10.1111/eva.12916

Giangregorio P (2018) Challenging the loss of genetic variability in Italian brown bears (*Ursus arctos*)-A genome-wide approach. PhD thesis, University of Bologna, Bologna, Italy.

Giangregorio P, Norman AJ, Davoli F, Spong G (2018) Testing a new SNP-chip on the Alpine and Apennine brown bear (*Ursus arctos*) populations using non-invasive samples. Conservation Genetics Resources 11(3): 355–363. https://doi.org/10.1007/s12686-018-1017-0

Glaubitz JC, Rhodes OE, Dewoody JA (2003) Prospects for inferring pairwise relationships with single nucleotide polymorphisms. Molecular Ecology Notes 12(4): 1039–1047. https://doi.org/10.1046/j.1365-294X.2003.01790.x

Groff C, Angeli F, Asson D, Bragalanti N, Pedrotti L, Rizzoli R, Zanghellini P (2016) Rapporto Orso 2015 del Servizio Foreste e fauna della Provincia Autonoma di Trento. https://grandicarnivori.provincia.tn.it/content/download/12386/226326/file/RapportoOrso_2015.pdf

Groff C, Angeli F, Asson D, Bragalanti N, Pedrotti L, Zanghellini P (2019) Rapporto Grandi carnivori 2018 del Servizio Foreste e fauna della Provincia autonoma di Trento. Provincia autonoma di Trento, Trento: Groff C, Angeli F, Asson D, N. B, L. P, R. R, P. Z (2016) Rapporto Orso 2015 del Servizio Foreste e fauna della Provincia Autonoma di Trento, 38 pp.

Gutierrez AP, Turner F, Gharbi K, Talbot R, Lowe NR, Penaloza C, McCullough M, Prodohl PA, Bean TP, Houston RD (2017) Development of a Medium Density Combined-Species SNP Array for Pacific and European Oysters (*Crassostrea gigas* and *Ostrea edulis*). G3 (Bethesda, Md.) 7(7): 2209–2218. https://doi.org/10.1534/g3.117.041780

Hauser L, Baird M, Hilborn R, Seeb LW, Seeb JE (2011) An empirical comparison of SNPs and microsatellites for parentage and kinship assignment in a wild sockeye salmon (Oncorhynchus nerka) population. Molecular Ecology Resources 11(Suppl 1): 150–161. https://doi.org/10.1111/j.1755-0998.2010.02961.x

Jones AG, Ardren WR (2003) Methods of parentage analysis in natural populations. Molecular Ecology 12(10): 2511–2523. https://doi.org/10.1046/j.1365-294X.2003.01928.x

Jones OR, Wang J (2010) COLONY: A program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources 10(3): 551–555. https://doi.org/10.1111/j.1755-0998.2009.02787.x

Kaiser SA, Taylor SA, Chen N, Sillett TS, Bondra ER, Webster MS (2017) A comparative assessment of SNP and microsatellite markers for assigning parentage in a socially monogamous bird. Molecular Ecology Resources 17(2): 183–193. https://doi.org/10.1111/1755-0998.12589

Kleinman-Ruiz D, Martínez-Cruz B, Soriano L, Lucena-Perez M, Cruz F, Villanueva B, Fernández J, Godoy JA (2017) Novel efficient genome-wide SNP panels for the conservation of the highly endangered Iberian lynx. BMC Genomics 18(1): 1–12. https://doi.org/10.1186/s12864-017-3946-5

Kohn M, Knauer F, Stoffella A, Schröder W, Pääbo S (1995) Conservation genetics of the European brown bear-a study using excremental PCR of nuclear and mitochondrial sequences. Molecular Ecology 4(1): 95–104. https://doi.org/10.1111/j.1365-294X.1995.tb00196.x

Kraus RH, Vonholdt B, Cocchiararo B, Harms V, Bayerl H, Kühn R, Förster DW, Fickel J, Roos C, Nowak C (2015) A single-nucleotide polymorphism-based approach for rapid and cost-effective genetic wolf monitoring in E urope based on noninvasively collected samples. Molecular Ecology Resources 15(2): 295–305. https://doi.org/10.1111/1755-0998.12307

Krofel M, Filacorda S, Jerina K (2010) Mating-related movements of male brown bears on the periphery of an expanding population. Ursus 21(1): 23–29. https://doi.org/10.2192/09SC015.1

Labuschagne C, Nupen L, Kotze A, Grobler PJ, Dalton DL (2015) Assessment of microsatellite and SNP markers for parentage assignment in ex situ African Penguin (*Spheniscus demersus*) populations. Ecology and Evolution 5(19): 4389–4399. https://doi.org/10.1002/ece3.1600

Labuschagne C, Dalton DL, Grobler JP, Kotze A (2017) SNP discovery and characterisation in White Rhino (*Ceratotherium simum*) with application to parentage assignment. Genetics and Molecular Biology 40(1): 84–92. https://doi.org/10.1590/1678-4685-gmb-2016-0058

Marth G, Yeh R, Minton M, Donaldson R, Li Q, Duan S, Kwok PY (2001) Single-nucleotide polymorphisms in the public domain: How useful are they? Nature Genetics 27(4): 371–372. https://doi.org/10.1038/86864

McLellan B, Proctor M, Huber D, Michel S (2016) Brown bear (Ursus arctos) isolated populations. supplementary material to Ursus arctos Redlisting account The IUCN red list of threatened species. https://www.iucnredlist.org/species/pdf/121229971/attachment

Moore JA, Draheim HM, Etter D, Winterstein S, Scribner KT (2014) Application of large-scale parentage analysis for investigating natal dispersal in highly vagile vertebrates:

a case study of American black bears (*Ursus americanus*). PLoS ONE 9: e91168. https://doi.org/10.1371/journal.pone.0091168

Morin PA, Luikart G, Wayne RK, the SNP workshop group (2004) SNPs in ecology, evolution and conservation. Trends in Ecology & Evolution 19(4): 208–216. https://doi.org/10.1016/j.tree.2004.01.009

Mustoni A, Carlini E, Chiarenzi B, Chiozzini S, Lattuada E, Duprè E, Genovesi P, Pedrotti L, Martinoli A, Preatoni P, Wauters LA, Tosi G (2003) Planning the brown bear (*Ursus arctos*) reintroduction in the Adamello Brenta Natural Park. A tool to estalish a metapopulation in the central-eastern Alps. Hystrix the Italian Journal of mammalogy 14: 3–27. https://doi.org/10.4404/hystrix-14.1-2-4313

Navidi W, Arnheim N, Waterman M (1992) A multiple-tubes approach for accurate genotyping of very small DNA samples by using pcr: Statistical considerations. American Journal of Human Genetics 50: 347.

Nielsen R, Mattila DK, Clapham PJ, Palsbøll PJ (2001) Statistical approaches to paternity analysis in natural populations and applications to the North Atlantic Humpback whale. Genetics 157(4): 1673–1682. https://doi.org/10.1093/genetics/157.4.1673

Norman AJ, Spong G (2015) Single nucleotide polymorphism-based dispersal estimates using noninvasive sampling. Ecology and Evolution 5: 3056–3065. https://doi.org/10.1002/ece3.1588

Norman AJ, Street NR, Spong G (2013) De novo SNP discovery in the Scandinavian brown bear (*Ursus arctos*). PLoS ONE 8(11): e81012. https://doi.org/10.1371/journal.pone.0081012

Norman AJ, Spong G (2015) Single nucleotide polymorphism-based dispersal estimates using noninvasive sampling. Ecology and Evolution 5(15): 3056–3065. https://doi.org/10.1002/ece3.1588

Peakall R, Smouse PE (2012) GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—an update. Bioinformatics 28(19): 2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Pemberton JM (2008) Wild pedigrees: The way forward. Proceedings of the Royal Society B, Biological Sciences 275(1635): 613–621. https://doi.org/10.1098/rspb.2007.1531

Pompanon F, Bonin A, Bellemain E, Taberlet P (2005) Genotyping errors: Causes, consequences and solutions. Nature Reviews. Genetics 6(11): 847–859. https://doi.org/10.1038/nrg1707

Raymond M, Rousset F (1995) GENEPOP (version 1.2): Population genetics software for exact tests and ecumenicism. The Journal of Heredity 86(3): 248–249. https://doi.org/10.1093/oxfordjournals.jhered.a111573

Riester M, Stadler PF, Klemm K (2009) FRANz: Reconstruction of wild multi-generation pedigrees. Bioinformatics 25(16): 2134–2139. https://doi.org/10.1093/bioinformatics/btp064

Robinson S, Simmons L, Kennington W (2013) Estimating relatedness and inbreeding using molecular markers and pedigrees: The effect of demographic history. Molecular Ecology 22(23): 5779–5792. https://doi.org/10.1111/mec.12529

Skrbinsek T, Jelencic M, Waits LP, Potocnik H, Kos I, Trontelj P (2012) Using a reference population yardstick to calibrate and compare genetic diversity reported in different studies: An example from the brown bear. Heredity 109(5): 299–305. https://doi.org/10.1038/hdy.2012.42

Stenglein JL, Waits LP, Ausband DE, Zager P, Mack CM (2011) Estimating gray wolf pack size and family relationships using noninvasive genetic sampling at rendezvous sites. Journal of Mammalogy 92(4): 784–795. https://doi.org/10.1644/10-MAMM-A-200.1

Stoch F, Genovesi P (2016) Manuali per il monitoraggio di specie e habitat di interesse comunitario (Direttiva 92/43/CEE) in Italia: specie animali. In: ISPRA SMelg, 141/2016 (Ed).

Taberlet P, Waits LP, Luikart G (1999) Noninvasive genetic sampling: Look before you leap. Trends in Ecology & Evolution 14(8): 323–327. https://doi.org/10.1016/S0169-5347(99)01637-7

Taylor HR (2015) The use and abuse of genetic marker-based estimates of relatedness and inbreeding. Ecology and Evolution 5(15): 3140–3150. https://doi.org/10.1002/ece3.1541

Tokarska M, Marshall T, Kowalczyk R, Wojcik JM, Pertoldi C, Kristensen TN, Loeschcke V, Gregersen VR, Bendixen C (2009) Effectiveness of microsatellite and SNP markers for parentage and identity analysis in species with low genetic diversity: The case of European bison. Heredity 103(4): 326–332. https://doi.org/10.1038/hdy.2009.73

Tosi G, Chirichella R, Zibordi F, Mustoni A, Giovannini R, Groff C, Zanin M, Apollonio M (2015) Brown bear reintroduction in the Southern Alps: To what extent are expectations being met? Journal for Nature Conservation 26: 9–19. https://doi.org/10.1016/j.jnc.2015.03.007

Valière N (2002) GIMLET: A computer program for analysing genetic individual identification data. Molecular Ecology Notes 2(3): 377–379. https://doi.org/10.1046/j.1471-8286.2002.00228.x-i2

Vignal A, Milan D, SanCristobal M, Eggen A (2002) A review on SNP and other types of molecular markers and their use in animal genetics. Genetics, Selection, Evolution. 34(3): 275–305. https://doi.org/10.1186/1297-9686-34-3-275

von Thaden A, Cocchiararo B, Jarausch A, Jungling H, Karamanlidis AA, Tiesmeyer A, Nowak C, Munoz-Fuentes V (2017) Assessing SNP genotyping of noninvasively collected wildlife samples using microfluidic arrays. Scientific Reports 7(1): 10768. https://doi.org/10.1038/s41598-017-10647-w

von Thaden A, Nowak C, Tiesmeyer A, Reiners TE, Alves PC, Lyons LA, Mattucci F, Randi E, Cragnolini M, Galian J, Hegyeli Z, Kitchener AC, Lambinet C, Lucas JM, Molich T, Ramos L, Schockert V, Cocchiararo B (2020) Applying genomic data in wildlife monitoring: Development guidelines for genotyping degraded samples with reduced single nucleotide polymorphism panels. Molecular Ecology Resources 20(3): 662–680. https://doi.org/10.1111/1755-0998.13136

Vonholdt BM, Stahler DR, Smith DW, Earl DA, Pollinger JP, Wayne RK (2008) The genealogy and genetic viability of reintroduced Yellowstone grey wolves. Molecular Ecology 17(1): 252–274. https://doi.org/10.1111/j.1365-294X.2007.03468.x

Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: Cautions and guidelines. Molecular Ecology 10(1): 249–256. https://doi.org/10.1046/j.1365-294X.2001.01185.x

Wang J (2019) Pedigree reconstruction from poor quality genotype data. Heredity 122(6): 719–728. https://doi.org/10.1038/s41437-018-0178-7

Webster MS, Reichart L (2005) Use of Microsatellites for Parentage and Kinship Analyses in Animals. 395: 222–238. https://doi.org/10.1016/s0076-6879(05)95014-3

Weinman LR, Solomon JW, Rubenstein DR (2015) A comparison of single nucleotide polymorphism and microsatellite markers for analysis of parentage and kinship in a cooperatively breeding bird. Molecular Ecology Resources 15(3): 502–511. https://doi.org/10.1111/1755-0998.12330

Wilson GA, Olson W, Strobeck C (2002) Reproductive success in wood bison (*Bison bison athabascae*) established using molecular techniques. Canadian Journal of Zoology 80(9): 1537–1548. https://doi.org/10.1139/z02-147

Wright B, Morris K, Grueber CE, Willet CE, Gooley R, Hogg CJ, O'Meally D, Hamede R, Jones M, Wade C, Belov K (2015) Development of a SNP-based assay for measuring genetic diversity in the Tasmanian devil insurance population. BMC Genomics 16(1): 791. https://doi.org/10.1186/s12864-015-2020-4

Zhao H, Li C, Hargrove JS, Bowen BR, Thongda W, Zhang D, Mohammed H, Beck BH, Austin JD, Peatman E (2018) SNP marker panels for parentage assignment and traceability in the Florida bass (*Micropterus floridanus*). Aquaculture (Amsterdam, Netherlands) 485: 30–38. https://doi.org/10.1016/j.aquaculture.2017.11.014

Supplementary material 1

Genetic and field data

Authors: Patrizia Giangregorio, Nadia Mucci, Anita J. Norman, Luca Pedrotti, Stefano Filacorda, Paolo Molinari, Göran Spong, Francesca Davoli

Data type: genetic and field data (word document)

Explanation note: Sample information, parentage relationships, marker summary statistics, FRANz v. 2 and Colony v. 2.0.6.4 output data.

Link: https://doi.org/10.3897/natureconservation.53.86739.suppl1

Nature Conservation 53: 125–155 (2023)
DOI: 10.3897/natureconservation.53.102810

Research Article

Dynamic change of habitat quality and its key driving factors in Ningxia Hui Autonomous Region, China

Ding Wang[1,2], Haiguang Hao[1], Hao Liu[1], Lihui Sun[1], Yuyang Li[1]

1 *Chinese Research Academy of Environmental Sciences, Beijing, No. 8 Dayangfang, Anwai Beiyuan, Chaoyang District, Beijing, 100012, China*
2 *China National Environmental Monitoring Centre, Beijing, No. 8 Dayangfang, Anwai Beiyuan, Chaoyang District, Beijing, 100012, China*
Corresponding author: Haiguang Hao (haohg@craes.org.cn)

Abstract

Habitat quality reflects the level of biodiversity, and habitat maintenance functions are related to human well-being and ecosystem stability. Ningxia Hui Autonomous Region is a typical ecologically fragile region in Western China with complex human-nature relationships. Maintaining good habitat is not only a fundamental requirement for biodiversity conservation but also a necessary path for sustainable regional development. In this study, we assessed and analysed the spatial and temporal patterns and changes in habitat quality in Ningxia from 2000 to 2020, and explored the driving factors of habitat quality using a geographically weighted regression (GWR) model. The results indicated: (1) The overall habitat quality level in Ningxia was low to intermediate, with an upwards and then downwards trend during the past 20 years, showing a small change in overall magnitude. (2) The high- and higher-level habitat quality patches in Ningxia were mainly distributed in areas with high vegetation cover, such as the Helan Mountain and Liupan Mountain. The patches of moderate-level habitat quality mainly included cultivated land, while the low- and lower-level patches were mainly distributed in areas subjected to more frequent human activities, such as cultivated land and construction land. (3) The spatial and temporal distribution patterns and changes in habitat quality in Ningxia from 2000 to 2020 were mainly influenced by fractional vegetation cover (FVC), soil moisture content (SMC), proportion of construction land area (PCL), and proportion of cultivated land area (CLP). Among them, FVC and SMC were positive driving factors, and PCL and CLP were negative driving factors. The results support that increasing vegetation cover and reducing anthropogenic disturbance to natural habitats are important measures to maintain fragile habitats and that key ecological function areas such as nature reserves are crucial for habitat quality protection in ecologically fragile areas.

Key words: Driving factors, geographically weighted regression (GWR), habitat quality, Ningxia Hui Autonomous Region, spatiotemporal pattern

This article is part of:
Remote Sensing Applications to Monitor Ecosystem Services
Edited by Javier Martinez-Lopez, Domingo Alcaraz, Simon Willcock, Javier Cabello, Francisco J. Bonet & Joris de Vente

Academic editor: F. J. Bonet García
Received: 1 March 2023
Accepted: 6 July 2023
Published: 16 August 2023

ZooBank: https://zoobank.org/AFD25A8E-D125-49A4-AF2C-8B0E714F0382

Citation: Wang D, Hao H, Liu H, Sun L, Li Y (2023) Dynamic change of habitat quality and its key driving factors in Ningxia Hui Autonomous Region, China. Nature Conservation 53: 125–155. https://doi.org/10.3897/natureconservation.53.102810

Introduction

Habitat quality refers to the ability of an ecological environment to provide suitable conditions for the sustainable survival and development of individuals, populations or communities, reflecting the richness of biodiversity in a region, and it is related to human well-being (Yohannes et al. 2021). The concept of habitat was first introduced by Grinnel in 1917 (Riedler and Lang 2018), and the study of habitat quality can be traced back to the 1960s (Goertz 1964; Rosenzweig and Winakur 1969; Janzen 1970) which fully developed by field surveys (Van Horne 1983; Congdon 1974), habitat indices (Berger and Hodge 1998), model simulations (Dunning et al. 1992; Roth et al. 1996) and other stages. Currently, since the introduction of the United Nations Sustainable Development Goals (SDGs) and the convening of the Conference of the Parties to the United Nations Convention on Biological Diversity (UN CBD-COP), governments and researchers have agreed to stabilize and enhance the global biodiversity level (Hale and Swearer 2016).

A favourable habitat condition means that various ecological factors in the ecosystem meet the needs of population survival and reproduction, and the orderly differentiation of biological ecological niches will achieve a balanced and stable ecosystem function. Additionally, habitat maintenance is an ecosystem service that is of great concern to humans (Celina et al. 2022), and habitat quality is a comprehensive representation of habitat maintenance capacity, which fully connects ecological processes with human needs. Habitat quality is deeply related to regional biodiversity levels, ecosystem service trade-offs and synergies, and ecological security patterns (Wu et al. 2013; Wang et al. 2022); habitat quality determines the balance of ecosystems, laying the foundation for sustainable development prospects of social-economic-natural complex systems (Wu et al. 2017). Managing and maintaining habitat functions and improving habitat quality can effectively maintain biodiversity and provide a good base of ecosystem services that ultimately meet the needs of the human economy and society (Peggy et al. 2021). Ecologically fragile areas are located in the cross-transition zone of different types of ecosystems, with weak system resistance to disturbance, sensitivity to global climate change, and generally low habitat quality as well as biodiversity levels (Prasad and Ramesh 2019). For ecologically fragile areas with poor ecological backgrounds, measures such as curbing urban expansion and maintaining landscape integrity can enhance regional habitat quality, thereby improving and enhancing ecosystem structure and function, which can promote a continuous supply of ecosystem services to human society (Ramachandra et al. 2019).

Habitat quality assessments include both ecological and geographic perspectives. Early studies focused on the substantial impacts of human activities on plant and animal habitats, and the research methods and contents were more biased towards natural and ecological properties (Dallimer et al. 2012). Such studies usually used relevant parameters obtained by field survey methods to construct indicator systems; additionally, hierarchical analysis, grey correlation models, and entropy weighting methods were used to synthesize habitat conditions, and the natural or anthropogenic driving factors of habitats of single or homogeneous species were analysed. These studies were mostly conducted on a small scale, such as small cities, rivers, and nature reserves, and usually used the sample strip method or sample method of investigation to obtain various parameters related to the quality of plant and animal habitats; moreover, these studies commonly used indicators including species richness, vegetation types, topographic indicators, and water quality (Harper and Everard 1998). The advantage of this method is that it can reflect the habits and habitat conditions of the evaluated objects more comprehensively, and the indicators are more sensitive and detailed. However, due to the high time and labour costs and the difficulty of obtaining data over long-time spans for dynamic analysis, such methods cannot be applied to habitat quality studies at larger spatial and temporal scales (Wang et al. 2017).

As land use/land cover (LULC) change has become the focus of global change research (van Vliet et al. 2015), habitat quality assessment models developed by remote sensing and GIS technologies have been widely used in practice, forming a paradigm for habitat quality research based on a geographic perspective (Romero-Calcerrada and Luque 2006). In recent years, many scholars have performed many studies on habitat quality at large-scale scales, such as across regions, from the perspectives of ecological service function, ecological risk and early warning and the relationship between urbanization development and ecosystem conservation (Nagendra et al. 2013; Chen et al. 2016; Gomes et al. 2021; Duan and Yu 2022). Commonly used mature habitat quality assessment models include Integrated Valuation of Ecosystem Services and Tradeoffs (InVEST) (Bao et al. 2015), Habitat Suitability Index Model (HSI) (Wang et al. 2009), Maximum Entropy Model (MaxEnt) (Radosavljevic and Anderson 2014), Artificial Intelligence for Ecosystem Services (ARIES) (Villa et al. 2009), and Multiscale Integrated Models of Ecosystem Services (MIMES) (Roelof et al. 2015), among others. Among them, the Habitat Quality module in the InVEST model is widely used (Kareiva et al. 2011) because of its low application cost and high assessment accuracy, and the evaluation results support the spatial visual representation of regional habitat distribution as well as habitat degradation.

Currently, humans are facing a serious biodiversity crisis, and habitat destruction is one of the most serious challenges threatening biodiversity conservation (Crooks et al. 2017). Population growth, economic development, and continuous changes in land use and its structure profoundly affect the material and energy flow circulation processes between habitat patches, which in turn change the distribution patterns and functions of regional habitats (Haddad N et al. 2015). The level of habitat quality is highly dependent on natural conditions and on its proximity to human land uses and the intensity of land use (Sobhani et al. 2022). Therefore, exploring the relationships among ecological factors, ecological processes and habitat quality is important for maintaining regional biodiversity and even ecosystem functions and provides a basis for improving regional ecological security patterns and the sustainable development of land resources (Kalacska et al. 2017; Hao et al. 2019).

The current methods used to investigate the factors affecting habitat quality mainly include spatial exploratory analysis, spatial econometric analysis, multiple regression analysis, grey correlation analysis, and Moran's I spatial autocorrelation index (Zhu and Alimujiang 2020; Moëzzi et al. 2022; Raimundo Lopes et al. 2022); moreover, these methods focus on the spatial and temporal patterns, multiscale change characteristics, and evolutionary mechanisms of habitat quality (Wu et al. 2021; Xiao et al. 2022). However, most of the studies have been conducted at the scale of administrative units, usually using methods such as indicator methods and spatial autocorrelation analysis, and they have failed to fully consider the heterogeneity of different geographic spaces and the spatial scales at which different influencing factors act (Chisholm et al. 2011; Smith et al. 2011). Based on Tobler's (1970) first law of geography, Brunsdon proposed the geographically weighted regression (GWR) model (Brunsdon et al. 1996), which allows for spatial heterogeneity in the coefficients of independent variables and can effectively detect the spatial nonstationarity characteristics of regression variables; this method is widely used in geography, economics, ecology and the environment (Zhu et al. 2020; Hu et al. 2022). The GWR model performs distance-weighted regression with the help of observations from neighbouring sample points, which can reveal the quantitative relationships between factors and impact factors more accurately, thus improving the goodness of fit of the model and solving the deficiency of using exploratory spatial data analysis (ESDA) tools that can solve only time-sectional data (Qin 2007).

In summary, this study selected the Ningxia Hui Autonomous Region (hereinafter referred to as Ningxia), a typical ecologically fragile region in Western China, as the study area, collected raw data on land use, NDVI, and temperature from 2000 to 2020, and analysed the spatial and temporal patterns of habitat quality and their changes in the past 20 years based on remote sensing and GIS analysis. Based on the objective fact that the distribution of habitat quality in Ningxia is spatially heterogeneous, the GWR model with optimal fitting parameters was finally used to investigate the key factors driving the distribution and changes in habitat quality in Ningxia.

Materials and methods

Study area

Ningxia (35°14'–39°23'N, 104°17'–107°39'E) (Fig. 1) is located in Western China in the middle and upper reaches of the Yellow. The climate is temperate continental, with an average annual temperature of 6–10 °C, an average annual precipitation of approximately 220 mm, and more than 3,200 h of sunshine. The topography is high in the south and low in the north, with an altitude of 1,100–1,200 m. The ecological geography is divided into 3 parts. The Yellow River flows through the northern irrigation area, which has a gentle topography and superior soil and water conditions. The central area is the arid wind-sand belt, which is subject to perennial drought and poor soil and water conditions; and the southern mountainous area is full of ravines and gullies, with complex topography and a cold and wet climate. Ningxia has a well-developed agriculture and animal husbandry industry, but water resources are concentrated with a small and uneven spatial distribution. Ningxia is located in the interlocking agricultural and pastoral areas of northern China, with a fragile ecological environment and simple species composition and ecosystem diversity. Due to its location in the transition zone of the arid and semiarid climate zones, Ningxia has become an important ecological security barrier in Western China. There was still 15,534.84 km^2 of soil erosion in the region, accounting for 23.40% of the total area of the region, and the contradiction between ecological and environmental problems and economic and social development was still relatively prominent.

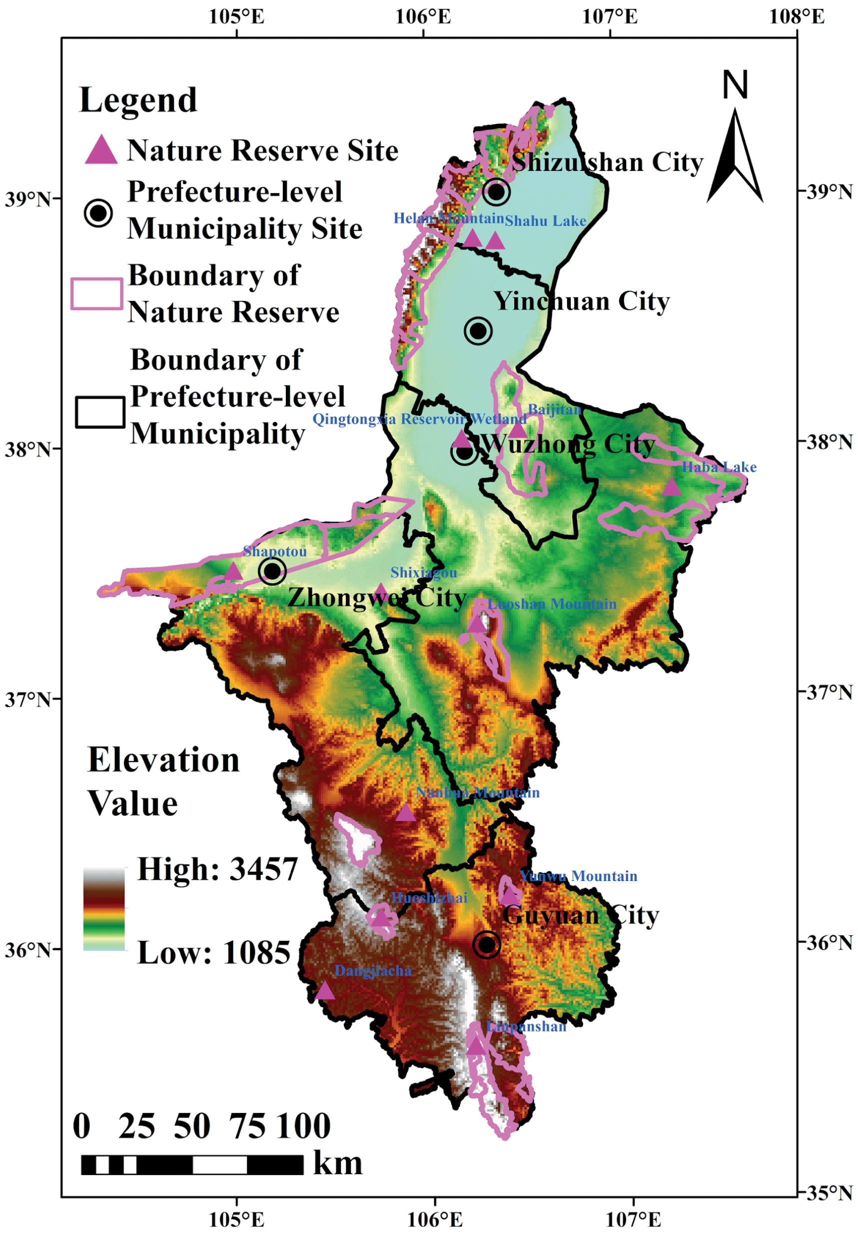


Figure 1. Topography, natural reserves and administrative division of Ningxia.

Research methods

Habitat quality assessment methods

In this study, the habitat quality module of the InVEST model was used to assess habitat quality in Ningxia, and the habitat quality index was calculated as follows:

$$Q_{xj} = H_j[1 - (\frac{D_{xj}^z}{D_{xj}^z + k^z})] \qquad (2.1.1)$$

where Q_{xj} is the habitat quality of raster x in land use type j; k is the half-saturation parameter, whose value is half of the resolution of the raster data in the study area and is generally 1/2 of the maximum value of habitat degradation; H_j is the habitat suitability of land use type j, whose value is usually 0~1; z is the normalization constant, which is usually set to 2.5; and D_{xj} is the level of stress to which raster *x* of land use type *j* is subjected, i.e., the degree of habitat degradation. The degree of habitat degradation is the intensity of habitat disturbance by threat sources and is calculated as follows:

$$D_{xj} = \sum_{r=1}^{R}\sum_{y=1}^{y_r}\left(\frac{\omega_r}{\sum_{r=1}^{R}\omega_r}\right) r_y i_{rxy} \beta_x S_{jr} \tag{2.1.2}$$

$$i_{rxy} = 1 - \left(\frac{d_{xy}}{d_{rmax}}\right) \text{ (Linear decay)} \tag{2.1.3}$$

$$i_{rxy} = \exp\left(\frac{-2.99 d_{xy}}{d_{rmax}}\right) \text{ (Exponential decay)} \tag{2.1.4}$$

where D_{xj} is the degree of habitat degradation; *R* is the number of stressors; *y* is the number of grids in the raster layer of stressor *r*; y_r is the number of grids occupied by stressors; w_r is the stressor weight; r_y is the stressor value of raster *y*; β_x is the accessibility level of raster *x*, which is not considered in this study; s_{jr} is the sensitivity of habitat type *j* to stressor *r*; i_{rxy} is the stress factor value r_y of raster *y* on the stress level of habitat raster *x*; d_{xy} is the linear distance between raster *x* and raster *y*; and d_{rmax} is the maximum stress distance of threat source *r*. The higher the calculated score is, the greater the threat level caused by the threat factor to the habitat and the higher the degree of habitat degradation.

Based on the InVEST model manual and with reference to previous research results on habitat quality in Ningxia and the arid and semiarid regions of Northwest China (Wu et al. 2020; Bao 2022; Ren et al. 2022), this study used paddy fields, drylands, urban land, rural settlements and other construction land as threat factors and determined the habitat suitability of habitat types and the sensitivity of different habitat types to stress factors (Tables 1, 2).

The rate of change in habitat quality was calculated using the terminal habitat quality minus the initial habitat quality with the following equation:

$$K_T = \frac{HQ_i - HQ_0}{HQ_i} \times 100\% \tag{2.2}$$

where K_T is the rate of change in habitat quality over time *T*. This study had a 5-year cycle; HQ_o is the size of habitat quality at the beginning of the study, HQ_i is the size of habitat quality at the end of the study, and the raster resolution is 30 m.

Spatial autocorrelation analysis

In this study, the global Moran's I index was used to describe whether habitat quality in the study area had a clustering effect on a global scale, and the local Moran's I index was used to reflect the spatial autocorrelation of habitat quality in the subregion. The spatial autocorrelation analysis was performed in ArcGIS 10.7 software.

$$\text{Global Moran's } I = \frac{n\sum_{i=1}^{n}\sum_{j=1}^{n}\omega_{ij}(x_i-\bar{x})(x_j-\bar{x})}{\sum_{i=1}^{n}(x_i-\bar{x})^2\left(\sum_i\sum_j\omega_{ij}\right)}(i \neq j) \tag{2.3.1}$$

$$\text{local Moran's } I = \frac{n(x_i-\bar{x})}{\sum_{i=1}^{n}(x_i-\bar{x})^2}\sum_{j=1}^{n}\omega_{ij}(x_i-\bar{x})(i \neq j) \tag{2.3.2}$$

where x_i and x_j are the values of variable *x* taken on neighbouring cells, *x* is the attribute value of the n location variables, $\bar{x}$ is the mean of the attribute values of the spatial variables, ω_{ij} is the spatial weight matrix of raster *i* and raster *j*, and *n* is the total number of rasters.

Table 1. Ecological threat factors and their maximum impact distances and weights.

Threat Factor	Impact Distance/km	Weight	Spatial Decline Type
Paddy Field	4	0.15	Linear Decline
Dryland	3	0.2	Linear Decline
Urban Land	5	0.3	Exponential Decline
Rural Settlements	4	0.3	Exponential Decline
Other Construction Land	8	0.2	Linear Decline

Table 2. Habitat suitability and relative sensitivity to threat factors.

Type	Habitat suitability	Paddy field	Dryland	Rural settlement	Urban land	Other construction land
Paddy Field	0.6	0.3	0.2	0.35	0.5	0.45
Dryland	0.4	0.3	0.2	0.35	0.5	0.4
Forested Land	1	0.8	0.7	0.85	1	0.6
Shrubland	1	0.4	0.3	0.45	0.6	0.4
Sparse Woodland	1	0.85	0.75	0.9	1	0.65
Other Forest Land	1	0.9	0.8	0.95	1	0.7
High Coverage Grassland	0.85	0.4	0.3	0.45	0.6	0.6
Medium Coverage Grassland	0.8	0.45	0.35	0.5	0.65	0.7
Low Coverage Grassland	0.75	0.5	0.4	0.55	0.7	0.8
Canal	1	0.7	0.6	0.75	0.9	0.5
Lake	1	0.7	0.6	0.75	0.9	0.5
Reservoir Pit	1	0.7	0.6	0.75	0.9	0.5
Beach Land	0.6	0.75	0.65	0.75	0.95	0.55
Urban Land	0	0	0	0.8	0	0
Rural Settlements	0	0	0	0	0	0
Other Construction Land	0	0	0	0	0	0
Unused Land	0	0	0	0	0	0

Geographically weighted regression

Pearson correlation regression, the least squares model (OLS), and geographically weighted regression models were used to explore the characteristics of driving factors acting on habitat quality in Ningxia. The GWR model is a local regression model that embeds the geographic location of the data into the regression parameters, allowing for local parameter estimation. In this study, the geographically weighted regression weight function was chosen as a Gaussian function (Adaptive Gaussian), and its calibration was performed using an adaptive approach (Adaptive).

$$y_i = \beta_0(\mu_i, v_i) + \sum_{i=1}^{k} \beta_k(\mu_i, v_i)\, x_{ik} + \varepsilon_i \qquad (2.4)$$

where y_i is the dependent variable at sample point *i*, x_{ik} is the observed value of the *kth* variable at the *ith* point, (μ_i, v_i)is the location coordinate of the ith point, $\beta_o(\mu_i, v_i)$ is the intercept, $\beta_k(\mu_i, v_i)$ is the regression coefficient of the *ith*, and ε_i is the error term.

Based on the results of previous studies on the driving factors of habitat quality in the Loess Plateau and Western China and the actual characteristics of the ecological environment in Ningxia (Yang et al. 2021; Bai et al. 2020), 16 indicators of physical geographic and socioeconomic factors were selected to analyse the key driving factors and characteristics of their effects on the status and dynamic change of habitat quality in Ningxia, as shown in Table 3.

Data sources and processing

The data in this study included habitat quality assessment data and driving regression data, and the InVEST model habitat quality assessment mainly used five periods of land use dataset from 2000 to 2020. The dataset were obtained from the Resource and Environmental Science and Data Center of the Chinese Academy of Sciences (https://www.resdc.cn/) at a resolution of 30 m. NDVI data were obtained from the 30 m annual maximum NDVI dataset of China at the National Ecological Science Data Center of China (http://www.nesdc.org.cn/). Net primary productivity data were obtained from the MOD17A3HGF Version 6.0 product (https://www.earthdata.nasa.gov/). Geospatial information data included the 2021 version of 1:1 million public geographic basic information dataset (https://www.webmap.cn/commres.do?method=result100W) and ALOS 12.5 m DEM data (https://www.gscloud.cn/). GDP and Population data were obtained from the 1 km-grid GDP dataset of China (https://www.resdc.cn/) and the 1 km-grid population dataset of China (https://www.resdc.cn/). Soil water content data were obtained from the Soil Moisture in China dataset (2002–2018) (http://data.tpdc.ac.cn/zh-hans/). A 1-km monthly mean temperature dataset for China (1901–2021), a 1-km monthly precipitation dataset for China (1901–2022), and the Prolonged Artificial Nighttime-light Dataset of China (1984–2020) were obtained from the National Tibetan Plateau Scientific Data Center (http://www.tpdc.ac.cn/).

Table 3. Selection of regressors for habitat quality driving factors in Ningxia.

Indicators	Abbreviation	Unit
Net Primary Productivity	NPP	$gC/(m^2*a)$
Fractional Vegetation Cover	FVC	%
Mean Annual Precipitation	MAP	mm
Drainage Density	DRA	km/km^2
Elevation	ELE	m
Slope	SLP	°
Degree of Relief	DRF	m
Soil Moisture Content	SMC	m^3/m^3
Average Annual Temperature	AAT	°C
Proportion of Cultivated Land	PCL	%
The Proportion of Construction Land	CLP	%
Population Density	POP	$person/km^2$
Road Network Density	RND	km/km^2
Nighttime Light Index	NLI	DN
Regional GDP	GDP	10^4 Yuan (¥) $/km^2$
Closest Distance to Road Network	DRN	m

The original spatial raster data of river network density, road density, distance to the nearest road, elevation, slope, topographic relief, GDP, population density, nighttime lighting index, proportion of construction land area, and proportion of cultivated land area for the whole Ningxia region in 2000, 2005, 2010, 2015 and 2020 were obtained by processing the above datasets. Based on the zoning of Ningxia and the accuracy of the data, a suitable 5 km × 5 km fishing grid was built, excluding the grid with null values, to obtain the final 2290 grids. The raw data were partitioned in tabular form to obtain the final data results for each impact factor as well as the raw data results for habitat quality for the period 2000–2020, with 17 categories and 85 datasets in five periods.

Results and analysis

Spatial and temporal patterns of habitat quality in Ningxia

The habitat quality of Ningxia was classified into five levels: low level (0–0.25), lower level (0.25–0.4), moderate level (0.4–0.6), higher level (0.6–0.75), and high level (0.75–1.0) (Fig. 2). The results showed that the proportion of high-level habitat quality patches in Ningxia in 2020 was the highest (29.27%), followed by lower-level (25.08%) and higher-level (22.62%) patches, while the proportions of low-level and moderate-level habitat quality patches were lower, at 13.41% and 9.61%, respectively. The proportion distribution of habitat quality patches in Ningxia in 2000, 2005, 2010 and 2015 was similar to that in 2020, which showed the distribution of high level > lower level > higher level > low level > moderate. From 2000 to 2020, the average habitat quality of the whole region of Ningxia was approximately 0.58, which was at a moderate-good level. Combined with the proportional distribution of high-level and higher-level habitat quality patches, the habitat maintenance function in Ningxia has been relatively healthy over the past 20 years.

In terms of spatial distribution (Fig. 3a–f), the high-level habitat quality patches in Ningxia from 2000 to 2020 were mainly distributed in nature reserves with high forest cover and excellent ecological conditions, such as in the south, Helan Mountain in the north, Shixia Gorge in the east and Luoshan in the centre. The higher-level habitat quality patches were scattered around the higher-level habitat quality patches. The moderate-level habitat quality patches mainly included most of the cultivated land types. The lower-level habitat quality patches contained some cultivated land and were more randomly distributed. The low-level habitat quality patches mainly included most of the construction land and unused land with harsh natural conditions, such as the central wind-sand region area, the sandpots in the west, and the foothills of the Helan Mountain and the edge of the Ningxia Plain, which are strongly disturbed by humans.

The results of spatial autocorrelation analysis showed (Fig. 4) that the distribution of habitat quality in Ningxia from 2000 to 2020 was significantly autocorrelated geographically and spatially, with Moran indices of 0.619, 0.604, 0.588, 0.581, and 0.144 ($P < 0.01$), all with a 90%+ credibility level. The results of local spatial autocorrelation coefficients showed (Fig. 5a–e) that the habitat quality aggregation in Ningxia mainly included two types of high-high value aggregation and low-low value aggregation, while the high-low value aggregation and low-high value aggregation types had poor significance levels and more random distributions.

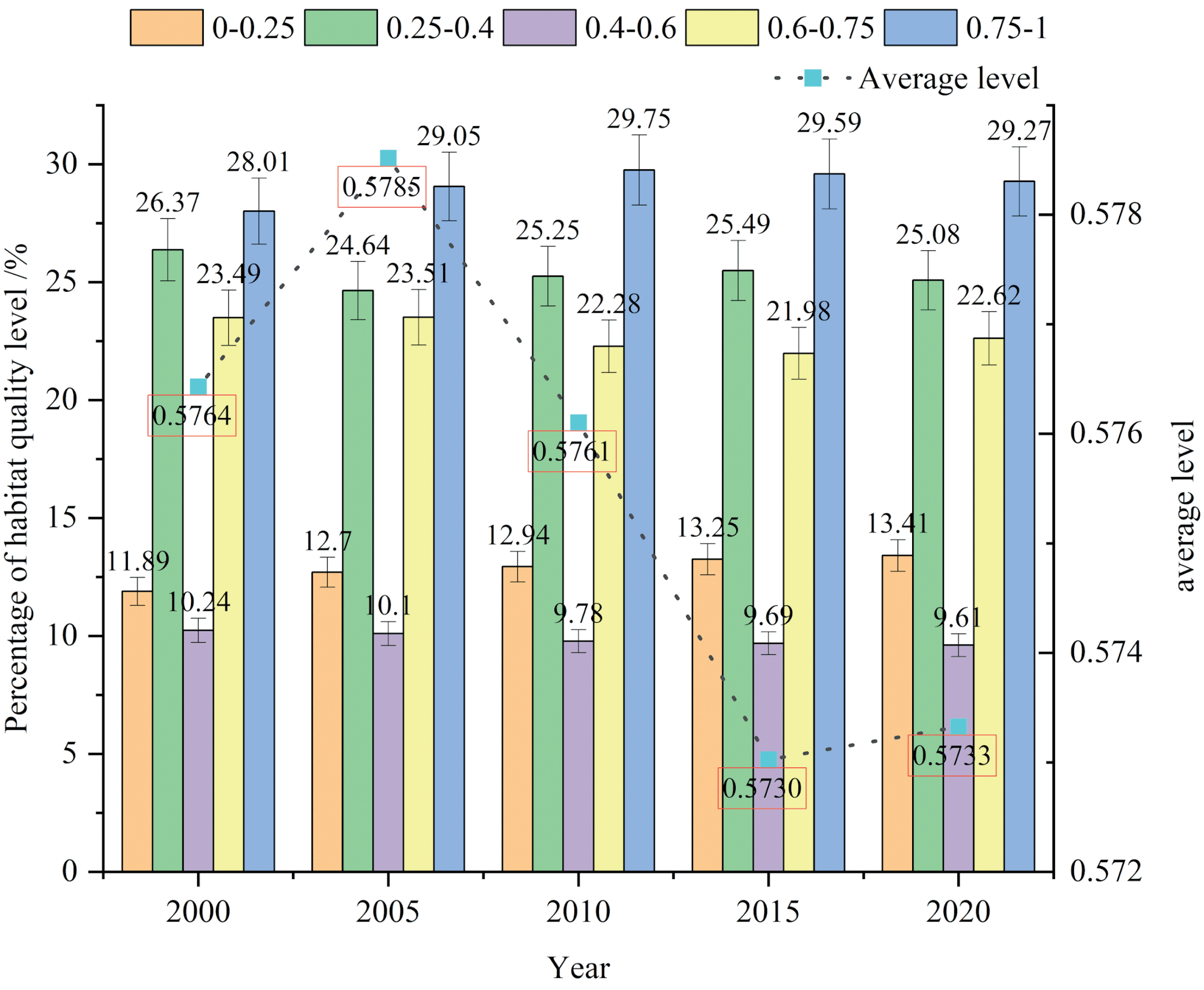


Figure 2. Proportion of habitat quality levels in Ningxia from 2000–2020.

Combining the distribution of patches with the different habitat quality levels in Ningxia, we found that habitat quality in Ningxia was closely related to patch type and was influenced by both natural conditions and human activities. Our study found that habitat quality levels were highest in primary forest reserves that were not disturbed by human activities, where precipitation, temperature, topography and elevation were suitable for the survival and reproduction of organisms. In contrast, habitat quality was significantly lowest in construction sites strongly disturbed by human activities, where the climate is arid, vegetation is sparse, and land use changes are frequent, i.e., they lacked the basic conditions needed to meet biological survival. In addition, although cultivated land is used as an artificial landscape, it possesses a moderate level of habitat maintenance function, and the habitat quality was generally categorised at the moderate level. Nature reserves concentrate the most fully functional ecosystems, which are crucial for protecting habitat quality and improving biodiversity levels. By identifying key areas and delineating priority protection areas, it will further contribute to the stability and improvement of regional biodiversity levels.

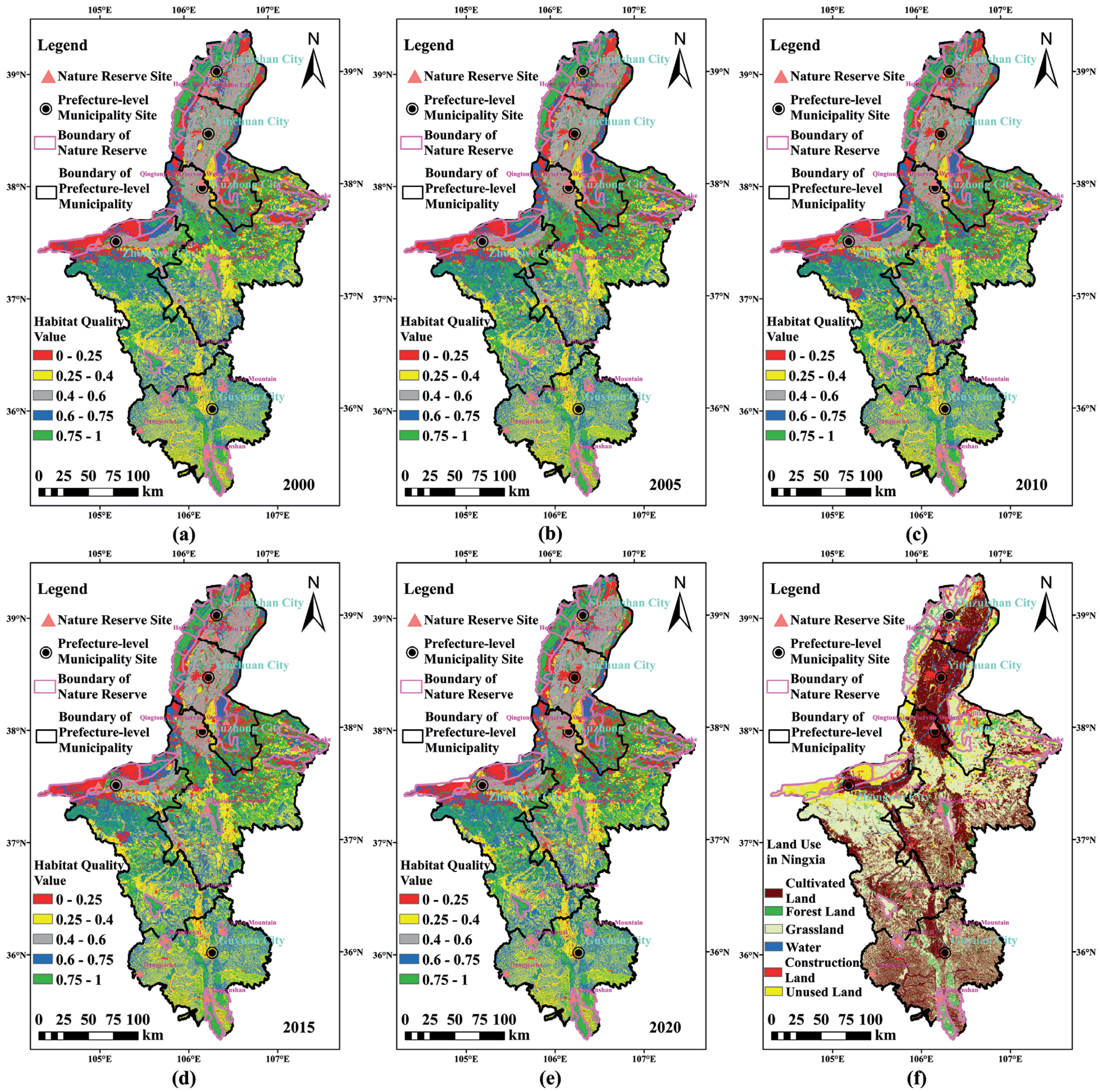


Figure 3. Spatial distribution of habitat quality and nature reserves in Ningxia from 2000 to 2020.

Dynamic changes of habitat quality in Ningxia

In terms of different levels of habitat quality (Fig. 6a), the proportion of high-level habitat quality patches in Ningxia increased by 1.26% from 2000 to 2020, while the low-level habitat quality patches also increased by 1.53%. The proportions of lower-level, moderate-level and higher-level habitat quality patch areas decreased by 1.29%, 0.62% and 0.87%, respectively. From different periods (Fig. 6a, b), the area of low-level habitat quality patches in Ningxia from 2000 to 2020 increased, the area of moderate-level habitat quality patches decreased, and the area of lower-level habitat quality patches was more volatile. While the area of higher-level habitat quality patches decreased and then increased, the high-level patches showed a trend of increasing and then decreasing. From 2000

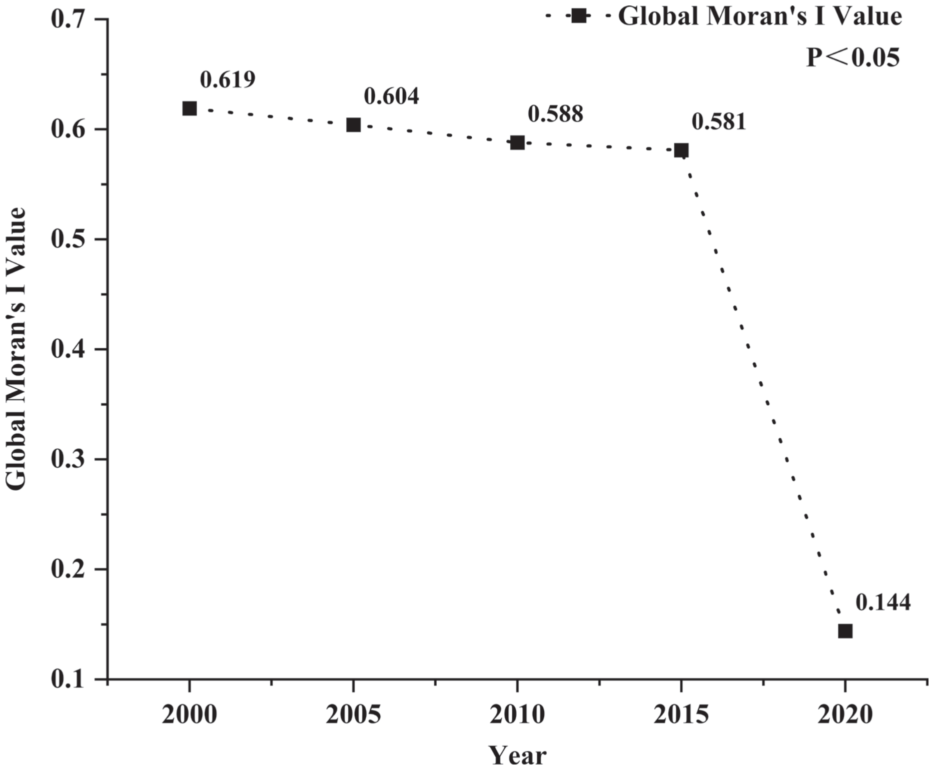


Figure 4. Spatial autocorrelation global Moran's I values of habitat quality in Ningxia from 2000–2020.

to 2020, the average habitat quality in Ningxia was maintained at a moderate level with small changes (<0.005) (Fig. 6b). On the one hand, ecological protection measures such as returning farmland to forests and grasses and ecological restoration have increased the habitat quality of some patches, but factors such as population growth and construction land expansion have caused the habitat quality of some patches to decrease, resulting in the contradiction between human activities and natural habitats remaining very prominent.

The spatial variation in habitat quality in Ningxia was divided into five classes: significantly decreasing (-1– -0.5), slightly decreasing (-0.5–-0.25), remaining stable (-0.25–0.25), slightly increasing (0.25–0.5), and significantly increasing (0.5–1) (Fig. 7a–f). In terms of spatial variation, habitat quality decreased more (-1–0.25) in areas such as Ningxia with a high density of construction land and some scattered cultivated land from 2000 to 2020. In contrast, significant increases in habitat quality were more concentrated in the central area where the cultivated land was returned to forest and grass (0.25–1). Habitat quality was stable in most areas of Ningxia over the 20-year period, with small changes (-0.25–0.25). In different periods (Figs 6a, b, 7a–f), the patches with decreasing habitat quality in Ningxia basically decreased continuously during the 20 years from 2000 to 2020. Most of the patches with habitat quality changes from 2000–2005 showed increases. From 2005 to 2010, habitat quality decreases were dominant. From 2010–2015, the decreasing trend remained severe. The decreasing trend improved only during 2015–2020.

The analysis of habitat quality in Ningxia showed (Fig. 7a–f) that the overall habitat quality of construction land and unused land was decreasing over the past 20 years, and these areas were severely disturbed by humans or had

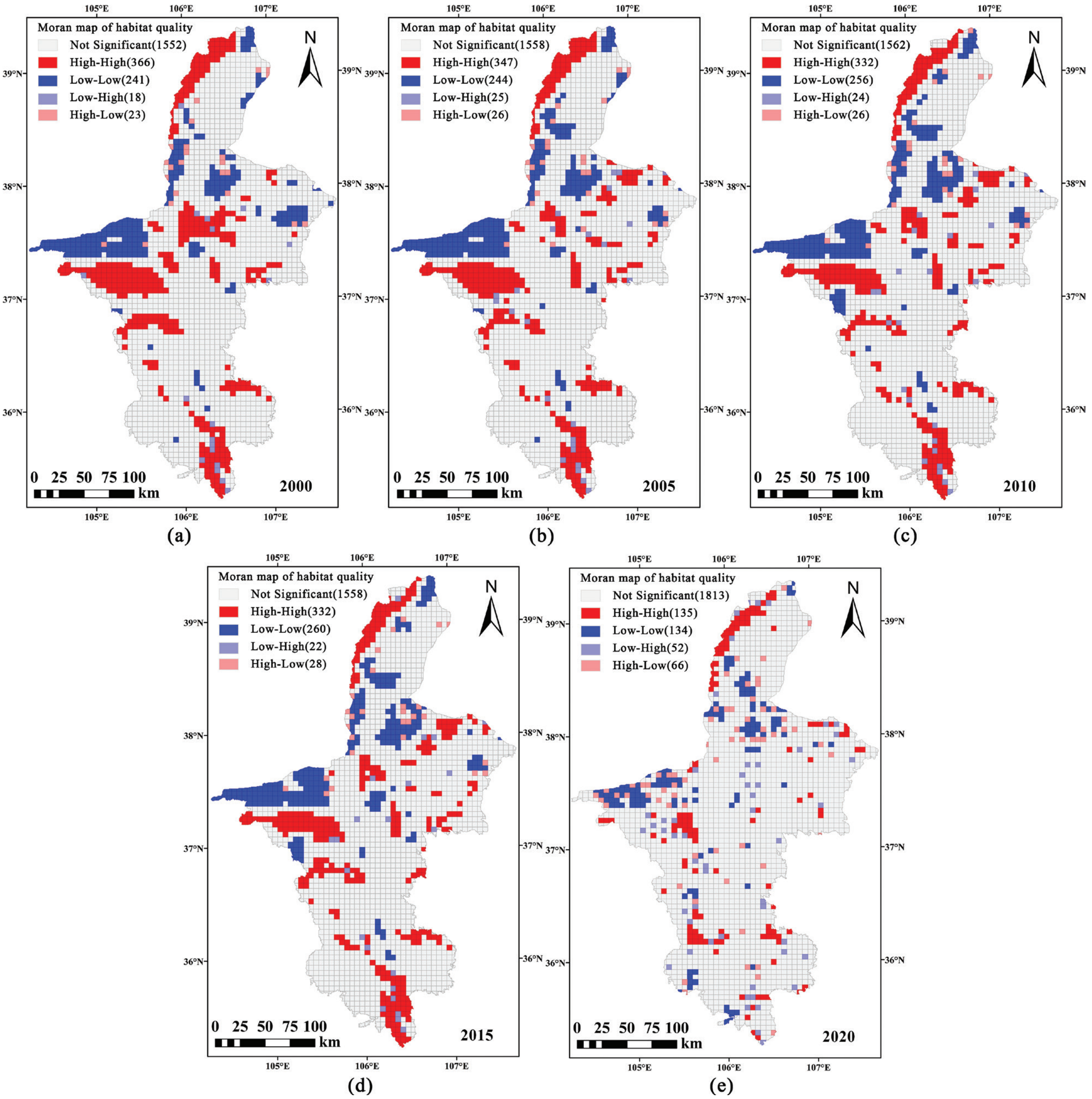
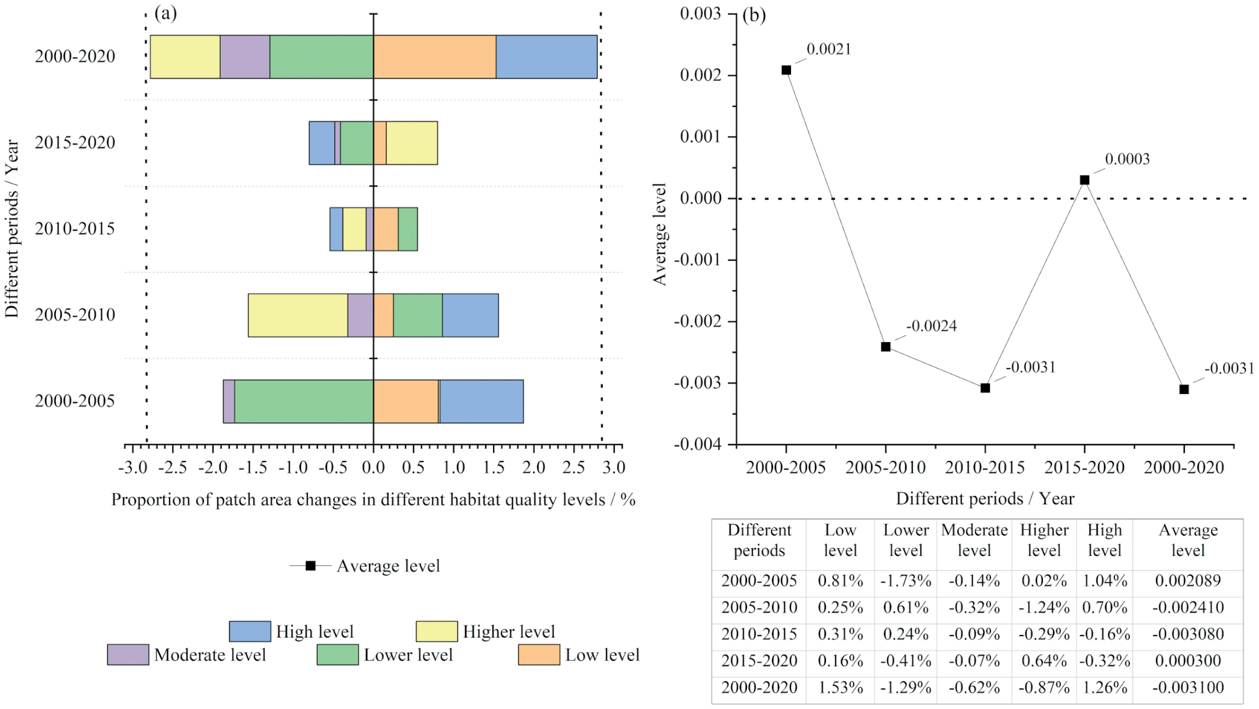


Figure 5. The results of spatial autocorrelation analysis of habitat quality in Ningxia from 2000 to 2020.

poor natural conditions. The patches with improved habitat quality were more randomly distributed, mostly in areas returned to forest and grass. By comparing the results with the land use change, we found that the changes from other types of patches to forest, grassland and water improved habitat quality, while the change from other types of patches to construction land, cultivated land and unused land obviously forced the habitat quality to decrease. When the habitat quality remained stable, the land use type remained the same over 20 years or the habitat quality remained the same after the transformation. Human activities not only cause damage to biological habitats, but also take proactive measures to control urban expansion and arable land development. The application of reasonable ecological restoration technologies and projects can promote habitat improvement. In this process, natural reserves, especially those designated for biodiversity, provide a stable and complete habitable environment, which has received significant attention and protection from humans, Integrating ecosystem functions and human needs.



Different periods	Low level	Lower level	Moderate level	Higher level	High level	Average level
2000-2005	0.81%	-1.73%	-0.14%	0.02%	1.04%	0.002089
2005-2010	0.25%	0.61%	-0.32%	-1.24%	0.70%	-0.002410
2010-2015	0.31%	0.24%	-0.09%	-0.29%	-0.16%	-0.003080
2015-2020	0.16%	-0.41%	-0.07%	0.64%	-0.32%	0.000300
2000-2020	1.53%	-1.29%	-0.62%	-0.87%	1.26%	-0.003100

Figure 6. Dynamic changes in the area proportion of patches with different levels of habitat quality in different periods in Ningxia from 2000 to 2020.

Analysis of the driving factors of habitat quality in Ningxia

Analysis of the applicability of the driving factor regression model

First, the analysis of 16 driving factors using Pearson's method found that the R^2 values for 2000–2020 (Fig. 8) were 0.327, 0.312, 0.325, 0.325, and 0.121, and the adjusted R^2 values were 0.322, 0.307, 0.320, 0.320, and 0.114, respectively ($P < 0.05$). From the sum of relative coefficients over the 20-year period (Fig. 9), factors such as AAT (-1.562), PCL (-1.302), and CLP (-0.895) had a strong negative effect on habitat quality in Ningxia, and factors such as RFI (2.158), SLP (2.142), ELE (1.604), and NPP (0.915) had a strong positive effect. Since the correlation analysis could not determine the covariance between the factors, OLS linear regression and GWR models were further adopted to explore the role of the 16 factors on habitat quality.

A comparative analysis (Table 4) revealed that the 2000–2020 GWR model AIC and AICc values were -4,365.10, -4,436.45, -4,302.96, -4,425.03, and -2,263.94 and -3,060.87, -2,542.82, -2,402.95, -2,525.42, and -1,769.53, respectively, which were significantly smaller than the OLS model AIC and AICc values. The R^2 and adjusted R^2 of the GWR model for 2000–2020 were 0.735, 0.779, 0.762, 0.777, and 0.202 and 0.692, 0.721, 0.700, 0.719, and 0.158, respectively, with a higher fitting effect and higher accuracy than the OLS model (0.70). In addition, OLS regressions of K(BP)-Prob and JB-Prob were significant ($P < 0.01$) in terms of regression coefficients and significance levels, indicating that the OLS regressions were robust and redundant in terms of the effect of each factor on habitat quality. The relative sum of OLS regression coefficients over the 20-year period indicated that habitat quality in Ningxia was strongly driven negatively

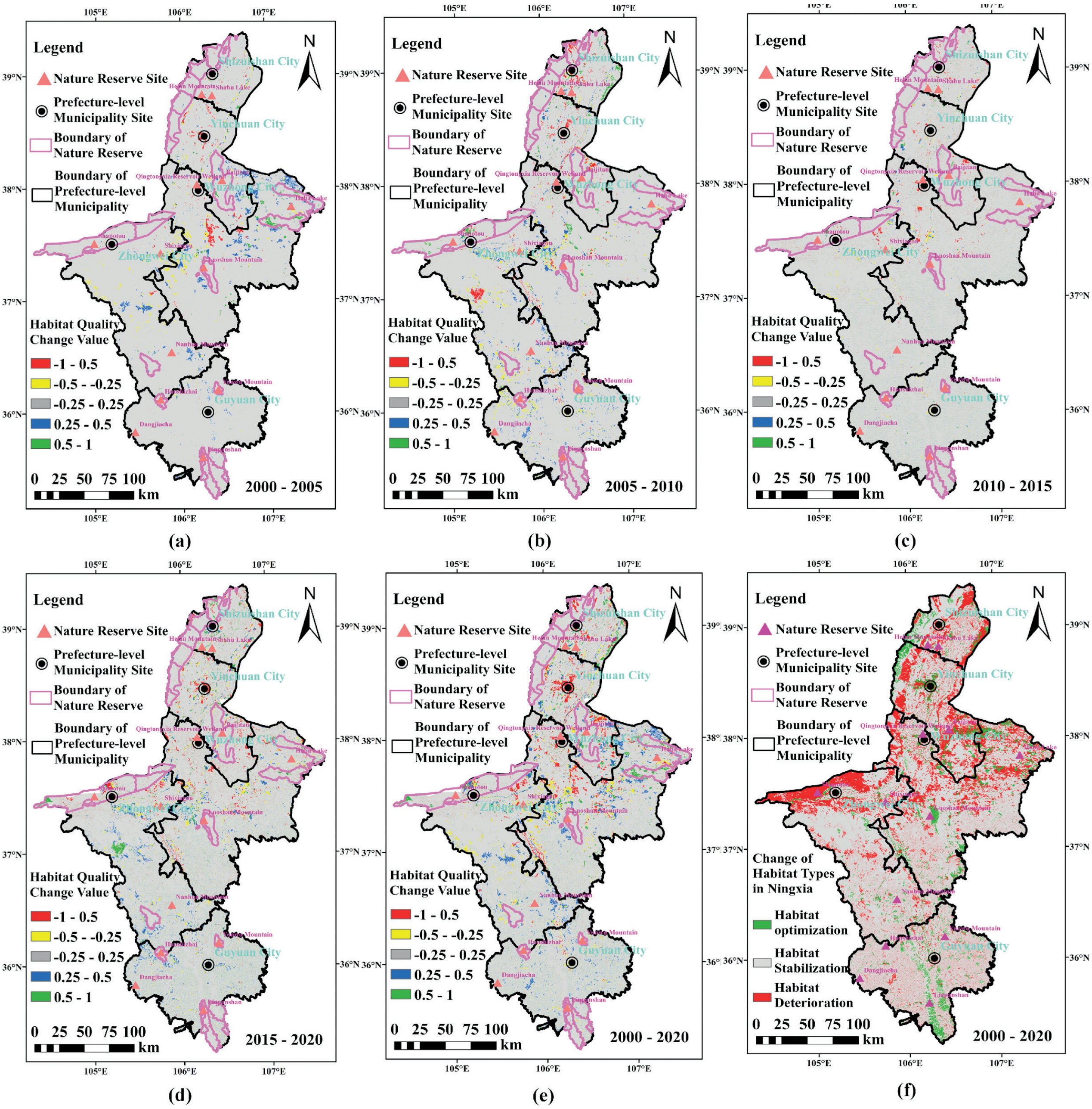


Figure 7. Spatial distribution of habitat quality and land use change in Ningxia in different periods from 2000 to 2020.

by PCL (-2.418), CLP (-0.485), and SLP (-0.083) and positively by SMC (1.215), NPP (1.109), and FVC (1.004). However, OLS regression is not applicable to the analysis of the driving effect of the factor random distribution due to the limitations of the small absolute values of the coefficients, the unmet significance level, and the significant aggregation of the residual results (Fig. 10a–e) (Global Moran's I = 0.618, 0.589, 0.591, 0.589, 0.086). Based on the results of Pearson correlation and OLS regression analysis (Fig. 11), the five driving factors with the strongest effects of multicollinearity (VIF: AAT, ELE, NPP, RFI, SLP > 7.5) were removed, the GWR model considering spatial heterogeneity was used to explore the driving effects of 11 factors including DRA, DRN, FVC, GDP, PCL, MPA, NLI, CLP, POP, RND and SMC on habitat quality in Ningxia.

Table 4. Comparison of regression test indicators between OLS and GWR models.

Year	AIC		AICc		R^2		Adj R^2	
	OLS	**GWR**	**OLS**	**GWR**	**OLS**	**GWR**	**OLS**	**GWR**
2000	-2,818.34	-4,365.10	-2,818.02	-3,060.87	0.327	0.735	0.322	0.692
2005	-2,754.45	-4,436.45	-2,754.14	-2,542.82	0.312	0.779	0.307	0.721
2010	-2,829.25	-4,302.96	-2,828.93	-2,402.95	0.325	0.762	0.320	0.700
2015	-2,832.13	-4,425.03	-2,831.81	-2,525.42	0.333	0.777	0.328	0.719
2020	-2,198.45	-2,263.94	-2,198.14	-1,769.53	0.121	0.202	0.114	0.158

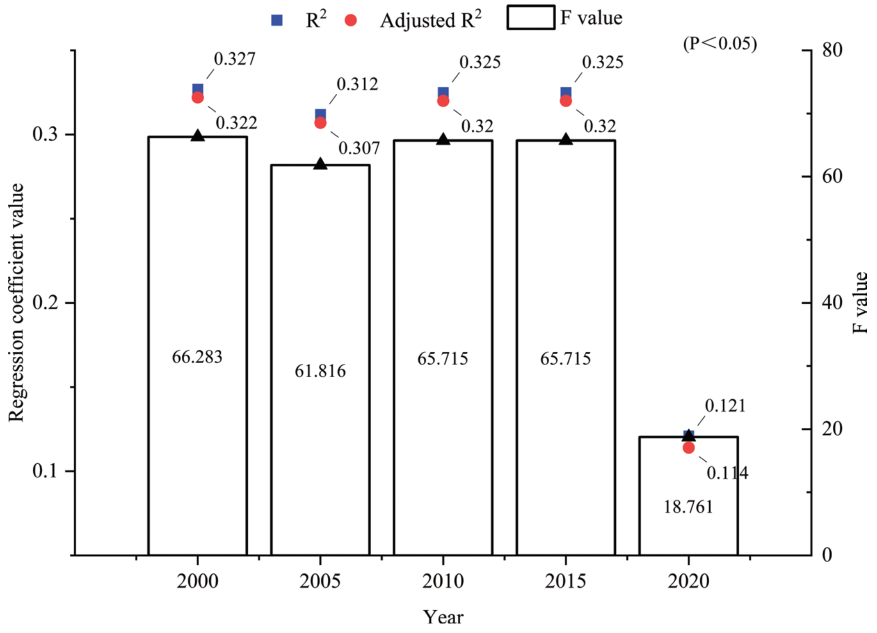


Figure 8. Pearson correlation significance results for each driving factor from 2000–2020.

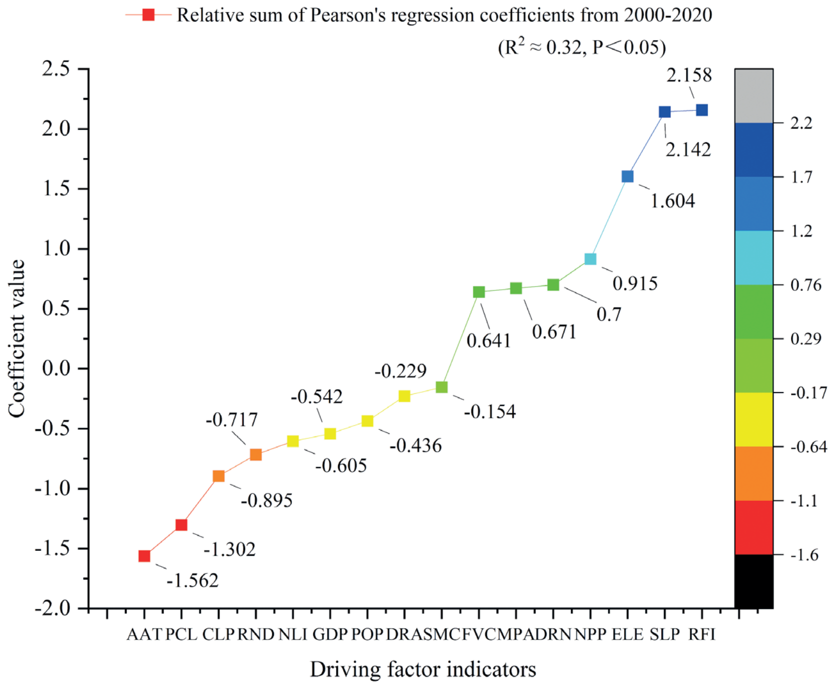


Figure 9. Relative sum of coefficients of Pearson correlation analysis of driving factors from 2000–2020.

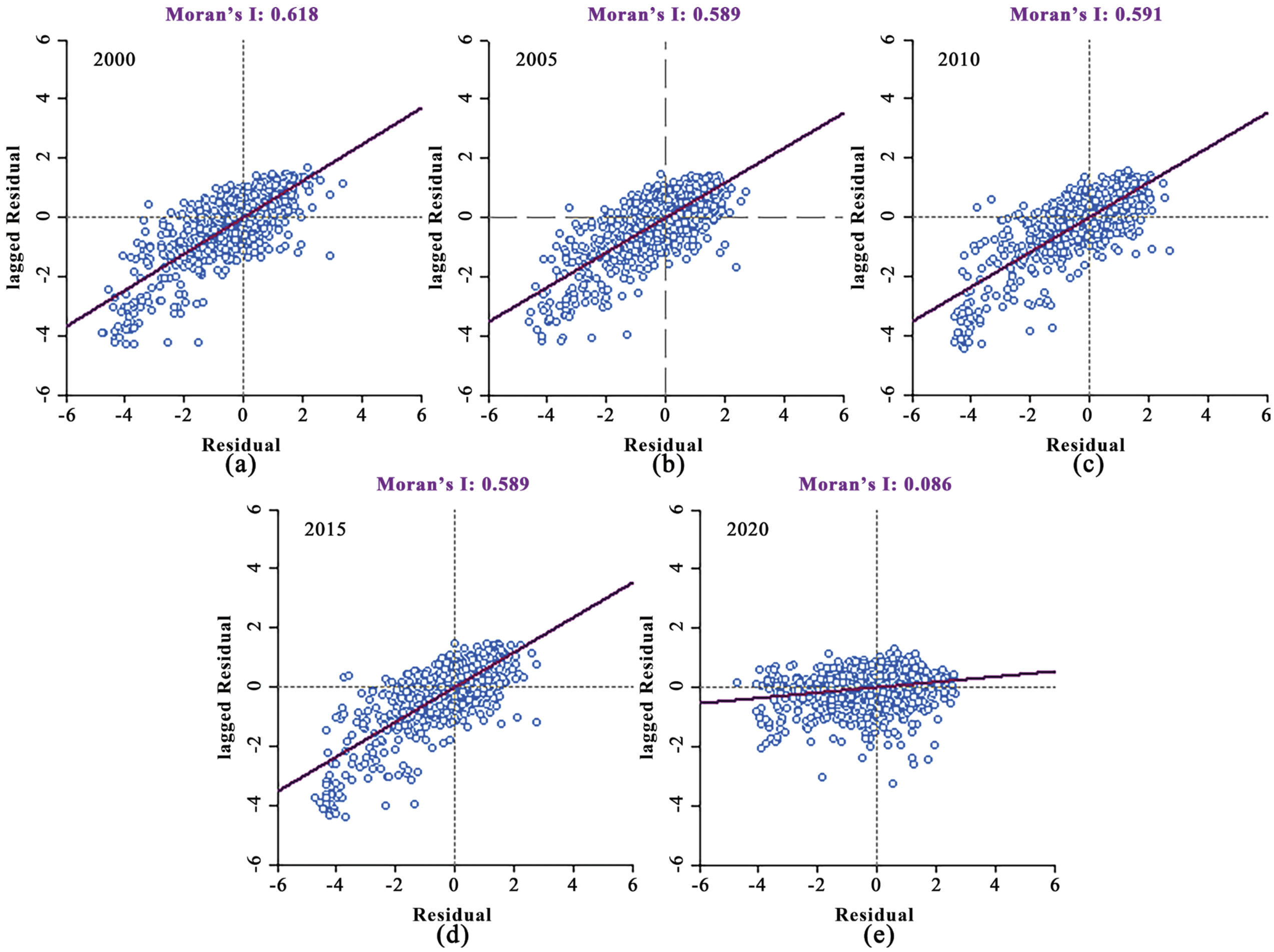


Figure 10. Results of spatial autocorrelation analysis of OLS regression residuals for habitat quality in Ningxia, 2000–2020.

Key driving factors of habitat quality in Ningxia

The results of GWR analysis showed (Table 5) that GDP (-0.001), NLI (-0.184), CLP (-1.413), and PCL (-3.260) negatively affected the habitat quality level in Ningxia, while FVC (1.765), SMC (0.619), DRA (0.023), POP (0.016), DRN (0.014), MPA (0.009), and RND (0.000) were positively correlated with habitat quality in Ningxia. Combining the absolute values of coefficients and driving effects over 20 years, we found that FVC (0.431, 0.392, 0.419, 0.256, 0.267), SMC (0.324, 0.097, 0.116, 0.089, -0.008), PCL (-0.687, -0.733, -0.687, -0.674, – 0.479), and CLP (-0.269, -0.356, -0.322, -0.327, -0.139) (Fig. 12) were the four factors with the strongest combined explanatory power for the spatial and temporal patterns and changes in habitat quality in Ningxia. Among them, FVC and SMC were positively correlated with habitat quality, PCL and CLP were negatively correlated with habitat quality, and the explanatory power of the remaining driving factors was weaker. This result indicates that higher vegetation cover and soil water content can significantly promote ecosystem habitat maintenance function. In contrast, a larger area of cultivated land and construction land within the unit grid will decrease the habitat quality.

From the local R^2 distribution map of Ningxia (Fig. 13a–e), it can be seen that from 2000 to 2005, the R^2 was higher in the Helan Mountain in northern Ningxia and in the Liupan Mountain in the south, while the R^2 was relatively smaller in the central and eastern regions, indicating that nature reserves such as the Helan Mountain and Liupan Mountain strongly affected habitat quality. Combined with the decreasing R^2 explanatory power of the key driving factors of FVC, SMC, PCL, CLP and habitat quality from 2000 to 2020, it was clear that the driving factors affecting habitat quality in Ningxia had complex nonlinear intersection characteristics during the 20 years, while the role of human disturbance became increasingly prominent.

Table 5. GWR regression coefficient values of habitat quality in Ningxia from 2000 to 2020.

Variable	2000	2005	2010	2015	2020	Relative sum of coefficients
Intercept	0.041	0.025	0.121	0.163	0.498	0.849
DRA	0.007	0.002	0.007	0.005	0.002	0.023
DRN	0.003	0.002	0.002	0.002	0.004	0.014
FVC	0.431	0.392	0.419	0.256	0.267	1.765
GDP	0.002	-0.001	0.000	-0.002	0.000	-0.001
PCL	-0.687	-0.733	-0.687	-0.674	-0.479	-3.260
MPA	0.002	0.002	0.001	0.001	0.002	0.009
NLI	-0.061	-0.054	-0.059	-0.010	0.000	-0.184
CLP	-0.269	-0.356	-0.322	-0.327	-0.139	-1.413
POP	0.001	0.004	0.001	0.010	0.000	0.016
RND	-0.002	-0.002	-0.001	0.000	0.004	0.000
SMC	0.324	0.097	0.116	0.089	-0.008	0.619

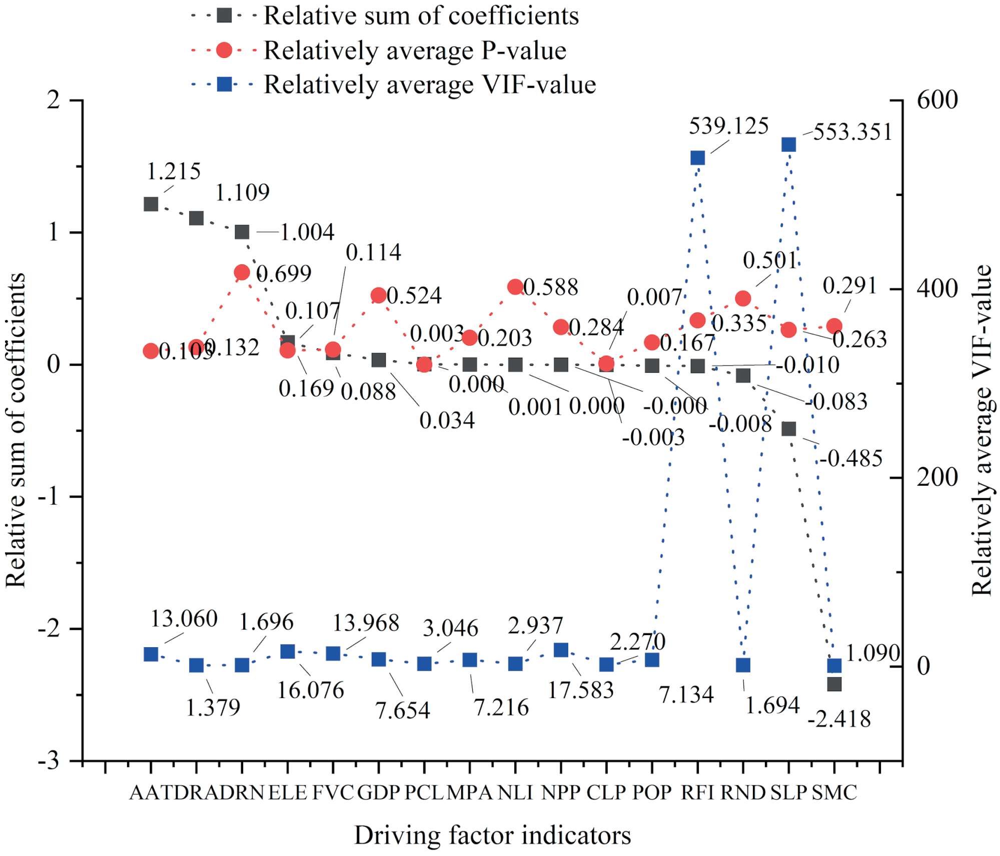


Figure 11. OLS regression coefficients, P values and VIF values of the driving factors from 2000 to 2020.

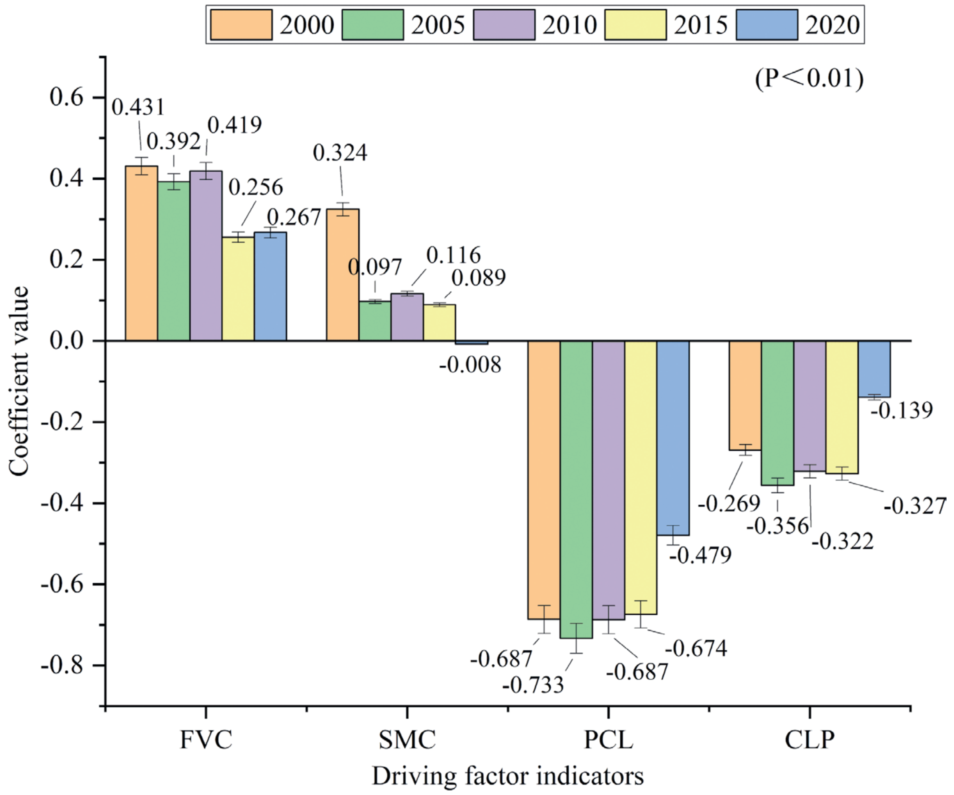


Figure 12. GWR regression coefficients of the main driving factors of habitat quality in Ningxia, 2000–2020.

Selecting the spatial distribution of the GWR regression coefficients of the main driving factors of habitat quality in 2020 as an example, spatially (Fig. 14a–d), the positive regression coefficients of FVC in Ningxia from 2000 to 2020 were concentrated in the central and northern Helan Mountain and the southern Liupan Mountain. These areas had a relatively high level of habitat quality, and the positive impact of FVC on habitat quality was very significant. The areas with positive SMC regression coefficients mainly included the central cultivated areas with good irrigation conditions and sufficient soil moisture, and a higher SMC had a stronger effect on habitat quality in these areas. The areas with negative PCL regression coefficients were mainly concentrated in the northern part of Ningxia with a high density of construction land and high developed urbanization level, and the habitat quality in these areas was generally low. The areas with negative CLP regression coefficients were mainly located in cultivated land in the yellow irrigation area, which retained part of the habitat maintenance function and had a moderate level of habitat quality. The spatial distribution of the regression coefficients of FVC, SMC, PCL, CLP and GWR of habitat quality had a small threshold of change during the 20 years, and the driving effects spread from the most accurate and close areas of their respective regression effects to the surrounding areas and gradually weakened, together with the interactions of other influencing factors, thus driving the distribution and evolution of habitat quality in Ningxia in the past 20 years. The driving effect of these key driving factors is particularly evident in the nature reserves of Ningxia, and the changes in key driving factors of habitat quality in nature reserves will largely drive changes in biodiversity, reflecting the importance of nature reserve delineation for biodiversity conservation under conditions of avoiding more human activities and sufficient ecological factors.

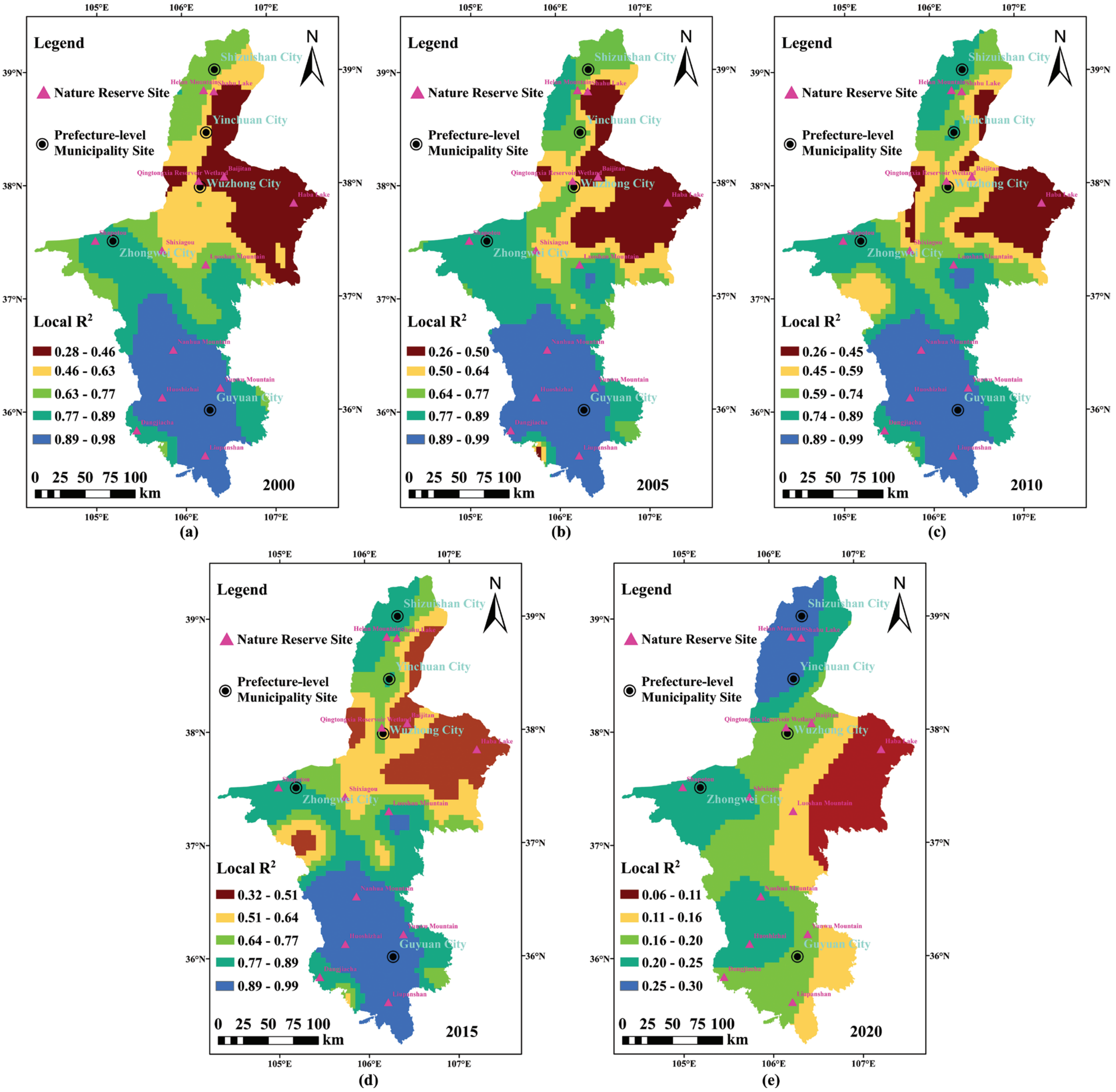
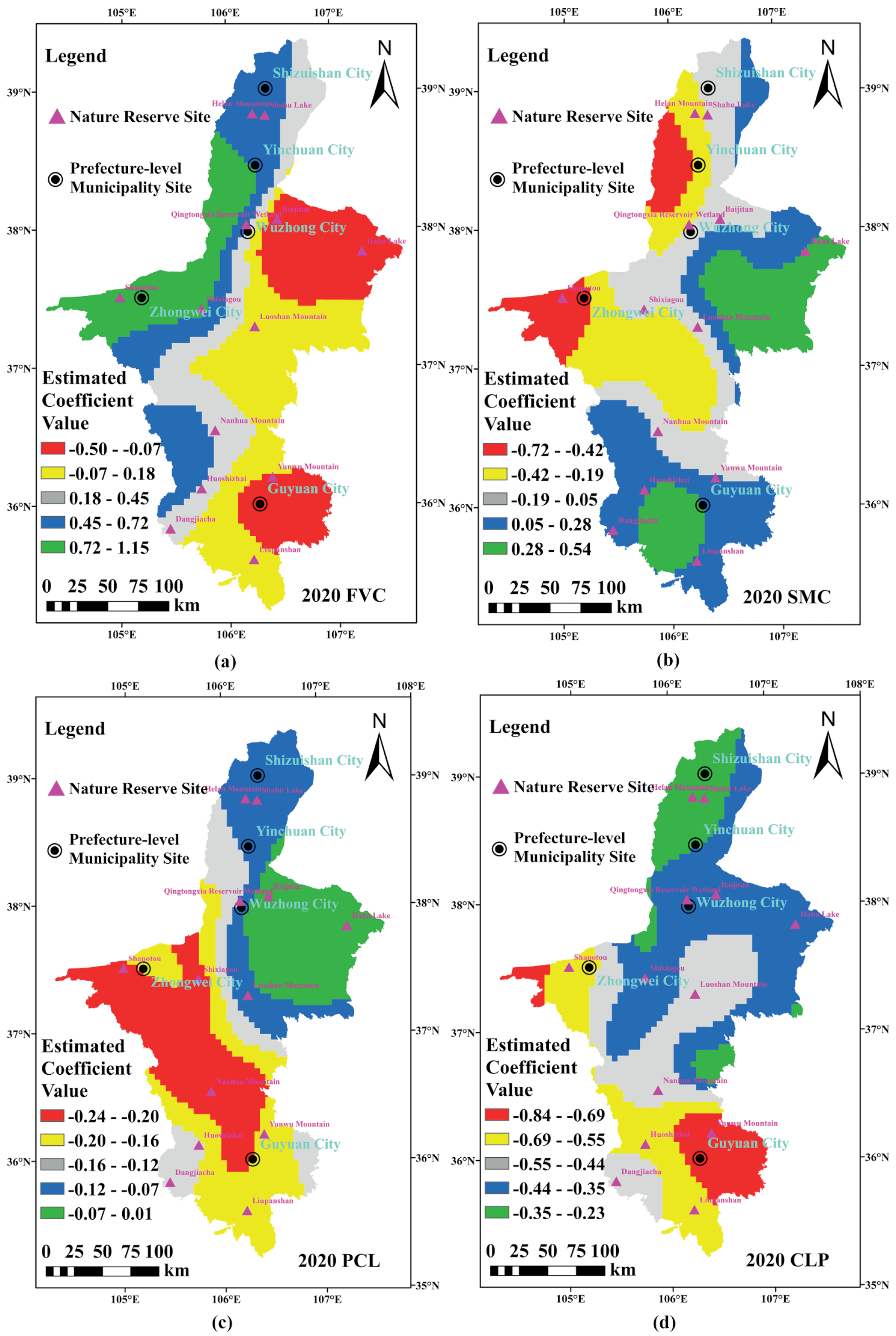


Figure 13. Local R^2 distribution of GWR regressions of the driving factors of habitat quality in Ningxia from 2000 to 2020.

Discussion

Habitat quality and biodiversity distribution in Ningxia

We conclude that the distribution and evolution of habitat quality in Ningxia were mainly driven by fractional vegetation cover, soil moisture content, cultivated land expansion, and construction land expansion, where high vegetation cover and soil moisture content were suitable for biological habitats; in contrast, cultivated land and construction land expansion reduced habitat suitability. The habitat quality of forestland, grassland, water area and some cultivated land in Ningxia was high, and these patches were in good condition as ecological source land and were far from human activity areas, so they were less disturbed by resource development and utilization. The habitat quality of areas such as urban land, which had a high intensity of human activities, was obviously extremely low. The



(a) (b) (c) (d)

Figure 14. Distribution of GWR regression coefficients for the main driving factors of habitat quality in Ningxia from 2000 to 2020.

relationships between soil moisture content, some cultivated land, pasture land and habitat quality were more specific. On the one hand, higher soil water content in the natural state means lush vegetation, and the expansion of cultivated land is not conducive to habitat maintenance. However, on the other hand, due to the construction of artificial cultivated land and artificial wetland, the investment of human and material resources, green funds and ecological technology can maintain the fragile habitat to a certain extent. Therefore, there is uncertainty regarding the role of factors such as cultivated land and soil water content on habitat quality. In response to the weak natural foundation, from 2000 to 2020, Ningxia continuously improved its vegetation cover through the project of returning farmland to forest and grass, and the level of habitat quality in nature reserves such as Liupanshan and Luoshan increased significantly. Due to frequent industrial and agricultural activities such as food production and mineral extraction, the habitat quality fluctuated in some areas of Ningxia over the course of 20 years, and the results showed a decrease in habitat function. In conclusion, urban and cultivated land expansion are the most critical factors reducing habitat suitability in Ningxia, and protecting and utilizing grassland, vegetation, wetland and other ecosystems can effectively improve habitat suitability in Ningxia.

In addition, although other factors, such as topographic relief, had insufficient explanatory power for habitat quality in Ningxia, related studies have shown that rainfall, slope, elevation, and temperature are important conditions that influence the distribution and ecological niche of organisms within small-scale habitats (Rahbek et al. 2019; Chase et al. 2020). Combining field surveys and indoor ecological experiments to obtain detailed habitat data for all or key species in a region is essential in driving factors studies of habitat quality (Forister et al. 2015). Therefore, in specific habitats and typical ecosystems such as grasslands, deserts, and forests, we need more refined work to obtain the total number of various ecological factors and their thresholds required for birth, death, migration, dispersal, and reproduction during the life cycles of key species in the region to determine the causes of habitat quality heterogeneity within small and medium scales (Fahrig 2017). Additionally, these elements are the focus of ecological niche, biodiversity, and ecosystem conservation studies (Xiao et al. 2004).

GWR modelling can fully reflect the spatial non-smoothness of the region and select the optimal spatial weights in combination with spatial heterogeneity; the results have higher accuracy and credibility than Pearson correlation analysis and OLS regression for exploring the role of driving factors at large spatial and temporal scales. Although, the results are consistent with the trend of habitat quality changes in Ningxia over the past 20 years. However, some studies have shown that GWR is essentially a one-dimensional linear regression with parameters that fail to consider multivariate and correlational settings (Fotheringham and Oshan 2016; Fotheringham et al. 2017), while the occurrence of some human irresistible factors, causing the Moran Index and the R^2 results after regression in 2020 in this study were less satisfactory. To improve the exploration method of driving factors, future research can utilize multivariate nonlinear regression, image-by-image correlation analysis, and artificial neural network models (Fotheringham et al. 2015; Li and Fotheringham 2020). Meanwhile, the sampling accuracy of the unit grid was improved, which eventually made the results match the actual influence of driving factors (McGarigal et al. 2016).

Habitat maintenance and ecosystem protection in ecologically fragile areas

Ningxia is a typical ecologically fragile area with relatively poor natural foundations, and the habitat quality is at an intermediate level with a wide scope for improvement. This study explored the driving effects of habitat quality in Ningxia based on 16 physical geographic, economic and social factors and found that habitat quality tended to be higher in areas with higher vegetation cover and lower in patches with a high proportion of construction land and cultivated land. The results demonstrate that for ecologically fragile areas with similar characteristics to Ningxia's ecological environment, enhancing vegetation cover can effectively improve habitat structure and function. For ecologically fragile areas, poor conditions such as low precipitation, loose soils, and low biodiversity, coupled with the frequent use of natural resources due to economic development, have led to further anthropogenic damage to the already fragile habitats. Ecologically fragile areas often lack biologically beneficial ecological factors, and due to the harsh natural conditions and prominent human-land conflicts, humans must sacrifice habitat quality in exchange for improved well-being, thus leading to a vicious circle of economic and social development and ecosystem decline in ecologically fragile areas (Tong and Long 2003). Therefore, it is of great practical significance and scientific value to find a way to maintain habitat quality, ecological environmental protection and sustainable and healthy economic and social development in both directions in ecologically fragile areas (Len and Liu 1999; Aksoy and Bayram Arli 2020). Of course, achieving a synergistic promotion of natural systems and economic society depends on rich biodiversity and on the frequency and cost of investment of time, money, technology, and methods by government, society, the public, and researchers and conservation agencies.

In this study, we considered 16 factors that are critical for influencing biodiversity levels in ecologically fragile areas, and these factors fully reflected the natural climatic conditions and human activity disturbances in ecologically fragile areas. The results can be used as a reference for the conservation of ecosystems in other ecologically fragile areas internationally. In the future, studies on biodiversity conservation and habitat quality in ecologically fragile areas should combine model simulations and biodiversity field surveys to summarize the distribution and change characteristics of habitat quality over a long time series. It is also important to understand the habitats of specific species from the key areas of habitat quality maintenance, such as nature reserves and ecological functional areas, which will give more full play to the natural stability (resistance and resilience) and human maintenance of the habitat systems in ecologically fragile areas (Geldmann et al. 2013; Gray et al. 2016). For example, Ningxia has designated a priority area for biodiversity conservation (24,409.7 km^2), which accounts for 47.1% of the national territory, effectively covering the typical ecosystems, biodiversity hotspots, and important ecological function areas within the territory and will play a leading role in long-term biodiversity conservation. How to build ecological barriers, ecological corridors and ecological nodes using nature reserves, nature protection areas and other ecosystems with strong habitat suitability to protect the landscape diversity and functional integrity of ecologically fragile areas for biological survival and reproduction will be important for future policy-makers and academic research. Our research supports that focusing on important natural conditions such as vegetation and precipitation in ecologically fragile areas and reducing excessive resource claims will benefit the natural vitality and well-being of human in ecologically sensitive and fragile areas.

Global changes such as climate change and cultivated land expansion have increased the instability of ecosystems in ecologically fragile areas (Garcia et al. 2014; Hautier et al. 2015; Venter et al. 2016; Newbold 2018), leading to a deterioration in biodiversity levels. Studies on global biodiversity suggest (Waldron et al. 2013; Gray et al. 2016; Lepczyk et al. 2017; Wintle et al. 2019; Maxwell et al. 2020; Xu et al. 2021) that the main measures that can be applied to biodiversity conservation in ecologically fragile areas are (1) the use of small patches to protect the minimum suitable habitat for very small populations and in this way consolidate the basis of biodiversity; (2) strengthening the intensification of cultivated land, the construction of nature reserves and the investment of green funds at the regional scale and focusing on the creation of good biodiversity landscapes in urban spaces; and (3) relying on international cooperation such as the Convention on Biological Diversity, in which we will link biodiversity and ecosystem services and improve human well-being to enhance genetic, species, and ecosystem diversity. Under the above opportunities, the identification of habitat suitability and ecological factors that cause habitat changes, the introduction of nature-based solutions to biodiversity dilemmas, and the realization of the systematic assessment, monitoring and management of biodiversity and ecosystems in multiple spatial and temporal sequences will further contribute to the achievement of the UN CBD goals.

Summary

This study evaluated the habitat quality of Ningxia from 2000 to 2020 based on the InVEST model, analysed the spatial and temporal patterns and changes during the 20-year period, and explored the role of driving factors on habitat quality using correlation analysis, the OLS model, and the GWR model in combination with 16 physical-geo-socioeconomic factors. The main results of this study are as follows:

(1) From 2000 to 2020, the average habitat quality in Ningxia was 0.576,428, 0.578,517, 0.576,102, 0.573,025, and 0.573,325, respectively, which increased and then decreased over 20 years, with a small overall decrease. The high-level habitat quality patches in Ningxia were mainly distributed in areas with high vegetation cover, while the low-level habitat quality patches were mainly distributed in areas subject to more frequent human activities, such as construction land and cultivated land. The habitat quality level in Ningxia had significant high-high value aggregation and low-low value aggregation characteristics, which basically overlapped with the distribution of high-level habitat quality and low-level habitat quality, respectively, while the area of patches with low-high value and high-low value aggregation characteristics was small.

(2) From 2000 to 2020, the regression results R^2 of 16 driving factors and the GWR model of habitat quality in Ningxia were 0.691,66, 0.721,169, 0.699,633, 0.718,556, and 0.158,344, respectively. By comparing R^2, AIC, AICc and other test indicators, we found that the GWR regression model in this study was able to fit the driving effects of different factors on habitat quality in Ningxia at different spatial scales. The results showed that FVC, SMC, PCL and CLP were the most important driving factors affecting the spatial distribution and evolutionary characteristics of habitat quality in Ningxia. Among them, high vegetation cover and soil water content positively promoted habitat suitability, and construction land and cultivated land expansion negatively affected habitat suitability.

(3) The integrity of habitats can ensure the ecological factors required for biological survival, while the multifunctionality of landscapes enriches the diversity of biological evolution. In natural ecosystems and socio-economic systems, the positive or negative role played by human activities is increasingly becoming an important driving factor for the quality of habitats. Whether policy formulation and public participation prioritize, coordinate, or lag the development of natural biodiversity, it determines the indispensable harmony between human and nature in the region. By integrating the distribution, evolution and driving factors of habitat quality in Ningxia, we found that for the conservation of biodiversity and habitat quality in ecologically fragile areas, it is first necessary to maintain the integrity of the original natural habitats as much as possible and increase the multifunctionality of the landscape so that organisms can survive, reproduce and spread smoothly. Second, it is necessary to minimize human interference with the natural landscape and to carry out human activities such as urban construction and cultivated land production in an appropriate and reasonable manner to avoid habitat fragmentation and improve the connectivity of habitat patches. Finally, it is necessary to protect already fragile natural habitats by delineating nature reserves and to develop suitable ecological environmental protection policies for targeted protection and restoration of habitat-sensitive and fragile areas.

Acknowledgments

We appreciate the constructive comments and suggestions from the reviewers that helped improve the quality of this manuscript.

Additional information

Conflict of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Ethical statement

No ethical statement was reported.

Funding

This research was funded by the National Natural Science Foundation of China (Grant Number 41871196).

Author contributions

H.H. constructed the concept and overall framework of the research and provided the necessary funding sources and other funding for the research; D.W. collected and sorted out the research data and designed and visualized the charting of the research results; L.S. and H.L. helped D.W., to skillfully use relevant software and charts; D.W., has written and reviewed the first draft, and Y.L. has given great creativity in polishing and improving the article; H.H., L.S., H.L., and Y.L. have all made great contributions to the writing, revision, editing of articles and the management, investigation and implementation of projects. All authors have read and agreed to the published version of the manuscript.

Author ORCIDs

Ding Wang https://orcid.org/0000-0003-1748-8383
Haiguang Hao https://orcid.org/0000-0003-3726-7254
Hao Liu https://orcid.org/0000-0002-1495-2120
Lihui Sun https://orcid.org/0000-0001-6380-8232
Yuyang Li https://orcid.org/0000-0001-6352-271X

Data availability

Not applicable.

References

Aksoy F, Bayram Arli N (2020) Evaluation of sustainable happiness with sustainable development goals: Structural equation model approach. Sustainable Development 28(1): 385–392. https://doi.org/10.1002/sd.1985

Bai LM, Feng XH, Sun RF, Gao H (2020) Spatial and temporal responses of habitat quality to urbanization: A case study of Changchun City, Jilin Province, China. Chinese Journal of Applied Ecology 31(4): 1267–1277. https://doi.org/10.13287/j.1001-9332.202004.012

Bao YB (2022) Habitat assessment and ecological corridor construction of priority areas for biodiversity conservation in Ningxia based on InVEST model. Journal of Ningxia University 43(03): 318–324. https://kns.cnki.net/kns8/defaultresult/index [Natural Science Edition]

Bao YB, Liu K, Li T, Hu S (2015) Effects of land use change on habitat based on invest model – Taking yellow river wetland nature reserve in Shaanxi province as an example. Ganhanqu Yanjiu 32(03): 622–629. https://doi.org/10.13866/j.azr.2015.03.29

Berger AR, Hodge RA (1998) Natural change in the environment: A challenge to the pressure-state-response concept. Social Indicators Research 44(2): 255–265. https://doi.org/10.1023/A:1006888532080

Brunsdon C, Fotheringham AS, Charlton ME (1996) Geographically weighted regression: A method for exploring spatial nonstationarity. Geographical Analysis 28(4): 281–298. https://doi.org/10.1111/j.1538-4632.1996.tb00936.x

Celina A, Jens-Christian S, German T, Francesc B, Unai P (2022) Wildness and habitat quality drive spatial patterns of urban biodiversity. Landscape and Urban Planning 228: e104570. https://doi.org/10.1016/j.landurbplan.2022.104570

Chase JM, Blowes SA, Knight TM, Gerstner K, May F (2020) Ecosystem decay exacerbates biodiversity loss with habitat loss. Nature 584(7820): 238–243. https://doi.org/10.1038/s41586-020-2531-2

Chen Y, Qiao F, Jiang L (2016) Effects of land use pattern change on regional scale habitat quality based on InVEST model – a case study in Beijing. Acta Scientiarum Naturalium Universitatis Pekinensis (3): 553–562. https://doi.org/10.13209/j.0479-8023.2016.057

Chisholm C, Lindo Z, Gonzalez A (2011) Metacommunity diversity depends on connectivity and patch arrangement in heterogeneous habitat networks. Ecography 34(3): 415–424. https://doi.org/10.1111/j.1600-0587.2010.06588.x

Congdon J (1974) Effect of habitat quality on distributions of three sympatric species of desert rodents. Journal of Mammalogy 55(3): 659–662. https://doi.org/10.2307/1379557

Crooks KR, Burdett CL, Theobald DM, King SRB, Di Marco M, Rondinini C, Boitani L (2017) Quantification of habitat fragmentation reveals extinction risk in terrestrial mammals. Proceedings of the National Academy of Sciences of the United States of America 114(29): 7635–7640. https://doi.org/10.1073/pnas.1705769114

Dallimer M, Irvine KN, Skinner AMJ, Davies ZG, Rouquette JR, Maltby LL, Warren PH, Armsworth PR, Gaston KJ (2012) Biodiversity and the feel-good factor: Understanding associations between self-reported human well-being and species richness. Bioscience 62(1): 47–55. https://doi.org/10.1525/bio.2012.62.1.9

Duan H, Yu X (2022) Land-use change, habitat connectivity, and conservation gaps: A case study of shorebird species in the Yellow River Delta of China using the InVEST model and network analysis. Remote Sensing 14(24): e6191. https://doi.org/10.3390/rs14246191

Dunning JB, Danielson BJ, Ronald-Pulliam H (1992) Ecological processes that affect populations in complex landscapes. Oikos 65(1): 169–175. https://doi.org/10.2307/3544901

Fahrig L (2017) Ecological responses to habitat fragmentation per se. Annual Review of Ecology, Evolution, and Systematics 48(1): 1–23. https://doi.org/10.1146/annurev-ecolsys-110316-022612

Forister ML, Novotny V, Panorska AK, Baje L, Basset Y, Butterill PT, Cizek L, Coley PD, Dem F, Diniz IR, Drozd P, Fox M, Glassmire AE, Hazen R, Hrcek J, Jahner JP, Kaman O, Kozubowski TJ, Kursar TA, Lewis OT, Lill J, Marquis RJ, Miller SE, Morais HC, Murakami M, Nickel H, Pardikes NA, Ricklefs RE, Singer MS, Smilanich AM, Stireman JO, Villamarín-Cortez S, Vodka S, Volf M, Wagner DL, Walla T, Weiblen GD, Dyer LA (2015) The global distribution of diet breadth in insect herbivores. Proceedings of the National Academy of Sciences of the United States of America 112(2): 442–447. https://doi.org/10.1073/pnas.1423042112

Fotheringham AS, Oshan TM (2016) Geographically weighted regression and multicollinearity: Dispelling the myth. Journal of Geographical Systems 18(4): 303–329. https://doi.org/10.1007/s10109-016-0239-5

Fotheringham AS, Crespo R, Yao J (2015) Geographical and Temporal Weighted Regression (GTWR). Geographical Analysis 47(4): 431–452. https://doi.org/10.1111/gean.12071

Fotheringham AS, Yang W, Kang W (2017) Multiscale Geographically Weighted Regression (MGWR). Annals of the Association of American Geographers 107(6): 1247–1265. https://doi.org/10.1080/24694452.2017.1352480

Garcia RA, Cabeza M, Rahbek C, Araújo MB (2014) Multiple dimensions of climate change and their implications for biodiversity. Science 344(6183): e1247579. https://doi.org/10.1126/science.1247579

Geldmann J, Barnes M, Coad L, Craigie ID, Hockings M, Burgess ND (2013) Effectiveness of terrestrial protected areas in reducing habitat loss and population declines. Biological Conservation 161: 230–238. https://doi.org/10.1016/j.biocon.2013.02.018

Goertz JW (1964) The influence of habitat quality upon density of Cotton Rat populations. Ecological Monographs 34(4): 359–381. https://doi.org/10.2307/2937068

Gomes E, Inácio M, Bogdzevič K, Kalinauskas M, Karnauskaitė D, Pereira P (2021) Future scenarios impact on land use change and habitat quality in Lithuania. Environmental Research 197: e111101. https://doi.org/10.1016/j.envres.2021.111101

Gray CL, Hill SLL, Newbold T, Hudson LN, Börger L, Contu S, Hoskins AJ, Ferrier S, Purvis A, Scharlemann JPW (2016) Local biodiversity is higher inside than outside terrestrial protected areas worldwide. Nature Communications 7(1): e12306. https://doi.org/10.1038/ncomms12306

Haddad N, Brudvig L, Clobert J, Davies K, Gonzalez A, Holt R, Lovejoy T, Sexton J, Austin M, Collins CD, Cook W, Damschen E, Ewers R, Foster B, Jenkins C, King AJ, Laurance W, Levey D, Margules C, Melbourne B, Nicholls AO, Orrock J, Song D, Townshend J (2015) Habitat fragmentation and its lasting impact on earth's ecosystems. Science Advances 1(2): e1500052. https://doi.org/10.1126/sciadv.1500052

Hale R, Swearer SE (2016) Ecological traps: Current evidence and future directions. Proceedings of The Royal Society B Biological Sciences 283(1824): e20152647. https://doi.org/10.1098/rspb.2015.2647

Hao Y, Zhang N, Du YJ, Wang YH, Zheng YD, Zhang CC (2019) Construction of ecological security pattern based on habitat quality in Tang County. Chinese Journal of Applied Ecology 30(03): 1015–1024. https://doi.org/10.13287/j.1001-9332.201903.007

Harper D, Everard M (1998) Why should the habitat-level approach underpin holistic river survey and management? Aquatic Conservation 8(4): 395–413. https://doi.org/10.1002/(SICI)1099-0755(199807/08)8:4<395::AID-AQC297>3.0.CO;2-X

Hautier Y, Tilman D, Isbell F, Seabloom EW, Borer ET, Reich PB (2015) Anthropogenic environmental changes affect ecosystem stability via biodiversity. Science 348(6232): 336–340. https://doi.org/10.1126/science.aaa1788

Van Horne B (1983) Density as a misleading indicator of habitat quality. The Journal of Wildlife Management 47(4): 893–901. https://doi.org/10.2307/3808148

Hu J, Zhang J, Li Y (2022) Exploring the spatial and temporal driving mechanisms of landscape patterns on habitat quality in a city undergoing rapid urbanization based on GTWR and MGWR: The case of Nanjing, China. Ecological Indicators 143: e109333. https://doi.org/10.1016/j.ecolind.2022.109333

Janzen DH (1970) Herbivores and the number of tree species in Tropical Forests. American Naturalist 104(940): 501–528. https://doi.org/10.1086/282687

Kalacska M, Arroyo-Mora JP, Lucanus O, Kishe-Machumu MA (2017) Land cover, land use, and climate change impacts on Endemic Cichlid Habitats in Northern Tanzania. Remote Sensing 9(6): e623. https://doi.org/10.3390/rs9060623

Kareiva P, Tallis H, Ricketts TH, Daily GC, Polasky S (2011) Natural Capital: Theory & Practice of Mapping Ecosystem Services. Ed, Oxford biology. Oxford University Press, Oxford [England], New York. https://doi.org/10.1093/acprof:oso/9780199588992.001.0001

Len SY, Liu YH (1999) The framework design of sustainable development index system for fragile ecological regions in China. China Population, Resources and Environment 02: 42–47.

Lepczyk CA, Aronson MF, Evans KL, Goddard MA, Lerman SB, MacIvor SJ (2017) Biodiversity in the City: Fundamental Questions for Understanding the Ecology of Urban Green Spaces for Biodiversity Conservation. Bioscience 67(9): 799–807. https://doi.org/10.1093/biosci/bix079

Li Z, Fotheringham AS (2020) Computational improvements to Multi-Scale Geographically Weighted Regression. International Journal of Geographical Information Science 34(7): 1378–1397. https://doi.org/10.1080/13658816.2020.1720692

Maxwell SL, Cazalis V, Dudley N, Hoffmann M, Rodrigues ASL, Stolton S, Visconti P, Woodley S, Kingston N, Lewis E, Maron M, Strassburg BBN, Wenger A, Jonas HD, Venter O, Watson JEM (2020) Area-based conservation in the twenty-first century. Nature 586(7828): 217–227. https://doi.org/10.1038/s41586-020-2773-z

McGarigal K, Wan HY, Zeller KA, Timm BC, Cushman SA (2016) Multi-scale habitat selection modeling: A review and outlook. Landscape Ecology 31(6): 1161–1175. https://doi.org/10.1007/s10980-016-0374-x

Moëzzi F, Poorbagher H, Eagderi S, Feghhi J, Dormann CF, Nergi SK, Amiri K (2022) Modelling habitat preference of Caspian Kutum, *Rutilus kutum*, using non-linear habitat suitability indices and generalized additive models. Regional Studies in Marine Science 56: e102715. https://doi.org/10.1016/j.rsma.2022.102715

Nagendra H, Lucas R, Honrado JP, Jongman RHG, Tarantino C, Adamo M, Mairot P (2013) Remote Sensing for conservation monitoring: Assessing protected areas, habitat extent, habitat condition, species diversity, and threats. Ecological Indicators 33: 45–59. https://doi.org/10.1016/j.ecolind.2012.09.014

Newbold T (2018) Future effects of climate and land-use change on terrestrial vertebrate community diversity under different scenarios. Proceedings of the Royal Society B, Biological Sciences 285(1881): e20180792. https://doi.org/10.1098/rspb.2018.0792

Peggy RM, Tridoyo K, Luky A, Achmad F (2021) Valuing habitat quality for managing mangrove ecosystem services in coastal Tangerang District, Indonesia. Marine Policy 133: e104747. https://doi.org/10.1016/j.marpol.2021.104747

Prasad G, Ramesh MV (2019) Spatio-temporal analysis of land use/land cover changes in an ecologically fragile area – Alappuzha District, Southern Kerala, India. Natural Resources Research 28(S1): 31–42. https://doi.org/10.1007/s11053-018-9419-y

Qin WZ (2007) The basic theoretics and application research on geographically weighted regression. PhD Thesis, Tongji University, Shanghai, China. https://kns.cnki.net/KCMS/detail/detail.aspx?dbname=CDFD0911&filename=2007222843.nh

Radosavljevic A, Anderson RP (2014) Making better maxent models of species distributions: Complexity, overfitting and evaluation. Journal of Biogeography 41(4): 629–643. https://doi.org/10.1111/jbi.12227

Rahbek C, Borregaard MK, Colwell RK, Dalsgaard B, Holt BG, Morueta-Holme N, Nogues-Bravo D, Whittaker RJ, Fjeldså J (2019) Humboldt's enigma: What causes global patterns of mountain biodiversity? Science 365(6458): 1108–1113. https://doi.org/10.1126/science.aax0149

Raimundo Lopes ND, Li TX, Qian DY, Matomela N, Sá RM (2022) Factors influencing coastal land cover change and corresponding impact on habitat quality in the North-Western Coastline of Guinea-Bissau (NC-GB). Ocean & Coastal Management 224: e106181. https://doi.org/10.1016/j.ocecoaman.2022.106181

Ramachandra TV, Bharath S, Vinay SA (2019) Visualisation of impacts due to the proposed developmental projects in the ecologically fragile regions- Kodagu district, Karnataka. Progress in Disaster Science 3: e100038. https://doi.org/10.1016/j.pdisas.2019.100038

Ren J, Zhao XY, Xu XC, Ma PY, Du YX (2022) Spatial-temporal evolution, tradeoffs and synergies of ecosystem services in the middle Yellow River. Journal of Earth Environment 13(4): 477–490. https://doi.org/10.7515/JEE222019

Riedler B, Lang SA (2018) A spatially explicit patch model of habitat quality, integrating spatio-structural indicators. Ecological Indicators 94: 128–141. https://doi.org/10.1016/j.ecolind.2017.04.027

Roelof B, Joe R, Irit A, Les K (2015) The multiscale integrated model of ecosystem services (MIMES): Simulating the interactions of coupled human and natural systems. Ecosystem Services 12: 30–41. https://doi.org/10.1016/j.ecoser.2015.01.004

Romero-Calcerrada R, Luque S (2006) Habitat quality assessment using Weights-of-Evidence based GIS modelling: The case of Picoides Tridactylus as species indicator of the biodiversity value of the Finnish Forest. Ecological Modelling 196(1): 62–76. https://doi.org/10.1016/j.ecolmodel.2006.02.017

Rosenzweig ML, Winakur J (1969) Population ecology of desert rodent communities: Habitats and environmental complexity. Ecology 50(4): 558–572. https://doi.org/10.2307/1936246

Roth NE, Allan JD, Erickson DL (1996) Landscape influences on stream biotic integrity assessed at multiple spatial scales. Landscape Ecology 11(3): 141–156. https://doi.org/10.1007/BF02447513

Smith AC, Fahrig L, Francis CM (2011) Landscape size affects the relative importance of habitat amount, habitat fragmentation, and matrix quality on forest birds. Ecography 34(1): 103–113. https://doi.org/10.1111/j.1600-0587.2010.06201.x

Sobhani P, Esmaeilzadeh H, Barghjelveh S, Sadeghi SMM, Marcu MV (2022) Habitat integrity in protected areas threatened by LULC changes and fragmentation: A case study in Tehran Province, Iran. Land 11(1): 1–6. https://doi.org/10.3390/land11010006

Tong YQ, Long HL (2003) Study on the sustainable development of poor areas under the coupling of fragile ecological environment. China Population, Resources and Environment 02: 50–54.

van Vliet J, de Groot HLF, Rietveld P, Verburg PH (2015) Manifestations and underlying drivers of agricultural land use change in Europe. Landscape and Urban Planning 133: 24–36. https://doi.org/10.1016/j.landurbplan.2014.09.001

Venter O, Sanderson EW, Magrach A, Allan JR, Beher J, Jones KR, Possingham HP, Laurance WF, Wood P, Fekete BM, Levy MA, Watson JEM (2016) Sixteen years of change in the global terrestrial human footprint and implications for biodiversity conservation. Nature Communications 7(1): e12558. https://doi.org/10.1038/ncomms12558

Villa F, Bagstad K, Johnson G, Krivov S, Ceroni M (2009) ARIES (Artificial Intelligence for Ecosystem Services): A new tool for ecosystem services assessment, planning, and valuation. 11th Annual Bioecon Conference on Economic Instruments to Enhance the Conservation and Sustainable Use of Biodiversity, Venice (Italy), September, 2009.

Waldron A, Mooers AO, Miller DC, Nibbelink N, Redding D, Kuhn TS, Roberts J, Gittleman JL (2013) Targeting global conservation funding to limit immediate biodiversity declines. Proceedings of the National Academy of Sciences of the United States of America 110(29): 12144–12148. https://doi.org/10.1073/pnas.1221370110

Wang ZQ, Chen ZC, Hao CY (2009) Breeding habitat suitability evaluation of red-crown crane in Zhalong national nature reserve by the method of habitat suitability index. Wetland Science 7(03): 197–201. https://doi.org/10.13248/j.cnki.wetlandsci.2009.03.002

Wang Q, Lu C, Li FY, Fan ZP (2017) River habitat quality assessment based on principal component analysis and entropy weight in Qinghe River as a case. Ecologic Science 36(4): 185–193. https://doi.org/10.14108/j.cnki.1008-8873.2017.04.025

Wang Z, Xiao L, Yan H, Qi Y, Jiang Q (2022) Optimization of the ecological network structure based on scenario simulation and trade-offs/synergies among ecosystem services in Nanping. Remote Sensing 14(20): e5245. https://doi.org/10.3390/rs14205245

Wintle BA, Kujala H, Whitehead A, Cameron A, Veloz S, Kukkala A, Moilanen A, Gordon A, Lentini PE, Cadenhead NCR, Bekessy SA (2019) Global synthesis of conservation studies reveals the importance of small habitat patches for biodiversity. Proceedings of the National Academy of Sciences of the United States of America 116(3): 909–914. https://doi.org/10.1073/pnas.1813051115

Wu JS, Zhang LQ, Peng J, Feng Z, Liu HM, He SB (2013) The integrated recognition of the source area of the urban ecological security pattern in Shenzhen. Acta Ecologica Sinica 33(13): 4125–4133. https://doi.org/10.5846/stxb201208081123

Wu JS, Mao JY, Qian L, Li JC (2017) Urban growth boundary based on the evaluation of habitat quality: Taking the Yangtze River delta as an example. Dili Kexue 37(1): 28–36. https://doi.org/10.13249/j.cnki.sgs.2017.01.004

Wu D, Li H, Ai N, Huang T, Gu JS (2020) Predicting spatiotemporal changes in land use and habitat quality based on CA-Markov: A case study in central Ningxia, China. Chinese Journal of Eco-Agriculture 28(12): 1969–1978. https://doi.org/10.13930/j.cnki.cjea.200221

Wu J, Li X, Luo Y, Zhang D (2021) Spatiotemporal effects of urban sprawl on habitat quality in the Pearl River Delta from 1990 to 2018. Scientific Reports 11(1): 1–15. https://doi.org/10.1038/s41598-021-92916-3

Xiao Y, Ouyang ZY, Zhu CQ, Zhao JZ, He GJ, Wang XK (2004) An assessment of giant panda habitat in Minshan, Sichuan, China. Acta Ecologica Sinica 07: 1373–1379.

Xiao PN, Zhou Y, Li MY, Xu J (2022) Spatiotemporal patterns of habitat quality and its topographic gradient effects of Hubei Province based on the InVEST model. Environment, Development and Sustainability 11: e13981. https://doi.org/10.1007/s10668-022-02310-w

Xu H, Cao Y, Yu D, Cao MC, He YX, Gill M, Pereira HM (2021) Ensuring effective implementation of the post-2020 global biodiversity targets. Nature Ecology & Evolution 5(4): 411–418. https://doi.org/10.1038/s41559-020-01375-y

Yang J, Xie BP, Zhang DG (2021) Spatial-temporal evolution of habitat quality and its influencing factors in the Yellow River Basin based on InVEST model and GeoDetector. Journal of Desert Research 41(4): 12–22. https://doi.org/10.7522/j.issn.1000-694X.2021.00026

Yohannes H, Soromessa T, Argaw M, Dewan A (2021) Spatio-temporal changes in habitat quality and linkage with landscape characteristics in the Beressa Watershed, Blue Nile Basin of Ethiopian Highlands. Journal of Environmental Management 281: e111885. https://doi.org/10.1016/j.jenvman.2020.111885

Zhu ZY, Alimujiang K (2020) Spatial-temporal evolution of habitat quality in Yili Valley based on geographical detector and its influencing factors. Chinese Journal of Ecology 39(10): 3408–3420. https://doi.org/10.13292/j.1000-4890.202010.009

Zhu C, Zhang X, Zhou M, He S, Gan M, Yang L, Wang K (2020) Impacts of urbanization and landscape pattern on habitat quality using OLS and GWR models in Hangzhou, China. Ecological Indicators 117: e106654. https://doi.org/10.1016/j.ecolind.2020.106654

Nature Conservation 53: 157–164 (2023)
DOI: 10.3897/natureconservation.53.110961

Letter To The Editor

Has climate change hijacked the environmental agenda?

Cássio Cardoso Pereira[1], Daniel Negreiros[2], Milton Barbosa[3], Fernando Figueiredo Goulart[2], Rodrigo de Loyola Dias[1], Maria Clara Melillo[1], Flávio Camarota[1], Mariana Antunes Pimenta[1], Marina Cruz[1], Geraldo Wilson Fernandes[1,2]

1 *Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, Departamento de Genética, Ecologia e Evolução, Universidade Federal de Minas Gerais, 31270-901, Belo Horizonte, Minas Gerais, Brazil*
2 *Knowledge Center for Biodiversity, 31270-901, Belo Horizonte, MG, Brazil*
3 *Environmental Change Institute, School of Geography and the Environment, University of Oxford, South Parks Road, Oxford, OX1 3QY, UK*
Corresponding author: Cássio Cardoso Pereira (cassiocardosopereira@gmail.com)

OPEN ACCESS

Academic editor: Klaus Henle
Received: 10 August 2023
Accepted: 21 August 2023
Published: 5 September 2023

ZooBank: https://zoobank.org/B8C1DA5A-EFF8-46CA-944A-4507AC445DDB

Citation: Pereira CC, Negreiros D, Barbosa M, Goulart FF, Dias RL, Melillo MC, Camarota F, Pimenta MA, Cruz M, Fernandes GW (2023) Has climate change hijacked the environmental agenda? Nature Conservation 53: 157–164. https://doi.org/10.3897/natureconservation.53.110961

Abstract

Since the establishment of the Intergovernmental Panel on Climate Change (IPCC), decision-makers have realised that periodic assessments were needed to closely monitor climate change. Studies on it became widespread and include the science of greenhouse gas emissions, the composition of these gases and the extent to which humans have been responsible for climate change. In this sense, the United Nations summit has made significant progress since the Rio Conference (Eco 92), with the creation of the Conference of the Parties (COPs). However, governments should not solely focus on curbing greenhouse gas emissions into the atmosphere. In a society with broad and deep environmental problems, governments, the private sector and non-governmental organisations' (NGOs) efforts should include biodiversity conservation in their agenda. Solving a single problem, the climate crisis is honourable and urgently needed, but to constrain our ever-increasing land-use footprints on the planet needs the tackling of another equally challenging problem, the loss of biodiversity. The destruction of ecosystems undermines nature's ability to regulate greenhouse gas emissions and protect against extreme weather, thus accelerating climate change and increasing our vulnerability to it. Therefore, tackling environmental challenges means more than building electric cars, investing in "clean" energy and imposing fines on those who burn forests. To save the environment, scientists, industry, policy-makers and the wider society urgently need to look at other aspects of ecosystem conservation and restoration in the same way they look at the climate agenda.

Key words: Biodiversity crisis, climate change, Conference of the Parties, COP Bio, COP Climate, environmental agenda, global warming, integrated management

Global change myopia

Since the establishment of the Intergovernmental Panel on Climate Change (IPCC), decision-makers have realised that periodic assessments were needed to closely monitor climate change (O'Brien 1990). Studies on it became widespread and include the science of greenhouse gas emissions, the composition of these gases and the extent to which humans have been responsible for climate change (Bloomfield and Steward 2022). In this sense, the United Nations summit has made significant progress since the Rio Conference (Eco 92), with the creation of the Conference of the Parties (COPs) (Humphreys et al. 2019). However, governments should not solely focus on curbing greenhouse gas emissions into the atmosphere.

When we analyse the popularity and prestige of intergovernmental organisations created in favour of the environment, the IPCC completely overshadows the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) (Fig. 1A). When we analyse environmental treaties, this situation repeats itself. The United Nations Framework Convention on Climate Change (UNFCCC) is far better known than the Convention on Biological Diversity (CBD) (Fig. 1B). This is a reflection of increased public attention

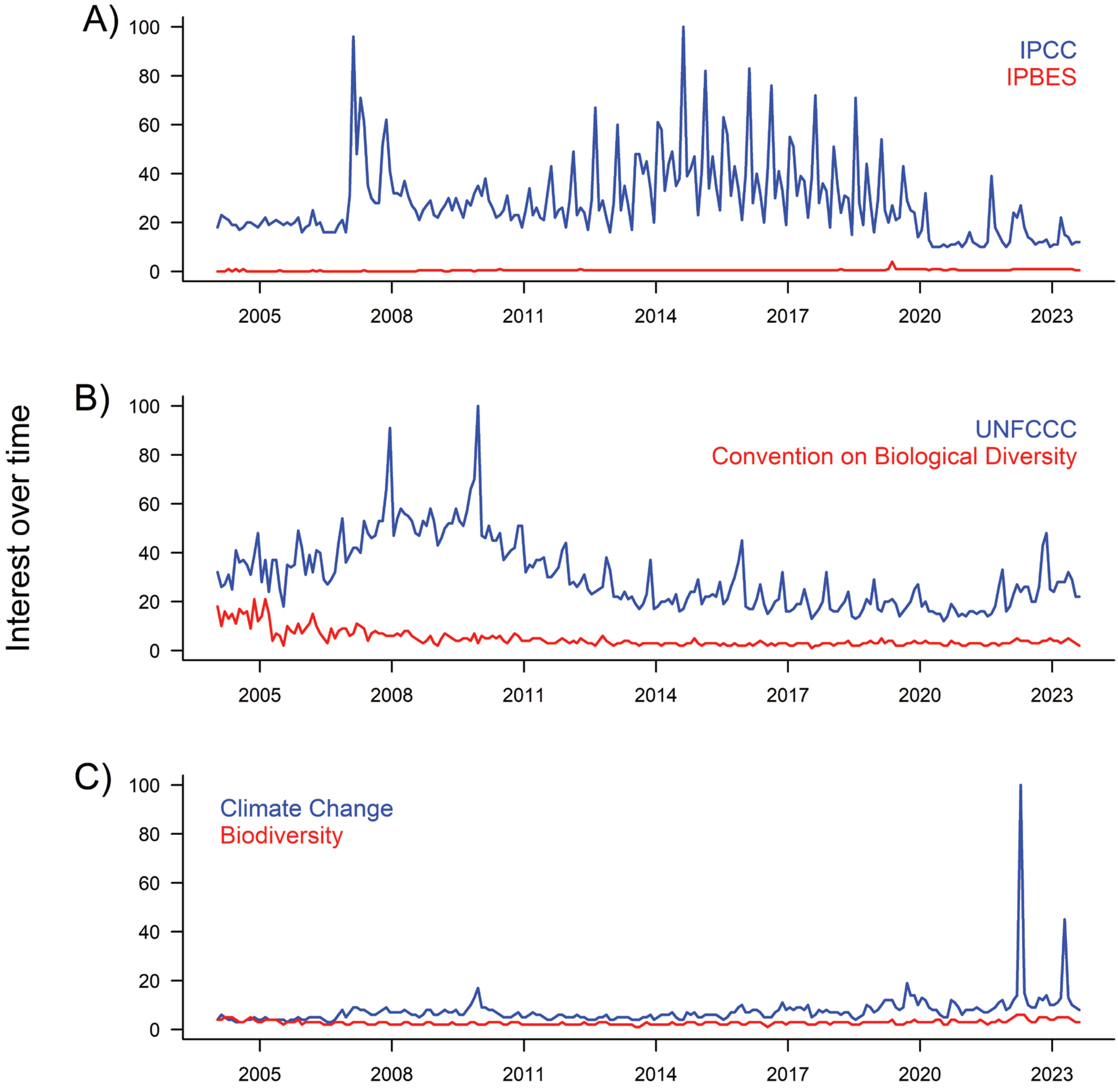


Figure 1. Web search interest for environmental topics around the world from 2004 to the present according to Google Trends™. Comparison of intergovernmental bodies (**A**), conventions (**B**) and terms (**C**) related to climate (blue) and biodiversity (red). Values represent the percentage of maximum (peak popularity). IPCC: Intergovernmental Panel on Climate Change; IPBES: Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services; UNFCCC: United Nations Framework Convention on Climate Change.

to climate change compared to other biodiversity issues (Hulme et al. 2018) (Fig. 1C) and may have contributed to a much higher number of COPs linked to climate change (27 COPs) relative to biodiversity (15 COPs) to this date. This asymmetry between environmental agendas can harm not only biodiversity, but also climate change, as environmental issues are inexorably interconnected (Rockström et al. 2021).

In a society with broad and deep environmental problems, governments, private sector and non-governmental organisations' (NGOs) efforts should include other dimensions of nature in their agenda (Chan et al. 2023; Schaubroeck 2023). Biodiversity, the unique variety of life on our planet, underpins our cultural, economic and social well-being (Cimatti et al. 2023). However, human-induced changes to ecosystems and the extinction of species have been more rapid in the past 50 years than at any time in human history (Cowie et al. 2022). Species are becoming extinct at about 1,000 times the average rate (Humphreys et al. 2019), as important habitats such as forests, wetlands, savannahs and coral reefs are plundered for human infrastructure (Torres et al. 2016). Soils are being deeply degraded (Ferreira et al. 2022), aquifers are being drained and polluted (Pereira and Fernandes 2022), corals are being bleached (Ainsworth et al. 2016), while fishing is being exploited at an unsustainable rate (Dulvy et al. 2021). The destruction of ecosystems undermines nature's ability to regulate greenhouse gas emissions and protect against extreme weather, further accelerating climate change and increasing vulnerability to it (Chan et al. 2022). Furthermore, around 50% of anthropogenic CO_2 emissions are removed by soil, vegetation and oceans each year, a free nature-based solution to climate change (Malhi et al. 2022). Therefore, it is puzzling that policy-makers are still over-focused on the climate component.

Challenges and perspectives

We argue here that climate change issue is an important and urgent matter; however, this problem must not be solved without considering the picture as a whole (Díaz et al. 2020). While many climate and biodiversity-friendly policies are aligned, as biomass accumulated by biodiverse ecosystems benefits both issues, some actions do not. The global carbon trade has been taken as an apple of the market's eye as a win-win solution that combines positive economic and environmental outputs. It is notable that, with some limitations, carbon trade has its climatic contribution (Xie et al. 2022). Nevertheless, plant biomass has much more to offer the world beyond acting as carbon sinks, as forests contribute to agriculture, medicine, energy and livelihoods for millions of people. As a consequence of this misguided focus on maintaining trees as carbon sinks, many tropical forests, savannahs and grasslands are being replaced by exotic *Pinus* or *Eucalyptus* monospecific stands for the sake of carbon sequestration (Veldman et al. 2015, 2019; Fernandes et al. 2016). In some tropical ecoregions, these plantations are amongst the major drivers of fragmentation, biodiversity and habitat loss, soil degradation and impact on non-climatic ecosystem services, such as water provision (Ricciardi et al. 2022). Finally, solar and wind farms from deforestation and bioenergy plantations (Seddon 2022), deep-sea mining for earth metals in energy batteries and photovoltaics (Lal and You 2023), and improper disposal of electric vehicle (EV) batteries (Lal and You

2023) are also harmful to biodiversity. Therefore, the climate agenda is unintentionally knocking down biodiversity conservation.

To fight climate change, we don't just depend on trees. Large animals aid climate change mitigation and adaptation through changes in fire regime, terrestrial albedo, increases in carbon stocks, trophic complexity, habitat heterogeneity, plant dispersal, resistance to abrupt change and microclimate modification (Johnson et al. 2018; Fricke et al. 2022; Malhi et al. 2022). We stress that if the fauna is not preserved, the populations of trees necessary for carbon sequestration will decrease (Fricke et al. 2022). Hunting mammals and birds, such as monkeys, tapirs and toucans, can reduce carbon storage in tropical forests (Fricke et al. 2022). This is because these animals spread the seeds of large trees, an important step for their reproduction (Galetti et al. 2013). Furthermore, the population decline of large grazing mammals may result in increased fires in savannahs, causing the release of CO_2 from ecosystems into the atmosphere (Johnson et al. 2018). It is also worth noting that preserved ecosystems act as natural buffers against extreme weather events, such as cyclones, floods and heat waves (Depietri et al. 2012). In this way, changes in land use must be integrated into climate models so that we can achieve a more detailed representation that increases our ability to predict how local impacts of change in land use will affect the future of biodiversity at a global level (Titeux et al. 2017).

Solving a single problem, the climate crisis, is honourable and urgently needed, but to constrain our ever-increasing land-use footprints on the planet needs the tackling of another equally challenging problem, the loss of biodiversity (Smith et al. 2022; Pörtner et al. 2023). Despite all the complexity of mitigating the climate change that we have been facing for decades, it can be contained, especially if there is enough biodiversity to purify the atmosphere and store carbon (Rockström et al. 2021; Sha et al. 2022). Finally, we emphasise that this path is necessary, but it is still winding. There is much to pass on to society in terms of ecological awareness (Peter et al. 2021). The spotlight is on climate change, at least in part, because climate action is something everyone already knows how to get involved in an accessible way. People can take simple steps to reduce emissions, for example, by watching their energy use or choosing to ride a bicycle instead of using a car. In addition, everyone can feel that the temperature is rising. However, the degradation of biodiversity can be difficult to notice (e.g. Régnier et al. 2009), especially for someone who does not get out and experience nature regularly. For example, not everyone in a city will notice that there are fewer bird species flying in an urban park. Therefore, a big question is how much we still have to learn about the various ecosystems around the planet, their delicate balance and interaction with their wider environment and indeed the climate (Mastrángelo et al. 2019).

Conclusion

Tackling environmental challenges means more than building electric cars, investing in "clean" energy and imposing fines on those who burn forests. To save the environment, scientists, industry, policy-makers and the wider society urgently need to look at other aspects of ecosystem conservation and restoration in the same way they look at the climate agenda.

Acknowledgements

The authors thank UFMG, PPG-ECMVS, CNPq, Fapemig and CAPES for their continuous support.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

Cássio Cardoso Pereira thanks Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Finance Code 001. G. Wilson Fernandes thanks Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for grant support.

Author contributions

Cássio Cardoso Pereira, Daniel Negreiros, Rodrigo Dias and G. Wilson Fernandes conceived the ideas; Cássio Cardoso Pereira, Daniel Negreiros and G. Wilson Fernandes led the writing of the manuscript; Rodrigo Dias made the figure. All authors contributed critically to the drafts and gave final approval for publication.

Author ORCIDs

Cássio Cardoso Pereira https://orcid.org/0000-0002-6017-4083
Daniel Negreiros https://orcid.org/0000-0002-4780-2284
Milton Barbosa https://orcid.org/0000-0003-4243-7895
Fernando Figueiredo Goulart https://orcid.org/0000-0002-6327-5285
Rodrigo de Loyola Dias https://orcid.org/0000-0003-2224-4834
Maria Clara Melillo https://orcid.org/0009-0008-1778-4661
Flávio Camarota https://orcid.org/0000-0003-4452-7755
Mariana Antunes Pimenta https://orcid.org/0000-0001-8764-5319
Marina Cruz https://orcid.org/0000-0001-6468-5609
Geraldo Wilson Fernandes https://orcid.org/0000-0003-1559-6049

Data availability

All of the data that support the findings of this study are available in the main text.

References

Ainsworth TD, Heron SF, Ortiz JC, Mumby PJ, Grech A, Ogawa D, Eakin CM, Leggat W (2016) Climate change disables coral bleaching protection on the Great Barrier Reef. Science 352(6283): 338–342. https://doi.org/10.1126/science.aac7125

Bloomfield J, Steward F (2022) Strategies for Climate Change Post COP26. The Political Quarterly 93(2): 278–287. https://doi.org/10.1111/1467-923X.13125

Chan S, Bauer S, Betsill MM, Biermann F, Boran I, Bridgewater P, Bulkeley H, Bustamente MMC, Deprez A, Dodds F, Hoffmann M, Hornidge A-K, Hughes A, Imbach P, Ivanova M, Köberle A, Kok MTJ, Lwasa S, Morrison T, Pörtner H-O, Sari AP, VanDeveer SD, Vollmer D, Widerberg O, Pettorelli N (2023) The global biodiversity framework needs a robust action agenda. Nature Ecology & Evolution 7(2): 172–173. https://doi.org/10.1038/s41559-022-01953-2

Cimatti M, Chaplin-Kramer R, Di Marco M (2023) The role of high-biodiversity regions in preserving Nature's Contributions to People. Nature Sustainability 13: 1–9. https://doi.org/10.1038/s41893-023-01179-5

Cowie RH, Bouchet P, Fontaine B (2022) The Sixth Mass Extinction: Fact, fiction or speculation? Biological Reviews of the Cambridge Philosophical Society 97(2): 640–663. https://doi.org/10.1111/brv.12816

Depietri Y, Renaud FG, Kallis G (2012) Heat waves and floods in urban areas: A policy-oriented review of ecosystem services. Sustainability Science 7(1): 95–107. https://doi.org/10.1007/s11625-011-0142-4

Díaz S, Zafra-Calvo N, Purvis A, Verburg PH, Obura D, Leadley P, Chaplin-Kramer R, De Meester L, Dulloo E, Martín-López B, Shaw MR, Visconti P, Broadgate W, Bruford MW, Burgess ND, Cavender-Bares J, DeClerck F, Fernández-Palacios JM, Garibaldi LA, Hill SLL, Isbell F, Khoury CK, Krug CB, Liu J, Maron M, McGowan PJK, Pereira HM, Reyes-García V, Rocha J, Rondinini C, Shannon L, Shin Y-J, Snelgrove PVR, Spehn EM, Strassburg B, Subramanian SM, Tewksbury JJ, Watson JEM, Zanne AE (2020) Set ambitious goals for biodiversity and sustainability. Science 370(6515): 411–413. https://doi.org/10.1126/science.abe1530

Dulvy NK, Pacoureau N, Rigby CL, Pollom RA, Jabado RW, Ebert DA, Finucci B, Pollock CM, Cheok J, Derrick DH, Herman KB, Sherman CS, VanderWright WJ, Lawson JM, Walls RHL, Carlson JK, Charvet P, Bineesh KK, Fernando D, Ralph GM, Matsushiba JH, Hilton-Taylor C, Fordham SV, Simpfendorfer CA (2021) Overfishing drives over one-third of all sharks and rays toward a global extinction crisis. Current Biology 31(21): 4773–4787.e8. https://doi.org/10.1016/j.cub.2021.08.062

Fernandes GW, Coelho MS, Machado RB, Ferreira ME, Aguiar LMDS, Dirzo R, Scariot A, Lopes CR (2016) Afforestation of savannas: An impending ecological disaster. Natureza & Conservação 14(2): 146–151. https://doi.org/10.1016/j.ncon.2016.08.002

Ferreira CSS, Seifollahi-Aghmiuni S, Destouni G, Ghajarnia N, Kalantari Z (2022) Soil degradation in the European Mediterranean region: Processes, status and consequences. The Science of the Total Environment 805: 150106. https://doi.org/10.1016/j.scitotenv.2021.150106

Fricke EC, Ordonez A, Rogers HS, Svenning J-C (2022) The effects of defaunation on plants' capacity to track climate change. Science 375(6577): 210–214. https://doi.org/10.1126/science.abk3510

Galetti M, Guevara R, Côrtes MC, Fadini R, Von Matter S, Leite AB, Labecca F, Ribeiro T, Carvalho CS, Collevatti RG, Pires MM, Guimarães Jr PR, Brancalion PH, Ribeiro MC, Jordano P (2013) Functional extinction of birds drives rapid evolutionary changes in seed size. Science 340(6136): 1086–1090. https://doi.org/10.1126/science.1233774

Hulme M, Obermeister N, Randalls S, Borie M (2018) Framing the challenge of climate change in Nature and Science editorials. Nature Climate Change 8(6): 515–521. https://doi.org/10.1038/s41558-018-0174-1

Humphreys AM, Govaerts R, Ficinski SZ, Nic Lughadha E, Vorontsova MS (2019) Global dataset shows geography and life form predict modern plant extinction and rediscovery. Nature Ecology & Evolution 3(7): 1043–1047. https://doi.org/10.1038/s41559-019-0906-2

Johnson CN, Prior LD, Archibald S, Poulos HM, Barton AM, Williamson GJ, Bowman DMJS (2018) Can trophic rewilding reduce the impact of fire in a more flammable world? Philosophical Transactions of the Royal Society B: Biological Sciences 373(1761): 20170443. https://doi.org/10.1098/rstb.2017.0443

Lal A, You F (2023) Will reshoring manufacturing of advanced electric vehicle battery support renewable energy transition and climate targets? Science Advances 9(24): eadg6740. https://doi.org/10.1126/sciadv.adg6740

Malhi Y, Lander T, Le Roux E, Stevens N, Macias-Fauria M, Wedding L, Girardin C, Kristensen JÅ, Sandom CJ, Evans TD, Svenning J-C, Canney S (2022) The role of large wild animals in climate change mitigation and adaptation. Current Biology 32(4): R181–R196. https://doi.org/10.1016/j.cub.2022.01.041

Mastrángelo ME, Pérez-Harguindeguy N, Enrico L, Bennett E, Lavorel S, Cumming GS, Abeygunawardane D, Amarilla LD, Burkhard B, Egoh BN, Frishkoff L, Galetto L, Huber S, Karp DS, Ke A, Kowaljow E, Kronenburg-García A, Locatelli B, Martín-López B, Meyfroidt P, Mwampamba TH, Nel J, Nicholas KA, Nicholson C, Oteros-Rozas E, Rahlao SJ, Raudsepp-Hearne C, Ricketts T, Shrestha UB, Torres C, Winkler KJ, Zoeller K (2019) Key knowledge gaps to achieve global sustainability goals. Nature Sustainability 2(12): 1115–1121. https://doi.org/10.1038/s41893-019-0412-1

O'Brien BJ (1990) IPCC's climate change mindset. Nature 348(6296): 9–9. https://doi.org/10.1038/348009a0

Pereira CC, Fernandes GW (2022) Cerrado conservation is key to the water crisis. Science 377(6603): 270–270. https://doi.org/10.1126/science.add4719

Peter M, Diekötter T, Höffler T, Kremer K (2021) Biodiversity citizen science: Outcomes for the participating citizens. People and Nature 3(2): 294–311. https://doi.org/10.1002/pan3.10193

Pörtner H-O, Scholes RJ, Arneth A, Barnes DKA, Burrows MT, Diamond SE, Duarte CM, Kiessling W, Leadley P, Managi S, McElwee P, Midgley G, Ngo HT, Obura D, Pascual U, Sankaran M, Shin YJ, Val AL (2023) Overcoming the coupled climate and biodiversity crises and their societal impacts. Science 380(6642): eabl4881. https://doi.org/10.1126/science.abl4881

Régnier C, Fontaine B, Bouchet P (2009) Not knowing, not recording, not listing: Numerous unnoticed mollusk extinctions. Conservation Biology 23(5): 1214–1221. https://doi.org/10.1111/j.1523-1739.2009.01245.x

Ricciardi L, D'Odorico P, Galli N, Chiarelli DD, Rulli MC (2022) Hydrological implications of large-scale afforestation in tropical biomes for climate change mitigation. Philosophical Transactions of the Royal Society B: Biological Sciences 377(1857): 20210391. https://doi.org/10.1098/rstb.2021.0391

Rockström J, Beringer T, Hole D, Griscom B, Mascia MB, Folke C, Creutzig F (2021) We need biosphere stewardship that protects carbon sinks and builds resilience. Proceedings of the National Academy of Sciences of the United States of America 118(38): e2115218118. https://doi.org/10.1073/pnas.2115218118

Schaubroeck T (2023) IPCC: Expand into a panel on sustainability. Nature 616(7958): 661–661. https://doi.org/10.1038/d41586-023-01392-4

Seddon N (2022) Harnessing the potential of nature-based solutions for mitigating and adapting to climate change. Science 376(6600): 1410–1416. https://doi.org/10.1126/science.abn9668

Sha Z, Bai Y, Li R, Lan H, Zhang X, Li J, Liu X, Chang S, Xie Y (2022) The global carbon sink potential of terrestrial vegetation can be increased substantially by optimal land management. Communications Earth & Environment 3(1): 1–10. https://doi.org/10.1038/s43247-021-00333-1

Smith P, Arneth A, Barnes DKA, Ichii K, Marquet PA, Popp A, Pörtner H, Rogers AD, Scholes RJ, Strassburg B, Wu J, Ngo H (2022) How do we best synergize climate mitigation actions to co-benefit biodiversity? Global Change Biology 28(8): 2555–2577. https://doi.org/10.1111/gcb.16056

Titeux N, Henle K, Mihoub J-B, Regos A, Geijzendorffer IR, Cramer W, Verburg PH, Brotons L (2017) Global scenarios for biodiversity need to better integrate climate and land use change. Diversity & Distributions 23(11): 1231–1234. https://doi.org/10.1111/ddi.12624

Torres A, Jaeger JAG, Alonso JC (2016) Assessing large-scale wildlife responses to human infrastructure development. Proceedings of the National Academy of Sciences of the United States of America 113(30): 8472–8477. https://doi.org/10.1073/pnas.1522488113

Veldman JW, Overbeck GE, Negreiros D, Mahy G, Le Stradic S, Fernandes GW, Durigan G, Buisson E, Putz FE, Bond WJ (2015) Tyranny of trees in grassy biomes. Science 347(6221): 484–485. https://doi.org/10.1126/science.347.6221.484-c

Veldman JW, Aleman JC, Alvarado ST, Anderson TM, Archibald S, Bond WJ, Boutton TW, Buchmann N, Buisson E, Canadell JG, Dechoum MDS, Diaz-Toribio MH, Durigan G, Ewel JJ, Fernandes GW, Fidelis A, Fleischman F, Good SP, Griffith DM, Hermann J-M, Hoffmann WA, Le Stradic S, Lehmann CER, Mahy G, Nerlekar AN, Nippert JB, Noss RF, Osborne CP, Overbeck GE, Parr CL, Pausas JG, Pennington RT, Perring MP, Putz FE, Ratnam J, Sankaran M, Schmidt IB, Schmitt CB, Silveira FAO, Staver AC, Stevens N, Still CJ, Strömberg CAE, Temperton VM, Varner JM, Zaloumis NP (2019) Comment on "The global tree restoration potential". Science 366(6463): eaay7976. https://doi.org/10.1126/science.aay7976

Xie Q, Hao J, Li J, Zheng X (2022) Carbon price prediction considering climate change: A text-based framework. Economic Analysis and Policy 74: 382–401. https://doi.org/10.1016/j.eap.2022.02.010

Nature Conservation

Nature Conservation 53: 165–182 (2023)
DOI: 10.3897/natureconservation.53.106406

Research Article

Conserving the threatened woody vegetation on dune slopes: Monitoring the decline and designing adaptive strategies for restoration

Amin U. Khan[1], Asad Abbas[1], Faiza Sharif[1], Asma Mansoor[2], Zafar Siddiq[3]

1 Sustainable Development Study Centre (SDSC), GC University (GCU), Lahore, 54000, Pakistan
2 Society for the Conservation of Indigenous Vegetation (SCIV), 13B Lake Road, Lahore, Pakistan
3 Department of Botany, GC University, Lahore, 54000, Pakistan
Corresponding author: Amin U. Khan (akeco1111@gmail.com)

Abstract

The southern tip of the Thal desert in Pakistan harbors the remnants of the original tropical thorn forest, amounting to two percent, which covered the province of Punjab a hundred years ago. In the past three decades, there has been a progressive decline in woody species cover on dunes, which is directly related to the increase in population in the surrounding area. Stabilized and destabilized dunes were subjectively selected followed by quantification of cover and diversity of woody species on the top and lower slopes. Dunes closely resembling the overall cover were grouped to suggest corresponding restoration measures. The results suggest that trends of decrease in cover and diversity of woody species were evident in the upper slopes of some stabilized dunes having less than 50% cover. The destabilized dunes with less than 20% cover are highly vulnerable to erosion. A general trend observed among dunes was that with a decrease in the cover of upper slopes, there is a decrease in the cover on lower slopes. The number of destabilized dunes is increasing without effective restoration measures against the prevailing trends of disturbances. Ranking dunes on the basis of cover could help in proposing simple restoration measures as a first step towards developing an understanding of designing adaptive strategies to restore the woody cover.

Key words: Anthropogenic factors, conservation, desertification, restoration, threatened tropical thorn forest

Academic editor: Ji-Zhong Wan
Received: 18 May 2023
Accepted: 12 August 2023
Published: 5 September 2023

ZooBank: https://zoobank.org/051B29F0-D2F0-46BF-9E90-61317317ABF5

Citation: Khan AU, Abbas A, Sharif F, Mansoor A, Siddiq Z (2023) Conserving the threatened woody vegetation on dune slopes: Monitoring the decline and designing adaptive strategies for restoration. Nature Conservation 53: 165–182. https://doi.org/10.3897/natureconservation.53.106406

Introduction

Although pervasive economic incentives to support human settlement are regarded as underlying causes of destruction and vulnerability in fragile arid lands, they are still being unabatedly pursued in developing countries (GOP 1992; Basso et al. 2000; Bakr et al. 2012). Aerodynamics models of interaction between wind, vegetation, and topography indicated that warmer temperatures can amplify the impacts of drought, making dunes more susceptible to mobilization (Wasson and Nanninga 1986; Lancaster 1988; Shumack et al. 2021; Kamel-Eddine 2022). Research in technology transfer, knowledge of local people, and governmental behavior are increasingly used at both spatial and temporal dimensions for characterizing the driving factors to control land use management (Ash and Wasson 1983; Lancaster and Baas 1998; Wolfe et al. 2010; D'Odorico et al. 2013; Siegal et al. 2013; Thomas and Redsteer 2016). Effective knowledge of vegetation dynamics has also played an important role in the identification of disturbance factors and developing protocols in categorizing biological features of dunes in terms of plant growth, stabilization process, and vegetation improvement (Aguiar and Sala 1999; Xiaodong et al. 2013; Yang et al. 2013; Zhang et al. 2013). Engineering and technological measures for combating desertification like chemical mulching and hydraulic solutions and many kinds of cementing materials have been used in improving sand dune stabilization (Fryrear 1985; Glen et al. 1998; Rezaie 2009; Gao et al. 2010; Khalili Moghadam et al. 2016) for prioritizing restoration in arid sandy land under the looming threats of climate change.

The bi-species association of *Salvadora oleoides* and *Prosopis cineraria* is considered to be the ultimate climax of the tropical thorn forests of the Indus Plain; stretching from the foothills of the Himalayas to the Arabian Sea (Khan 1994). These forests provided fodder and shelter to wildlife, medication, recreation, and most importantly, it was used as famine food in drought (Khan 1996). In Pakistan, 80% of land comes under arid and semi-arid climate, and at the turn of the twentieth century after the advent of the canal irrigation system, most of the native tropical thorn forest was gradually replaced by agriculture, and its remnants now survive in the deserts of Thal, Cholistan, and Thar. In these deserts uncontrolled extraction of colonizer species, and the use of *Leptadenia pyrotechnica* and *Calligonum polygonoides* as fuel wood, further eliminated the possibilities of natural succession and these trends of mismanagement of native woody vegetation are now threatening the last remnants of dry woodlands (GOP 1992; Khan 2003; Book Hut 2014; Gratzfeld and Khan 2015).

In addition, piloting restoration of four woody species on a sand ridge by enrichment planting showed 40% survival of *S. oleoides*, of the four woody species planted (Khan 2022). Simple amendments like the addition of loamy soils, which are freely available to local farmers after annual dredging of the irrigation canal, have augmented the growth of *S. oleoides* after the cessation of irrigation. It indicates that such an amendment might also help in stabilizing stressed plants on sandy dune slopes. This project also demonstrates that active restoration on a ridge over a period of six years costs nearly 100$ per plant, making it an expensive and time-consuming proposal and further strengthening the argument that the conservation of woody vegetation on dunes should be prioritized.

Most of the work conducted in the deserts of this region is based on investigating the characteristics of soil and vegetation parameters, and socio-economic and developmental factors (Hassan and Hassan 1998; Singh 2004; Arshad et al. 2008; Qureshi and Bhatti 2008; Sivaperuman et al. 2008). Little information is available on designing strategies to combat loss of diversity and assessment of intangible protective values of indigenous vegetation to the landscape stability. This paper copes with the knowledge gap between the vulnerability and sustainability of threatened vegetation on the fragile landscape by categorizing them into groups and suggesting choices of restoration strategies for their long-term sustainability on the dunes. In short, this is a last-ditch effort to propose the conservation of fast-declining climatic climax species on the dune tops. The paper also captures the historical ecology of the study area, and prevailing practices, and gives some policy insights to identify, categorize and rank relevant measures for practical implications for large-scale restoration.

Historical ecology of the study area

The Thal desert (Fig. 1) presented a complex geological and geomorphological pattern of alluvial deposition by a westerly drifting Indus river progressively, followed by wind resorting of the sediments into various forms of sand ridges or dunes (GOP 1968). Summer winds are from the south and winter winds from the north and they seldom increase in force to three in strength (12–19 km/h) on the Beaufort scale (GOP 1968). The desert originally extended over 25,809 km but most of it was claimed by agriculture after the advent of the canal irrigation system. Rakh Khairewala (RK), a state-controlled land (160 km^2) jointly looked after by the Rangeland and Livestock Departments, is located in the arid southern tip of Thal desert (mean annual temperature 26 °C; with a mean summer 32 °C, mean winter 19 °C, and average annual rainfall of less than 125 mm). The plant cover of RK comprises a two-phased mosaic; sandy loam flats and fine sand dunes (Fig. 2). Flats and ridges are dominated by bi-species groves of *S. oleoides* and *P. cineraria*, climatic climax of the region, colonizer shrub, *Calligonum polygonoides* and less common sub-climax species *Capparis decidua*. Ground cover is dominated by grasses; *Cenchrus ciliaris*, *Cymbopogon jawarancusa* and *Eleusine flagillefera*. The dunes have cover of woody patches, forming either single or clumpy plant communities composed of *S. oleoides*, *P. cineraria* and *C. polygonoides* with scanty cover of *Aristida mutabalis*, *C. jawarancusa* and *Aera javanica*.

Research (Khan 2010) on structural and functional status of bi-species groves of *P. cineraria* and *S. oleoides*, provided information on the coexistence

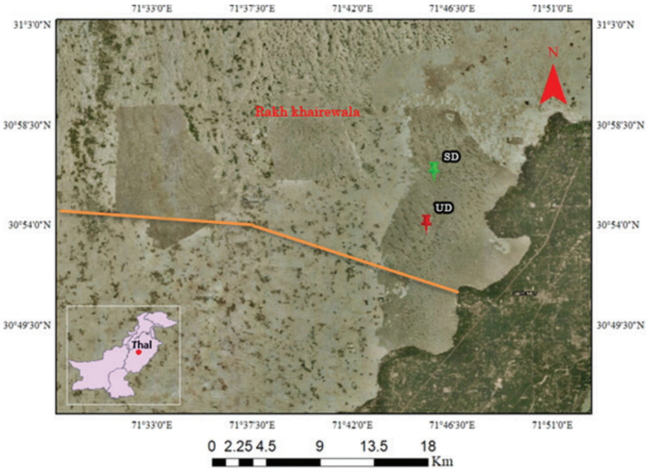


Figure 1. General view of RK and the surrounding landscape: dark grey color represent natural forest cover in which stabilized (SD) and destabilized dunes (DS) are located, and intervening light grey color shows the cultivated area with seasonal cropping. Dark grey patch on the left is the rangeland area in which the restoration site is located near the town of Chaubara. Green color shows the private arable canal irrigated zones. The road (orange) across the desert between two main towns. Map powered by google imagery 2023 CNES / Airbus, Maxar Technologies, Map data 2023.

Figure 2. General view of the KP with grove of *P. cineraria*, *S. oleoides* and *C. polygonoides* with grass cover on the flats and ridges, where sheep can be spotted, and a dune in the background showing slopes occupied by woody patches.

between species in the form of biomass allocation and root architecture. It also highlighted the impact of anthropogenic activities which have altered this association between species making them vulnerable on destabilized slopes. The livestock rearing and gram cultivation (*Cicer arientinum*) are the main sources of livelihood in this area; the latter is being extended on fragile sandy soils by leveling dunes and extraction of natural vegetation. These practices have made landscape more vulnerable to desertification and threatened the survival of the relict species of tropical thorn forest (Khan et al. 2016).

Studies (Khan 2010) on the contribution of litter by *S. oleoides*, *P. cineraria*, *C. polygonoides* and *C. decidua* indicated that these woody species are, in fact, acting as fertile islands (Chen et al. 2003) on the sandy substratum. Canopy cover and litter (composed mainly of twigs, barks, leaves, fruits and seeds) decreased on the upper slopes due to stressed status of species as compared to lower slopes. Among the four species, *S. oleoides* produced twice as much litter on both slopes and this higher litter accumulation of *S. oleoides* was attributed to its drought evading habit of growth; evergreen with surface feeder roots, and multi-stemmed drooping branches that protect and trap the litter from being dispersed. The low litter accumulation of *P. cineraria* was attributed to its arboreal and deciduous habit, whereas *C. polygonoides* and *C. decidua* are both leafless.

A survey (Khan et al. 2016) showed that woody patches on slopes are larger on stabilized dunes as compared to the destabilized dunes and their formation and spread seems to be related to the maintenance of balance between disturbance and availability of resources. Woody patches on slopes of stabilized dunes seem to be contributing to the build-up phases of natural processes, as they are more suited for extending the vegetative spread of woody species by basal branching forming above ground canopy size with lateral root spread beneath. Bottom slopes, on the other hand, (where they merge with flats), provide favorable microenvironment for the recruitment of grasses and herbs. Comparative analysis of surveys (Khan 1990; Khan et al. 2016) showed that the cultivated fields, which were about 10 km away from the southwestern boundary of RK, are now adjacent to its boundary; this is also evident from the satellite images taken during the period 1991–2021 (Google Earth Pro 2021). There has been a noticeable increase in the number of mud tracks and encounters with donkey load of extracted wood, even in remote areas, several kilometers away from the road. Moreover, RK is a state-controlled rangeland, therefore more land is cleared and reseeded, and rented for grazing on an annual basis.

Over the past two decades pumping of ground water by turbines has made agriculture more reliable in non-irrigated areas, resulting in an increase in the cultivated areas and a corresponding increase in population in the adjacent villages. In addition, this is because sugar cane is the most popular crop grown in irrigated areas and bagasse an agricultural by-product (Dotaniya et al. 2016) derived from the sugarcane milling process has become a popular source of organic fertilizer in crop production on the sandy soil in this region. When applied as a soil amendment it improved soil pH, BD, WHC, OM, macro- and micronutrients, tiller count, and, ultimately, yield (Bhadha et al. 2017; Chacha et al. 2019). It was available to the local farmer at 10 cents per 40 kg bag in 2021.

Studies conducted at RK (Khan 1990, 1994, 1996, 2010; Abbas 2015) revealed that the decline in groves of *P. cineraria* and *S. oleoides* is also linked with the general discriminatory perception about the two species among public and planners. The former species is officially protected because of its superior quality fuel and fodder over the latter, which is discarded as inferior quality fuel wood and home for vermin and its complete extraction is recommended prior to any agriculture or afforestation program. On the contrary, patterns of interaction of *S. oleoides* with environment showed that despite its low market value, it is ideal for stabilization of dunes. Similarly, there is no restriction on extraction of *C. polygonoides* and its scarcity in the vicinity of metaled roads is linked to its transportation to nearby towns where it is sold (by the trolley load), as a popular fuel wood. Research work being carried out at RK, covering a period of nearly three decades, also revealed that there are strong links between an increase in land under cultivation and a corresponding increase in unrestricted extraction of woody species, and an increase in the number of de-stabilized dunes. It is obvious that weak law enforcement on controlling the extraction of woody plants, which provided a protective cover to the sandy substratum for many centuries, has resulted in accelerating the pace of degradation.

Methodology

Study design for grouping dunes

Obtaining true replication was difficult at the landscape-scale level for this management-based study, therefore it was hoped that comparisons based on pseudoreplicates would effectively help in interpreting the trends of the decline of woody vegetation on the dune slopes. Priori categorization of dunes was made by selecting assemblages of one square kilometer area in stabilized and destabilized dunes located in less exploited and over-exploited areas, respectively. The assemblage of stabilized dunes was located about 3 km away from the metaled road connecting the two main towns whereas assemblage of destabilized dunes was located adjacent to the metaled road. The stabilized dunes generally have a greater height (>4 m) and dimension as compared to destabilized dunes (<3 m).

The criteria of selection of dunes were slightly different in the two assemblages. In the assemblages of stabilized dunes adjacent ten dunes having approximately 50 m^2 cover of woody species on both slopes were demarcated for study. In the assemblages of destabilized dunes where most upper slopes are devoid of woody species, dunes having at least 5 m^2 cover of woody species on upper slopes were selected. GPS coordinates of the selected dunes were noted. The cover value of the following woody species was made: *P. cineraria*, *S. oleoides*, *C. polygonoides*, and *C. decidua*. Grasses and herbs were not included in the cover estimates as they have poor representation on the slopes.

Upper and lower slopes were visually bifurcated; as the upper slope is the top of the dune and the steep slopes whereas lower slopes are gentle slopes before it merges with flats dominated by grasses and herbs. It varies from dune to dune; they are more defined in the stabilized dunes as compared to destabilized dunes.

A rope was used to demarcate a circle in order to estimate the cover of woody species. Since the cover of woody species was very scattered, ranging from a single plant to patches of various sizes, therefore measurements were based on the proportion of the ground cover occupied by the perpendicular projection of the aerial crown (Kershaw and Looney 1985).

Statistical analysis

Hierarchal cluster analyses (HCA) using Ward method was applied to provide classification of upper and lower slopes of both stabilized and destabilized dunes. Two-way ANOVA was applied to find out the statistical mean difference of vegetation cover between the upper and lower slope of both stabilized and destabilized dunes. Shannon Diversity Index was used to determine the vegetation diversity trends with cover among the groups. Linear regression analysis was applied for grouping dunes on the basis of cover. In this research, the dependent variable represented by average coverage of plant species and independent factor is the condition of the dune in terms of stabilized and destabilized dunes. And dunes having close resemblance of overall cover were grouped to suggest corresponding restoration measures. All statistical methods were applied using software SPSS (SPSS 21). Species nomenclature followed the taxonomic criteria of Stewart (1972).

Results

The results are analyzed on the basis of the cover of upper and lower slopes separately and also dunes as a unit by combining cover on upper and lower slopes and then grouping them in order to suggest appropriate techniques for restoration and conservation of the woody plant cover.

The result of vegetation hierarchical clustering (Fig. 3) on the horizontal axis shows the squared Euclidean Distance between units. At the cluster distance of 25, vegetation units (slopes) are separated into 2 clusters A (destabilized) and B (stabilized) consisting of 21 and 19 units respectively. Cluster A includes slopes having average cover of less than 20 m^2, B having greater than 48 m^2 cover. The cluster B at lower distance (5), further splits on the basis of cover into C (7) and D (14) units. Units in cluster C have average cover of 40 m^2, referring to upper slopes of some stabilized dunes, with the exception of one

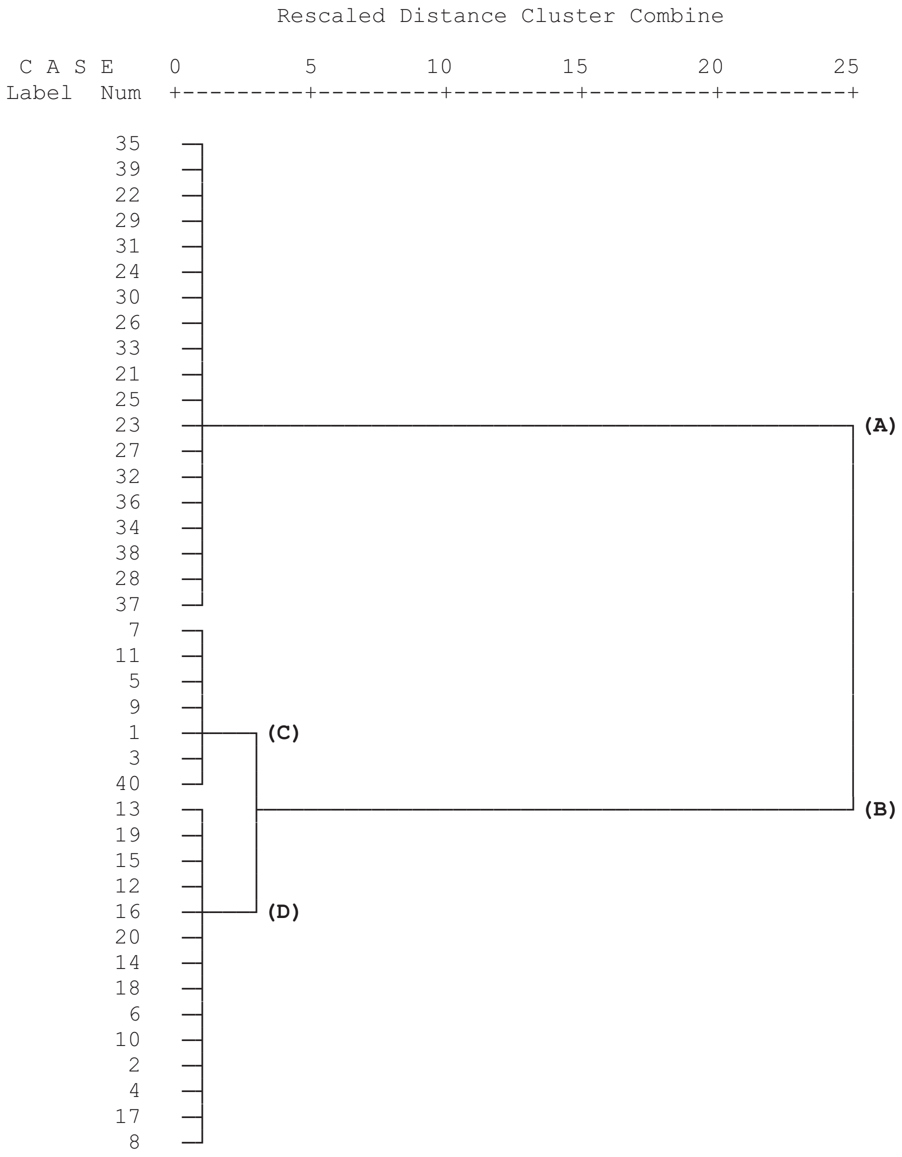


Figure 3. Hierarchial cluster analyses (HCA) showing classification of upper and lower slopes of both stabilized and destabilized dunes into distinct communities' groups. Slopes of destabized dunes were placed in group **A** where as slopes of stabilized slopes were placed in group **B** the group **B** at a lower distance splits into **C** representing upper slopes of stabilze dunes and **D** representing their lower slopes.

unit (40) from lower slope of destabilized dune with lower than average cover (28 m^2). Units in cluster D have average cover of 57 m^2, referring to lower slopes of all the stabilized dunes. The division of cluster groups showed that the cover is the most influenced factor in detecting the trends of decline of woody species and corresponding erosion which seems to be highest in lower slopes of stabilized dunes followed by upper slopes of stabilized dunes and finally destabilized dunes.

These units formed by cluster analysis also indicate a kind of interconnection among the upper and lower slopes of a dune as a decrease in the cover of upper slopes is reflected by trends of decrease on lower slopes. Therefore it was considered that by ranking dunes on the basis of overall cover and then suggesting restoration measures for the two slopes would be more practical in restoration of the woody plant cover. Results of two-way ANOVA show that the difference in woody cover (m^2) between upper and lower slopes is more significant in the stabilized dunes compared to the destabilized dunes. This trend is also obvious in the difference between mean cover of upper and lower slopes which is significantly higher in stabilized dunes compared to destabilized dunes (Fig. 4).

Similarly, linear regression showed that expected cumulative cover (Fig. 5) of stabilized dunes is higher than five, whereas it is less than three for the destabilized dunes. The dune 40 appeared to be an anomaly in the cluster analysis; its upper and lower slopes are located in two different clusters and its expected cumulative prob is less than 4, which indicates that it is a transition dune between the two clusters. It can be regarded as tipping point; where the decrease in cover of the upper slope is beginning to show its impact on the cover of lower slopes. The expected cumulative prob also indicates grouping within the stabilized dunes. Three dunes on the top have expected cumulative prob above nine corresponds to cluster D, followed by two groups having above seven and five

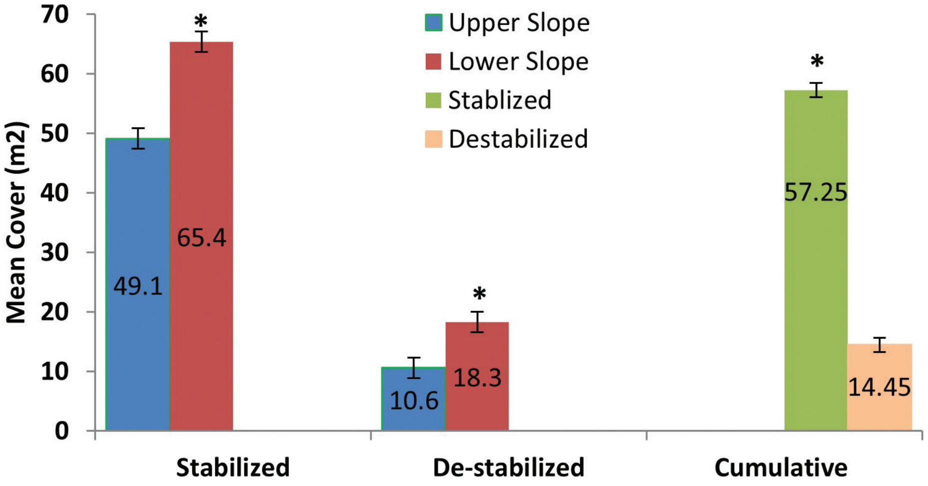


Figure 4. Mean cover (m^2) of woody vegetation on upper and lower slopes of stabilized and de-stabilized sand dunes. Upper bars show ± *S.E.* *Significant difference between means ($P < 0.01$), Two-way ANOVA.

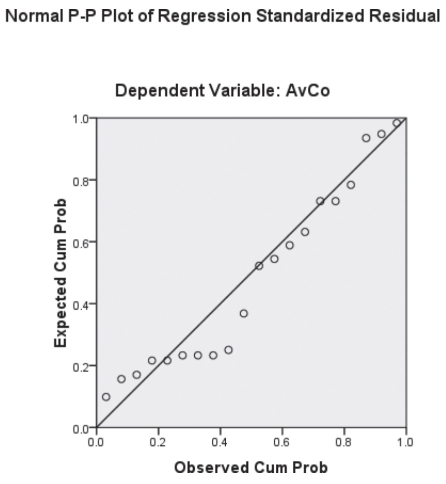


Figure 5. Linear regression analysis showed grouping of dunes on the basis of cover, destabilized groups have lower expected cum prob as compared to stabilize groups.

respectively. The latter two groups of dunes reflect the gradual decline of cover on upper slopes, which corresponds to the cluster C. These three groups clearly show that the difference in cover of upper and lower slopes is more significant in group 1 and 2 as compared to 3, showing how decrease in cover of upper slopes has gradually impacted on lower slopes. In addition, the Shannon diversity index applied to the five groups ranked on the basis of cover indicated trends of increase in diversity with increase in expected cumulative prob (Table 1).

Ranking dunes on the basis of cover

The following dune groups were segregated after ranking them on the basis of overall cover for both stabilized and destabilized dunes and corresponding restoration measures were suggested for the two slopes.

Stabilized dunes

Ranking of stabilized dunes on the basis of overall vegetation cover (Table 2) shows linear trends of steepness in the cover among the dunes (Fig. 5). The dunes were ranked and categorized into the following three groups and corresponding restoration measures were suggested for the two slopes of the dune (Fig. 6).

The group I includes dunes 1, 2 and 3, the overall cover is less than 55 m^2 although they have 60 m^2 cover on lower slopes but the upper slopes have less than 45 m^2. In addition, upper slopes have low diversity as compared to lower slopes. The presence of lone *P. cineraria* on dune 2 seems to be a vestige from the original cover. This group of dunes could naturally restore their cover on protection from further exploitation but at the same time active restoration based on enrichment planting of saplings *P. cineraria* and *C. polygonoides* on upper slopes by soil amendments could assist in increasing stability of the patch.

The group II with dunes 4, 5 and 10, have overall cover of less than 60 m^2 although lower slopes have 60 m^2 cover but the upper slopes have less than 50 m^2 (Fig. 7). Diversity of woody species on both slopes is similar. The presence of *P. cineraria* and *C. polygonoides* indicates that it is less exploited and retain the capacity to expand its cover by simple protection from exploitation. The presence of associate species indicates that simple soil amendments on upper slopes might help in stabilizing the cover.

The group III with dunes 6, 7, 8, and 9, have more than 60 m^2 overall cover; their upper slopes have slightly less than 60 m^2 cover. The presence of *C. decidua*, indicates that the woody patches are stabilized as they are harbouring species preferring more stable sandy loam soils. This group represents a low level of

Table 1. Shannon Diversity Index applied to the groups indicating trends of increase in diversity with increase in cover among the groups.

	Dune no.	Group	Upper	Lower
Stabilized	1,2,3	1	0	0.175
Stabilized	4,5,6	2	0.92	0.93
Stabilized	7,8,9,10	3	1.06	1.06
Destabilized	1,3,4,5,9	4	0	0.11
Destabilized	2,6,7,8,10	5	0.42	0.48

Table 2. Arranging stabilized dunes (SD) on the basis of cover.

Dune Rank and no ( )	Slope	Area (m^2)	Plant species (m^2)				Total cover (m^2)	Mean cover (m^2)
			S. oleoides	*P. cineraria*	*C. polygonoides*	*C. decidua*		
1 (SD 1)	US*	120.5	41	0	0	0	41	51.5
	LS**	156.7	62	0	0	0	62	
2 (SD 2)	US	125.6	44	0	0	0	44	53
	LS	206.2	54	8	0	0	62	
3 (SD 3)	US	121.1	43	0	0	0	43	54
	LS	288.5	65	0	0	0	65	
4 (SD 4)	US	130.5	35	5	8	0	48	54
	LS	250.7	49	1	10	0	60	
5 (SD 5)	US	140.9	25	11	6	0	42	54
	LS	192.7	40	14	12	0	66	
6 (SD 10)	US	109.4	24	9	13	0	46	57.5
	LS	201.9	36	12	19	2	69	
7 (SD 6)	US	108.9	29	12	14	0	55	60
	LS	192.8	38	19	8	0	65	
8 (SD 9)	US	131.5	28	11	13	0	52	60
	LS	226.6	37	16	15	0	68	
9 (SD 7)	US	162.2	30	10	21	2	63	64
	LS	289.1	34	10	18	3	65	
10 (SD 8)	US	185.1	26	8	23	0	57	64.5
	LS	311.7	35	11	26	0	72	
Total ± S.E.			775 ± 2.85	157 ± 1.32	206 ± 1.90	7 ± 0.19		
Frequency (%)	US		100	70	70	10		
	LS		100	80	70	20		

*Lower Slope ** Upper slope.

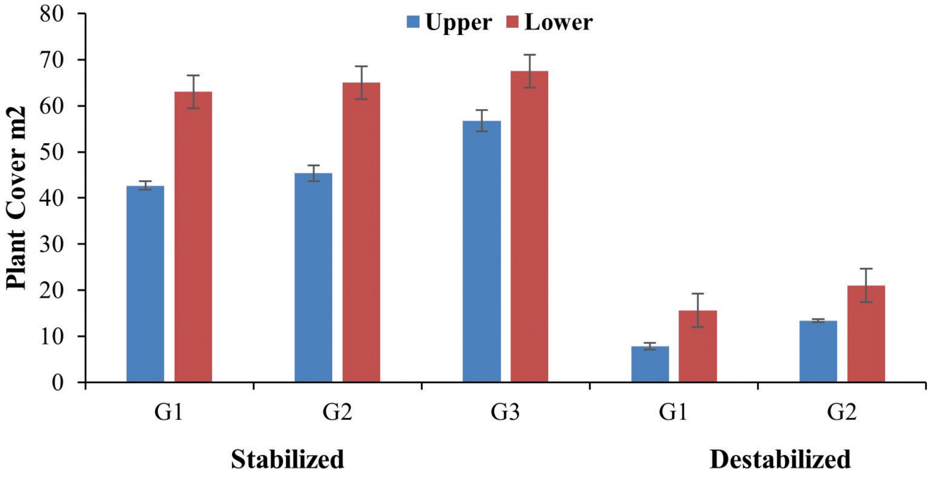


Figure 6. Mean overall cover of woody patches in the three groups on the upper and lower slopes of stabilized and destabilized dunes.

exploitation and are therefore less susceptible to natural disturbance. These dunes are recommended to be officially designated as model dunes, barring them from any form of extraction.

Figure 7. Close view of stabilized dune showing decrease in the cover of upper slopes while still retaining the diversity of species.

Destabilized dune

Ranking of destabilized dunes on the basis of overall vegetation cover (Table 3) shows a decrease in linear trends of steepness (Fig. 5) as compared to stabilized dunes. In addition, the absence of *C. polygonoides*, the scanty presence of *P. cineraria* and signs of stress on *S. oleoides*, indicates that the surviving woody species are susceptible to wind erosion. The dunes were ranked and categorized into the following two groups and corresponding restoration measures were suggested for the two slopes of the dune (Fig. 6).

The group I with dunes 1, 3, 4, 5, 9, have low cover (< 10 m^2) and surviving *S. oleoides* on upper slopes have either exposed root system (Fig. 8) or are partially buried. The dune 5 with lone *P. cineraria*, seems to be a relic from its original cover. It appears that with time this scanty cover would succumb to wind erosion, which has manifested on both slopes. In this group proposed adaptive management includes enhancing the growth of the stressed species by adding bagasse and even mulching to evade the impacts of wind erosion. Absence of colonizers on the destabilized dunes are warning signs of decline of woody cover therefore designing restoration should also be based on planting of strips of perennial colonizers, *Calligonum polygonoides* and *Cymbopogon jawarancusa*, after soil amendments. Both species were occasionally present on top slopes of sand dunes, and could help in soil binding around the stressed woody plants as they compete less with them and will prevent erosion around the plants.

The group II with dunes 2, 6, 7, 8 and 10 have higher cover (>18 m^2) and *S. oleoides* appears less stressed and presence of occasional *P. cineraria*, showed increase in diversity as compared to group I. Proposed amendments for upper slopes should be similar to those suggested for group I; at the same time enrichment planting of woody sampling on lower slopes with bagasse amendments could help to expand the woody patches.

Table 3. Arranging destabilized dunes (DD) on the basis of cover.

Dune Rank and no ( )	Slope	Area (m^2)	Plant species (m^2)		Total cover (m^2)	Mean cover (m^2)
			S. oleoides	*P. cineraria*		
1 (DD 1)	US*	91.9	8	0	8	10
	LS**	143.5	12	0	12	
2 (DD 5)	US	86.3	7	0	7	11
	LS	95.8	13	2	15	
3 (DD 9)	US	79.9	8	0	8	11
	LS	153.1	14	0	14	
4 (DD 3)	US	58.3	5	0	5	11.5
	LS	95.8	18	0	18	
5 (DD 10)	US	51.9	11	0	11	13
	LS	95.8	15	0	15	
6 (DD 4)	US	90.9	11	0	11	15
	LS	163.5	19	0	19	
7 (DD 6)	US	86.6	9	5	14	16
	LS	95.0	13	5	18	
8 (DD 7)	US	49.4	12	0	12	15.5
	LS	155.6	19	0	19	
9 (DD 8)	US	114.7	13	5	18	18
	LS	237.3	11	7	18	
10 (DD 2)	US	76.9	12	0	12	24
	LS	239.4	27	8	35	
Total ± *S.E.*			257 ± 1.12	32 ± 0.60		
Frequency (%)	US		100	20		
	LS		100	40		

*Lower Slope ** Upper Slope.

Figure 8. Exposed root system of *S. oleoides* on the upper slopes of destabilized dunes.

Discussion

Trends of decline of cover exhibited in the stabilized and destabilized dunes indicated that the woody patches on top of the slope are more vulnerable to destabilization and once its cover decreases below a minimum on the top slopes

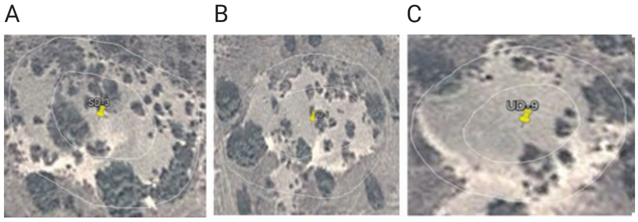


Figure 9. **A** stabilized dune, showing both slopes having more than 50% cover **B** stabilized dune showing decrease in the cover of upper slopes **C** destabilized dune showing decrease in cover on both slopes. Map powered by google imagery 2023 CNES / Airbus, Maxar Technologies, Map data 2023.

it could destabilize woody patches on the lower slopes. Signs of initiation of the process of destabilization are evident in the low ranked stabilized dunes in the form of decrease in cover and diversity on the upper as compared to lower slopes (Fig. 9A–C). But, in destabilized dunes the loss of cover on upper slopes has been effectively transferred down slope. It seems that the prevailing regime of anthropogenic extraction is further jeopardizing the basic processes of plant succession essential for the development of woody patches in this harsh environment. Higher cover and frequency of *S. oleoides* on dunes highlights its drought-evading attributes (Khan 2010) which has allowed it to adapt to burial and abrasion under increasing sand movement as compared to associate species, thus making it an ideal species for restoration of dunes. The restoration experiment on a sand ridge (Khan 2022), showed enhanced growth response of *S. oleoides* to addition of sandy loam soils, and therefore one can expect similar amendments on in situ stressed species on dunes would be a far better option cost- wise compared to active restoration. Similarly, if sandy loam with (5%) organic matter supplemented the growth and survival of species on sandy soil, naturally bagasse amendments (95% organic matter) with numerous other agronomic benefits, would further augment the growth of stressed species on the destabilized dunes.

This study shows that large woody patches on the slopes retain the ability to naturally expand their cover on sandy slopes but once they are destabilized they are increasingly impacted by sand mobilization and therefore cover seems the most influential factor in suggesting control measures. At the moment there is virtually no data available on the economic cost of wind erosion, including, site damage, loss of biodiversity and soil productivity in terms of organic matter removal. Neither have any measures ever been adopted to monitor and restore the woody patches. The simple restoration measures proposed here could be considered as a first step in developing an understanding towards designing adaptive strategies on conservation of the woody cover.

The decline of woody species on dune slopes reflects the lack of recognition and understanding of values and attributes of species in influencing physical, chemical and biological characteristics of fragile soils at societal and official levels. Effective knowledge of vegetation dynamics has played an important role in identification of disturbance factors and developing protocols in categorizing biological features of dunes in terms of plant growth, stabilization process and vegetation improvement, (Su et al. 2004; Thomas and Redsteer 2016). Furthermore, good governance in sandy deserts demands maximizing total benefits from natural vegetation irrespective of their market value and the only way forward for the planner is to implement a conservation and restoration program based on consumptive and productive use of natural vegetation (McNeely 1988; Akbari et al. 2020; You et al. 2021). Large areas on the verge of desertification due to human pressure were stabilized by community participation and ratification of anti-desertification movement (Levin and Ben-Dor 2004; Amiraslani and Dragovich 2011; Rubinstein et al. 2013; Xiaodong et al. 2013). Similarly, China has accumulated rich experiences in combating desertification by using various control measures which were innovated and developed with experience over the years (Down to Earth 2021). Unfortunately, developing countries are confronted by serious economic deficits and their policies are therefore geared to the interest of the present at the expense of future generations. These results in the enactment of policies indirectly accelerating the pace of extraction of woody species, leading to serious environmental problems in inherently fragile desert areas. In this era of global recession constructing a pilot project can help in further refining the proposals and its scaled implementation.

Conclusion

The significance of study findings in combating loss of threatened woody species on sand dunes emphasizes that management strategies in a spatially heterogeneous edaphic system, where the stability of dunes is of great significance to the basic ecology of this region, needs to be adapted to an understanding of the factors and processes. Suggesting high-ranked stabilized dunes as protected representative benchmarks is compatible with the objectives of the range land. This would discourage leveling of dunes as it would affect the productivity of grasses on flats and therefore can be adopted on a large-scale restoration plan. Whereas conserving stressed plants on destabilized dunes can be proposed to be implemented at a small scale to develop a protocol for large-scale restoration. In addition, the indigenous threatened species can be popularized as a productively chosen species by proposing it as a multiple-species shelter belt for sustainable agriculture, which will effectively control erosion and provide food and fodder, and pest control, and as a seed production area for recruitment. But in the absence of laws encompassing the conservation of natural vegetation on sandy land, these proposals seem to be ahead of their time.

Acknowledgments

We are also thankful to the rangeland officer and livestock officer at Rakh Khairewala for helping us during our visits to the site.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

No funding was reported.

Author contributions

Amin Khan conceived, designed the research and wrote the manuscript; Asad Abbas contributed in data collection and performing experiment; Asma Mansoor applied the all statistical analysis and edited the manuscript; Faiza Sharif and Zafar Siddiq reviewed the manuscript

Author ORCIDs

Amin U. Khan https://orcid.org/0000-0001-8521-2579
Faiza Sharif https://orcid.org/0000-0002-5142-9136
Asma Mansoor https://orcid.org/0000-0002-6786-3236
Zafar Siddiq https://orcid.org/0000-0002-1693-1649

Data availability

All of the data that support the findings of this study are available in the main text.

References

Abbas A (2015) Monitoring the status of native vegetation on the dunes of Rakh Kharewala and establishing a demonstration site for the stabilization of dunes with native vegetation. M.Phil Dissertation, Sustainable Development Study Center, Government college university Lahore Pakistan.

Aguiar MR, Sala OE (1999) Patch structure dynamics and implies for the functionality of arid ecosystem. Trends in Ecology & Evolution 14(7): 273–277. https://doi.org/10.1016/S0169-5347(99)01612-2

Akbari M, Shalamzari MJ, Memarian H, Gholami A (2020) Monitoring desertification processes using ecological indicators and providing management programs in arid regions of Iran. Ecological indicator 111: e106011. https://doi.org/10.1016/j.ecolind.2019.106011

Amiraslani F, Dragovich D (2011) Combating desertification in Iran over the last 50 years: An overview of changing approaches. Journal of Environmental Management 92(1): 1–13. https://doi.org/10.1016/j.jenvman.2010.08.012

Arshad M, Hassan AU, Ashraf MY, Noureen S, Moazzam M (2008) Edaphic factors and distribution of vegetation in the Cholistan desert, Pakistan. Pakistan Journal of Botany 40(5): 1923–1931.

Ash JE, Wasson RJ (1983) Vegetation and sand mobility in the Australian desert dune field. Zeitschrift für Geomorphologie 45(Supp.): 7–25.

Bakr N, Weindorf DC, Bahnassy MH, El-Badawi MM (2012) Multi-temporal assessment of land sensitivity to desertification in a fragile agro–ecosystem. Ecological Indicator 15(1): 271–280. https://doi.org/10.1016/j.ecolind.2011.09.034

Basso F, Bove E, Dumontet S, Ferrara A, Pisante M, Quaranta G, Taberner M (2000) Evaluating environmental sensitivity at the basin scale through the use of geographic information systems and remotely sensed data: An example covering the Agri basin (Southern Italy). Catena 40(1): 19–35. https://doi.org/10.1016/S0341-8162(99)00062-4

Bhadha JH, Capasso J, Khatiwada R, Swanson S, LaBorde C (2017) Raising Soil Organic Matter Content to Improve Water Holding Capacity: SL447 /Ss661. University of Florida Institute of Food and Agricultural Sciences, Gainesville. https://doi.org/10.32473/edis-ss661-2017

Book Hut (2014) Desertification in Pakistan and its Control. Retrieved From the Book Hut Website. http://www.bookhut.net/climate-change-desertification-in-pakistan/

Chacha MS, Andrew B, Vegi MR (2019) Amendment of Soil Water Retention and Nutrients Holding Capacity by Using Sugar Cane Bagasse. Current Agriculture Research Journal 7(2): 224–235. https://doi.org/10.12944/CARJ.7.2.10

Chen G, Zeng D, Chen F, Fan Z, Geng H (2003) A research review on “fertile islands” of soils under shrub canopy in arid and semi-arid regions. Ecological Indicator 14(12): 2295–2300.

D’Odorico P, Bhattachan A, Davis KF, Ravi S, Runyan CW (2013) Global desertification: Drivers and feedbacks. Advances in Water Resources 51: 326–344. https://doi.org/10.1016/j.advwatres.2012.01.013

Dotaniya ML, Datta SC, Biswas DR, Dotaniya CK, Meena BL, Rajendiran S, Lata M (2016) Use of sugarcane industrial by-products for improving sugarcane productivity and soil health. International Journal of Recycling of Organic Waste in Agriculture 5(3): 185–194. https://doi.org/10.1007/s40093-016-0132-8

Down to Earth (2021) How communities in China helped keep desertification at bay. Retrieved May 7. https://www.downtoearth.org.in/news/climate-change/how-communities-in-china-helped-keep-desertification-at-bay-66542

Fryrear DW (1985) Soil cover and wind erosion. Transactions of the ASAE. American Society of Agricultural Engineers 28(3): 781–0784. https://doi.org/10.13031/2013.32337

Gao Z, Li C, Yang X, Ci L (2010) Engineering and Technological Measures for Combating Desertification. Desertification and Its Control in China. Springer, Berlin. https://doi.org/10.1007/978-3-642-01869-5_9

Glen E, Smith MS, Squires V (1998) On our failure to control desertification: Implications for global change issues, and a research agenda for the future. Environmental Science & Policy 1(2): 71–78. https://doi.org/10.1016/S1462-9011(98)00007-0

Google Earth Pro (2021) Thal Desert. https://earth.google.com/web/search/South+Thal,+punjab+pakistan/@31.2281263,71.8098613,160.94733295a,4662.46777133d,35y [Accessed: 23 Dec 2022]

GOP [Govt. of Pakistan] (1968) Reconnaissance Soil Survey Thal South. Directorate of soil survey of Pakistan, Lahore.

GOP [Govt. of Pakistan] (1992) The Pakistan National Strategy. Government of Pakistan and International Union for Conservation of Nature (IUCN), Karachi.

Gratzfeld J, Khan AU (2015) Dry Woodlands in Pakistan’s Punjab province-Piloting restoration of unique yet vanishing natural assets. Botanic Gardens Conservation International, Richmond.

Hassan A, Hassan Z (1998) Thardeep Rural Development program. The story of the thar Rural development project from a donor supported relief effort to a national NGO. United Nations International Children’s Emergency Fund, Karachi, Pakistan.

Kamel-Eddine B (2022) Vegetation and landscape dynamics of the Guerbes-Benazouz dune cordon in Skikda, Algeria. Ukrainian Journal of Ecology 12(3): 36–45.

Kershaw KA, Looney JHH (1985) Quantitative and dynamic plant ecology (3rd edn.). Edward Arnold. Victoria, USA.

Khalili Moghadam B, Jamili T, Nadian H, Shahbazi E (2016) The influence of sugarcane mulch on sand dune stabilization in Khuzestan, the southwest of Iran. Iran Agricultural Research 34(2): 71–80. https://doi.org/10.22099/IAR.2016.3446

Khan A (1990) A report on the identification, comparative assessment & rationale for conserving the remnants of natural tropical thorn forest in Punjab. WWF-Pakistan, Lahore.

Khan AU (1994) History of decline and present status of natural tropical thorn forest in Punjab. Biological Conservation 67(3): 205–210. https://doi.org/10.1016/0006-3207(94)90611-4

Khan AU (1996) Appraisal of ethno-ecological incentives to promote conservation of Salvadora oleoides Decne: The case for creating a resource area. Biological Conservation 75(2): 187–190. https://doi.org/10.1016/0006-3207(95)00056-9

Khan AU (2003) Conservation of Wild Natural Resources and the need for good Governance in Pakistan. Lead, Pakistan.

Khan AU (2010) Monitoring structural assets of bi-species groves according to land use types: A case study from arid plains. Environmental Monitoring and Assessment 168(1–4): 121–131. https://doi.org/10.1007/s10661-009-1096-1

Khan AU (2022) Piloting restoration of four woody species on a sand ridge (unpublished). SCIV bulletin 2022.

Khan AU, Sharif F, Siddique Z, Hayat MU (2016) Conserving dry-land ecosystem in the Indus plains of Pakistan (unpublished). SDSC-BGCI Report.

Lancaster N (1988) Development of linear dunes in the southwestern Kalahari, southern Africa. Journal of Arid Environments 14(3): 233–244. https://doi.org/10.1016/S0140-1963(18)31070-X

Lancaster N, Baas A (1998) Influence of vegetation cover on sand transport by wind: Field studies at Owens Lake, California. Earth Surface Processes and Landforms 23(1): 69–82. https://doi.org/10.1002/(SICI)1096-9837(199801)23:1<69::AID-ESP823>3.0.CO;2-G

Levin N, Ben-Dor E (2004) Monitoring sand dune stabilization along the coastal dunes of Ashdod-Nizanim, Israel, 1945–1999. Journal of Arid Environments 58(3): 335–355. https://doi.org/10.1016/j.jaridenv.2003.08.007

McNeely JA (1988) Economics and Biological Diversity: Developing and Using Economic Incentives to Conserve Biological Resources. International Union for the Conservation of Nature, Gland.

Qureshi R, Bhatti GR (2008) Diversity of micro-habitats and their plant resources in Nara desert Pakistan. Pakistan Journal of Botany 40(3): 979–992.

Rezaie SA (2009) Comparison between Polylatice polymer and petroleum mulch on seed germination and plant stabilizement in sand dune fixation. Iranian Journal of Range and Desert Reseach 16: 124–136.

Rubinstein Y, Groner E, Yizhaq H, Svoray T (2013) An eco-spatial index for evaluating stabilization state of sand dunes. Aeolian Research 9: 75–87. https://doi.org/10.1016/j.aeolia.2012.08.007

Shumack S, Fisher A, Hesse PP (2021) Refining medium resolution fractional cover for arid Australia to detect vegetation dynamics and wind erosion susceptibility on longitudinal dunes. Remote Sensing of Environment 265: e112647. https://doi.org/10.1016/j.rse.2021.112647

Siegal Z, Tsoar H, Karnieli A (2013) Effects of prolonged drought on the vegetation cover of sand dunes in the NW Negev Desert: Field survey, remote sensing and conceptual modeling. Aeolian Research 9: 161–173. https://doi.org/10.1016/j.aeolia.2013.02.002

Singh AK (2004) Endangered economic species of Indian desert. Genetic Resources and Crop Evolution 51(4): 371–380. https://doi.org/10.1023/B:GRES.0000023452.91250.52

Sivaperuman C, Baqri QH, Ramaswamy G, Naseema M (2008) Faunal ecology and conservation of the Great Indian Desert. Springer Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-87409-6

Stewart RR (1972) An annotated catalogue of the vascular plants of West Pakistan. In: Nasir E, Ali SI (Eds) Flora of Pakistan. University Publications, Karachi, Pakistan.

Su YZ, Zhao HL, Li YL, Cui JY (2004) Influencing mechanisms of several shrubs on soil chemical properties in semiarid Horqin Sandy Land, China. Arid Land Research and Management 18(3): 251–263. https://doi.org/10.1080/15324980490451339

Thomas KA, Redsteer MH (2016) Vegetation of semi-stable rangeland dunes of the Navajo Nation, Southwestern USA. Arid Land Research and Management 30(4): 400–411. https://doi.org/10.1080/15324982.2016.1138157

Wasson RJ, Nanninga PM (1986) Estimating wind transport of sand on vegetated surfaces. Earth Surface Processes and Landforms 11(5): 505–514. https://doi.org/10.1002/esp.3290110505

Wolfe S, Bond J, Lamothe M (2010) Dune stabilization in central and southern Yukon in relation to early Holocene environmental change, northwestern North America. Quaternary Science Reviews 30(3): 324–334. https://doi.org/10.1016/j.quascirev.2010.11.010

Xiaodong G, Jinren N, Zhenshan L, Ronggui H, Xin M, Qing Y (2013) Quantifying the synergistic effect of the precipitation and land use on sandy desertification at county level: A case study in Naiman Banner, northern China. Journal of Environmental Management 123: 34–41. https://doi.org/10.1016/j.jenvman.2013.02.033

Yang X, Wang X, Liu Z, Li H, Ren X, Zhang D, Scuderi L (2013) Initiation and variation of the dune fields in semi-arid China–with a special reference to the Hunshandake Sandy Land, Inner Mongolia. Quaternary Science Reviews 78: 369–380. https://doi.org/10.1016/j.quascirev.2013.02.006

You Y, Zhou N, Wang Y (2021) Comparative study of desertification control policies and regulations in representative countries of the Belt and Road Initiative. Global Ecology and Conservation 27: e01577. https://doi.org/10.1016/j.gecco.2021.e01577

Zhang Y, Cao C, Han X, Jiang S (2013) Soil nutrient and microbiological property recoveries via native shrub and semi-shrub plantations on moving sand dunes in Northeast China. Ecological Engineering 53: 1–5. https://doi.org/10.1016/j.ecoleng.2013.01.012

Nature Conservation 53: 183–221 (2023)
DOI: 10.3897/natureconservation.53.106923

Research Article

Assessment of the threat status of reptile species from Vietnam - Implementation of the One Plan Approach to Conservation

Lilli Stenger[1], Anke Große Hovest[1], Truong Quang Nguyen[2,3], Cuong The Pham[2,3], Anna Rauhaus[4], Minh Duc Le[5,6,7], Dennis Rödder[8], Thomas Ziegler[4,9]

1 *University of Cologne, Cologne, Germany*
2 *Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology, Hanoi, Vietnam*
3 *Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Hanoi, Vietnam*
4 *Cologne Zoo, Cologne, Germany*
5 *Faculty of Environmental Sciences, University of Science, Vietnam National University, Hanoi, Vietnam*
6 *Central Institute for Natural Resources and Environmental Studies, Vietnam National University, Hanoi, Vietnam*
7 *Department of Herpetology, American Museum of Natural History, New York, USA*
8 *LIB, Museum Koenig Bonn, Leibniz Institute for the Analysis of Biodiversity Change, Bonn, Germany*
9 *Institute of Zoology, University of Cologne, Cologne, Germany*
Corresponding author: Thomas Ziegler (ziegler@koelnerzoo.de)

Abstract

Since the world is currently in the midst of a major biodiversity crisis, scientists have assigned high conservation priority to 36 biodiversity hotspots around the world. As part of one of the leading hotspots in terms of species richness and local endemism, Vietnam is considered a country with high conservation priority. The reptile fauna of Vietnam is known for its high level of diversity and an outstanding number of endemic species. Vietnamese reptiles are highly threatened due to habitat loss and overharvesting for domestic and international trade, traditional medicine and food, making them a group of great conservation concern. As a baseline for improved reptile conservation in Vietnam, we conducted a conservation assessment of Vietnamese reptile species by evaluating data from a variety of sources. Our study results show that approximately 32.9% (n = 159) of the total reptile species (n = 484) present in Vietnam are endemic to the country, of which more than half are only known from their type locality and about one-third restricted to a particular subregion, making the species particularly vulnerable to threats. Furthermore, 33.5% (n = 53) of 158 endemic taxa included in the protected area analysis have not yet been recorded from any protected area. Among all 418 Vietnamese reptile species listed on the IUCN Red List, 17.7% (n = 74) are threatened with extinction, 46.0% (n = 34) of the total 74 threatened species are endemic to Vietnam. The fact that 135 species are either listed as DD or have not yet been evaluated by the IUCN highlights the urgency of further research. Moreover, only very few species are protected by national or international legislation, and further assessments are needed to protect reptiles of particular concern. A Zoological Information Management System (ZIMS) analysis revealed that 22.5% (n = 109) of all reptiles occurring in Vietnam and only 6.3% (n = 10) of the endemic Vietnamese reptiles are currently kept in zoos worldwide. Although 60.8% (n = 45) of the threatened reptiles (n = 74) from Vietnam are currently held in zoos, only 23.5 (n = 8) of the endemic threatened species (n = 34) are held there. Following the IUCN CPSG`s One Plan Approach to Conservation, it is therefore recommended to increase the number of threatened and endemic species in breeding stations and zoos to maintain assurance populations, suitable for restocking measures.

Academic editor: Md Mizanur Rahman
Received: 24 May 2023
Accepted: 5 August 2023
Published: 5 September 2023

ZooBank: https://zoobank.org/F7D034BA-196F-45FF-B7F3-092A1EC16DC3

Citation: Stenger L, Große Hovest A, Nguyen TQ, Pham CT, Rauhaus A, Le MD, Rödder D, Ziegler T (2023) Assessment of the threat status of reptile species from Vietnam - Implementation of the One Plan Approach to Conservation. Nature Conservation 53: 183–221. https://doi.org/10.3897/natureconservation.53.106923

Despite ongoing efforts in Vietnam, further conservation measures are required. We therefore also identify areas of highest reptile diversity and with the largest number of threatened species and provide a list of 50 most threatened species (10% of total species) as a guide for further research and conservation action in Vietnam.

Key words: Conservation breeding, diversity, endemic species, protected area coverage, reptile conservation, threat analysis, Vietnam

Introduction

The world is currently in the midst of a major biodiversity crisis, associated with significant biodiversity loss and extinction rates far outpacing normal background extinction rates. While some predict a sixth mass extinction, others fear that we may be right in the midst of it already (Barnosky et al. 2011; Pievani et al. 2014; Ceballos et al. 2015; McCallum 2015; Ceballos et al. 2017). Our planet's biodiversity is changing immensely, and at a pace that would not have occurred without humankind's influence (Pimm et al. 1995; Cowie et al. 2022; Rull 2022). At this rate, up to one million plant and animal species could disappear (IPEBS 2019; Tollefson 2019), and extinction rates are expected to be even higher due to numerous species still remaining unidentified (Lees and Pimm 2015; Melville et al. 2021).

As the number of threatened species in need of conservation efforts is greater than the available resources, it is important to prioritize most threatened taxa and areas with the greatest number of endemic and threatened species. To this end, regions with high levels of biodiversity and facing critical anthropogenic threats have been identified (Myers et al. 2000). To date, 36 global biodiversity hotspots characterized by significant habitat loss have been assigned a high priority for conservation (Myers et al. 2000). Many of them may face significant future threats due to increased anthropogenic pressure and climate change effects (Cremene et al. 2005; Malcolm et al. 2006; Habel et al. 2019).

One of the leading hotspots in terms of endemism is the Indo-Burma region, which is composed of southern China and the mainland of Southeast Asia, including Vietnam (Myers et al. 2000). Due to its high level of endemism combined with the accelerating rate of habitat loss and overexploitation, Vietnam is considered a country with top conservation priority (Myers et al. 2000; Sterling et al. 2006). The country possesses a broad variety of rare and endemic species and its herpetofauna has been recognized as one of the most diverse in the world (Stolton et al. 2004; Adler 2009). A vast number of reptile and amphibian species have been discovered from Vietnam over the past decades, with many microendemic taxa including those known only from type localities so far (e.g., Bain and Hurley 2011; Ngo et al. 2022). With decreasing range, the risk of extinction generally increases (Chichorro et al. 2019). Therefore, species occuring exclusively in small areas or which are restricted to their type locality are particularly threatened and thus require more protection (Meiri et al. 2017).

To protect both biodiversity and natural habitats, the government of Vietnam established the first protected area, Cuc Phuong National Park, in 1962 (Sterling et al. 2006). Since then, the number of national parks (NP) has grown to 34, accounting for approximately 3% of the total land area, with the last one being established in 2020, i.e., Song Thanh NP. Additionally, the country maintains 88 nature reserves, 22 marine protected areas, 1 wetland protected area and 8 Ramsar sites (Wetlands of International Importance) (Stolton et al. 2004; Le et al. 2018; VEA 2020; Protected Planet 2022).

However, many species still suffer from habitat loss and degradation, overexploitation, invasive species, disease, climate change, and pollution, and are highly vulnerable to extinction (Sterling et al. 2006; Drury 2011; Blair et al. 2017; Blair et al. 2022). To reverse the trend, *in situ* conservation reinforced by *ex situ* measures has been proposed in the One Plan Approach to Conservation (OPA) developed by the Conservation Planning Specialist Group (CPSG) of the International Union for Conservation of Nature (IUCN) Species Survival Commission (SSC). In order to reduce the gap between the management of wild and *ex situ* populations, reintroduction of certain species into their natural habitat can take place when the protection level improves to prevent targeted species from extinction (Conde et al. 2013; Seddon et al. 2014a, b). In this regard, modern zoos can play a crucial role by not only conducting or financially supporting *in situ* conservation projects, but also by protecting species from extinction through the build-up of conservation breeding programs for subsequent release (Gilbert et al. 2017; Gusset 2019; Wahle et al. 2021; Krzikowski et al. 2022). This approach aims to bring together all responsible parties and considers the *in situ* and *ex situ* populations as a single unit with a view to developing a conservation plan for the entire population (Gusset 2019; Traylor-Holzer et al. 2019; Wahle et al. 2021). As modern ark, the "conservation zoo" provides space, expertise, time and funds for threatened species.

Globally, reptiles have been considered a group of special conservation concern (Böhm et al. 2013; Stanford et al. 2020; Cox et al. 2022). They play an important role in almost all ecosystems and often have relatively small distribution ranges, making them vulnerable to anthropogenic threats. While about 11% of the 11,460 reptile species described so far (Uetz et al. 2022) have still not been assessed by the IUCN, an additional 14.7% of the 10148 species assessed are listed as Data Deficient. Approximately 21% of the reptile species assessed up to now are considered to be threatened with extinction (Cox et al. 2022). In particular, agricultural expansion poses the greatest threat to reptiles. The resulting habitat loss is, among other places, particular evident in the mainland of Southeast Asia (Böhm et al. 2013).

As Vietnam's herpetofauna is among the richest in the world (Adler et al. 2009; Nguyen et al. 2009) and especially threatened due to habitat loss and being overharvested for traditional medicine, trade, and food (Van Schingen et al. 2015; Janssen and Indenbaum 2019; Pham et al. 2019a, b; Le et al. 2020), it is critical to assess the conservation status of Vietnamese species. Following the recently published study on amphibians in Vietnam (Krzikowski et al. 2022) we herein provide a detailed assessment of Vietnam's reptile fauna in light of the One Plan Approach. In order to establish a baseline for improved reptile conservation in Vietnam, an up-to-date list of all reptile species extant in Vietnam was compiled and they were then evaluated individually for their IUCN Red List status, listing in national legislations and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), distribution range, potential occurrence in protected areas, and whether an ex situ component in zoos or other institutions already exists. Finally, we provide a list of those species that are particularly important to be considered for further OPA measures in the future.

Methods

Species list

The list of extant reptile species in Vietnam was based on Nguyen et al. (2009). The species list also comprised sea snakes and sea turtles (except for the protected area coverage analysis, see below). We then cross-checked each account with the reptile database (https://reptile-database.reptarium.cz/, Uetz et al. 2022) and new publications were included using the search engine Google Scholar (https//:scholar.google.com) to document taxonomic changes and species records in Vietnam after 2009 (see Suppl. material 1: tables S1–S5).

We generally followed the taxonomy of Nguyen et al. (2009). However, while Homalopsinae, Pareatinae, Psammophiinae, and Xenodermatinae were classified as subfamilies of Colubridae (Serpentes) in Nguyen et al. (2009), this study followed Pyron et al. (2011) in placing Psammophiinae in the family of Lamprophiidae and elevating the other subfamilies to the family level. In addition, the genus *Psammodynastes*, including the species *P. pulverulentus*, was transferred from the subfamily Natricinae (Colubridae: Serpentes) to the family Lamprophiidae (Pyron et al. 2011).

Conservation status

The extinction risk assessment of the reptile species extant in Vietnam was undertaken using the IUCN Red List of Threatened Species on the 5th of January 2022 (IUCN 2022) using automatized searches via the rredlist package for R 4.2.2 (Chamberlain 2022). We considered species either Not-threatened, which compromise Least Concern (LC) and Near Threatened (NT), threatened with extinction, which compromise Vulnerable (VU), Endangered (EN) and Critically Endangered (CR), or unclassifiable, which compromise Data Deficient (DD) and Not Evaluated (NE). No species on the list were listed as Extinct (EX) or Extinct in the Wild (EW). We further analysed the recorded species with respect to inclusion in the three appendices (I-III) of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES)(CITES 2021a, b).

National regulations were examined by evaluating the appendices of national decrees and the listing in the Vietnam Red Data Book. In Vietnam, species are primarily protected by two national decrees. While the first one, Decree No. 64/2019, lists species with highest conservation priority, the second, Decree 84/2021, largely follows CITES in listing species that are threatened by trade and/or overexploitation. Threatened species are also listed in the Vietnam Red Data Book which uses the IUCN Red List Categories. If a species is endemic to Vietnam, its status in the Vietnam Red Data Book may differ from the global IUCN Red List status. The latest version of the Vietnam Red Data Book has been published in 2007 (Tran et al. 2007) and is thus outdated, but an updated version is already under preparation (Krzikowski et al. 2022).

Vietnam and its biogeographic subregions

The study follows Bain and Hurley (2011) in dividing Vietnam into 13 different geographic subregions, namely Northwest Uplands (NWU), Northeast Uplands (NEU), Northern Annamites (NAN), Northeast Lowlands (NEL), Northern Coast

(NC), Northern Islands (NIS), Central Annamites (CAN), Central-South Vietnam Lowlands (CSL), Central Coast (CC), Southern Annamites (SAN), Mekong Delta (MEK), Southern Coast (SC) and Southern Islands (SIS) (see Fig. 1). Originally, Bain and Hurley (2011) referred their study area as Indochina, which was defined as Laos, Cambodia, and Vietnam, and split it into 19 subregions based on topographic and geographic criteria, including locations on the coast, island groups, mountain ranges and associated lowland regions and major river systems and their deltas. However, six of these subregions do not occur in Vietnam and are therefore irrelevant for this study. The 13 subregions occurring in Vietnam are assigned to different regions: Uplands, Lowlands, Coasts and Islands, all characterized by distinct climatic conditions and vegetation characteristics. Uplands are defined as locations above 450 meters, and locations below 450 meters are considered lowlands (Bain and Hurley 2011).

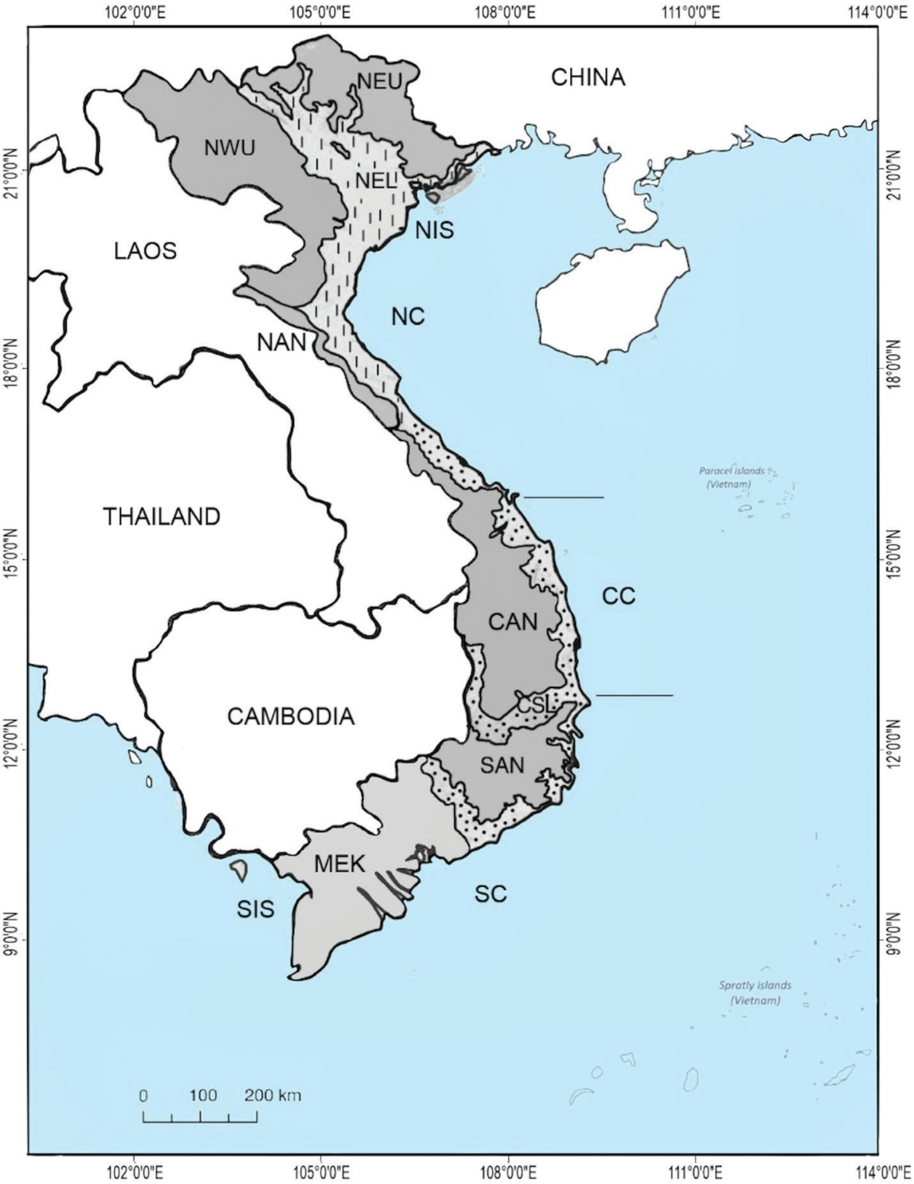


Figure 1. Map of Vietnam with its 13 subregions; adopted and modified from Bain and Hurley (2011).

Distribution

Information on the current distribution of each species extant in Vietnam was obtained based on the most recent species list by Nguyen et al. (2009), entries in the Reptile Database, distribution data from the IUCN Red List and the following publications: Bain and Hurley (2011), Wang et al. (2018, 2021), Nguyen LT et al. (2016, 2018a, b, 2020), Thao (2020), Nguyen, TQ et al. (2010b, 2017, 2018a, b), Hoang et al. (2018), Luu et al. (2015a, b, 2020a,c), Ngo et al. (2016, 2018, 2019a, b, 2021, 2022), Orlov et al. (2008, 2021), Grismer et al. (2015, 2019a, b, 2020, 2021a, b), Tung et al. (2018), Ostrowski et al. (2021), Murdoch et al. (2019), Pham et al. (2015, 2019a, b, 2020), Neang et al. (2020), Meiri et al. (2018), Ziegler et al. (2010, 2014, 2015a, b, c, d, 2019b, 2020a, b, c, d), Hecht et al. (2013), Le DT et al. (2018, 2020), Le DO et al. (2021), Richmond et al. (2021), Linh et al. (2019), Poyarkov Jr et al. (2019a, b, c), Siler et al. (2018), Wang et al. (2013), Van et al. (2014), Mallik et al. (2020), Le et al. (2021), Van Nguyen et al. (2019), Holden et al. (2021), Geissler et al. (2011a) Ren et al. (2018), Do et al. (2017), Nguyen SN et al. (2016, 2017), Amarasinghe et al. (2015), Rasmussen et al. (2011, 2012), Kurniawan et al. (2021), Ding et al. (2020), Li et al. (2021), Miller et al. (2020), Farkas et al. (2019), TTWG (2021), Chen et al. (2021), David et al. (2008), Sy (2019), Tan et al. (2019).

Species endemic to Vietnam and the Indochinese Region were identified and their level of endemism was further analysed, viz. whether these species are endemic to a specific subregion, region or on a macroregional or local level. The three macroregions were identified as North, Central or South, each representing up to four regions including uplands (elevations above 450m), lowlands (elevations below 450m), coasts or islands. For this purpose, the 13 subregions according to Bain and Hurley (2011) (n = 13: Northwest Uplands (NWU), Northeast Uplands (NEU), Northern Annamites (NAN), Northeast Lowlands (NEL), Northern Coast (NC), Northern Islands (NIS), Central Annamites (CAN), Central-South Lowlands (CSL), Central Coast (CC), Southern Annamites (SAN), Mekong Delta (MEK), Southern Coast (SC), Southern Islands (SIS)) were used as distribution measures.

For each species, we extracted detailed information on preferred habitats from the IUCN Red List using the rredlist package for R (Chamberlain 2022). Gridded information on habitat availability with a spatial resolution of 100 m was obtained from Jung et al. (2020) and intersected with the range information. Subsequently, we intersected the presence-absence maps of all terrestrial and limnic species (n = 454, excluding the 30 marine species, see Suppl. material 1: table S16) with the protected area network and extracted the potential of each species to occur in a given reserve. Furthermore, we created species richness maps by stacking the single presence-absence maps. Areas of high local endemism were identified using the corrected weighted endemism approach of Crisp et al. (2001).

Vietnamese reptiles in global ex situ facilities

For this analysis, the Zoological Information Management System (ZIMS) was used to identify which reptile species extant in Vietnam are currently kept in zoos worldwide and which species are currently managed in studbooks or other coordinated breeding programs. In addition, data on the number of held individuals and the number of keeping institutions was recorded for each species (ZIMS 2022). By using this approach, we could examine which of the species extant in Vietnam were already represented in *ex situ* facilities and how many of the species were considered threatened or were endemic to Vietnam. Based on the results, the proportion of threatened and endemic species extant in Vietnam not yet kept in zoos or other institutions globally was calculated. Since participation in ZIMS is voluntary and some data may not be up to date, some held populations may have been omitted. In order to increase coverage, the data obtained from ZIMS were compared with those available on the website “Zootierliste” (ZTL, List of Zoo Animals – Zootierliste 2022). Nevertheless, the database includes only facilities from Europe. ZTL also does not have other detailed information such as breeding success and thus only was checked for additional species holdings, viz. those not available in ZIMS.

Diversity analysis

In order to identify major geographic patterns in the distribution of zoos keeping Vietnamese taxa, we computed for each facility the Shannon index (Weaver and Shannon 1949) taking both the number of different taxa and number of indiviudals per species into acount. The georeferenced localities of each facility were subsequently mapped and coded according to the number of individuals and the respective indices.

Top 50 list

As a guideline for further conservation action regarding reptiles in Vietnam, we compiled a Top 50 list of species likely to benefit most from conservation efforts based on the data of this study. For this purpose, a rating system was established and points were assigned for 1) IUCN Red List status, 2) year of latest assessment, 3) level of endemism 4) *ex situ* populations, 5) inclusion in legislation and 6) no occurrence in protected areas. For more detailed information about the categories and the evaluation, see Suppl. material 1: table S19.

Results

Reptile diversity

With a total of 484 reptile species, representing all orders, Vietnam harbors 4.2% of the global reptile diversity (Table 1). The order Testudines was the highest represented, comprising 8.6% (n = 31) of the global species richness, followed by Serpentes with 6.2% (n = 244), Crocodylia with 3.7% (n = 1) and Sauria with 2.9% (n = 208) (see Table 1). The order Squamata, with its suborders Sauria and Serpentes, accounted for 93.4% (n = 452) of all 484 reptile species recorded in Vietnam. Specifically, more than half of the squamates were snakes (54%, n = 244), the others belonged to the suborder of lizards (n = 208). Roughly 6.4% (n = 31) of all species in Vietnam (n = 484) belonged to the order Testudines and 0.2% (n = 1) to Crocodylia, which is represented by only one extant species. All of the species belonged to 28 families and 123 genera. The most speciose family was Colubridae with 134 species, followed by Gekkonidae with 93 species. Containing a total of 50 species, the genus *Cyrtodactylus* had the highest species richness (Sauria, Gekkonidae) (13 most species rich genera, Suppl. material 1: table S6).

Table 1. Reptile fauna worldwide, in Vietnam and Vietnamese endemics (percentage compared to global scale). Data for the number of reptiles worldwide were compiled using the Reptile Database (Uetz et al. 2022).

(Sub)order	Worldwide	Vietnam	Vietnamese endemics
Crocodylia	27	1 (3.7%)	0 (0%)
Sauria	7144	208 (2.9%)	119 (1.7%)
Serpentes	3956	244 (6.2%)	37 (0.9%)
Testudines	360	31 (8.6%)	3 (0.8%)
Total	11460	484 (4.2%)	159 (1.4%)

Out of the total 484 reptile species extant in Vietnam, 38.2% (n = 185) were endemic to the Indochinese region, whereas the proportion of Vietnamese endemic species was 32.9% (n = 159). With 74.8% (n = 119), the order Sauria possessed the most species among Vietnamese endemic species, followed by Serpentes and Testudines with 23.2% (n = 37) and 2% (n = 3), respectively.

IUCN Red List status

An IUCN Red List status was available for 418 out of 484 reptile species reported from Vietnam, representing a total of 86.4% (See Fig. 2). Percentage-wise, 13.6 (n = 66) of the 484 Vietnamese reptile species had not yet been assessed by the IUCN, including 29 lizard species, 34 snakes and three turtles.

Among all 418 reptile species assessed by the IUCN, 17.7% (n = 74) were threatened with extinction. 20 of these species were classified as Critically Endangered (CR), 22 species as Endangered (EN), and 32 species as Vulnerable (VU) (Table 2). Measured in absolute numbers, lizards were found to be the most threatened order (31 species), followed by turtles with 27 threatened species, snakes with 15 threatened species, and one crocodile species classified as Critically Endangered. In terms of relative proportions of threatened and non-threatened species within each order, Crocodylia was the most threatened

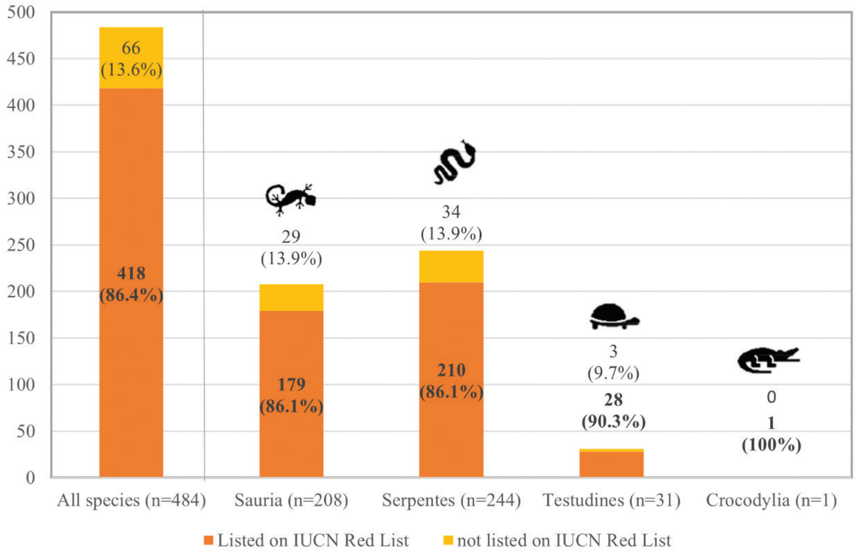


Figure 2. Listing on the IUCN Red List of threatened species. Total number of Vietnamese reptile species listed and not listed in total and separated by order.

Table 2. Vietnamese reptile species and their IUCN Red List status, including endemics.

	IUCN Red List status						No status (NE)	Total
	CR	EN	VU	NT	LC	DD		
All Vietnamese reptile species								
Crocodylia	1	0	0	0	0	0	0	1
Sauria	4	9	18	7	110	31	29	208
Serpentes	0	4	11	4	153	38	34	244
Testudines	15	9	3	1	0	0	3	31
Total	20	22	32	12	263	69	66	484
Endemics								
Sauria	4	8	15	4	35	28	25	119
Serpentes	0	2	2	1	0	15	17	37
Testudines	3	0	0	0	0	0	0	3
Total	7	10	17	5	35	43	42	159

order with 100% (n = 1), immediately followed by Testudines, with 96.4% threatened species (27 out of 28 listed turtle species). Lizards with 17.3% (31 out of 179 listed species) and snakes with 7.1% (15 out of 210 listed species) were much less threatened. More than half (61.5%; n = 110) of the lizards listed (n = 179) and two-thirds (72.9%; n = 153) of the snakes (n = 210) were considered LC, and about one-fifth of each of these orders was listed as DD.

In the IUCN Red List, 73.6% (n = 117) of all endemic species from Vietnam (n = 159) were included. While 36.8% (43 species) were classified as DD, 29.1% (34 species) were considered threatened with extinction and the remaining 34.1% (40 species) non-threatened (see Table 2, see examples in Fig. 3). While the endemic species from Vietnam evaluated by the IUCN accounted for 28% (n = 117) of all assessed species (n = 418), they also covered 45.9% (34 of 74 species) of all species assessed as threatened. They made up 35.0% (n = 7) of all CR species (n = 20), 45.5% (n = 10) of all EN species (n = 22), and 53.1% (n = 17) of all VU species (n = 32).

Listing in CITES and Vietnam's decrees

Appendix I of CITES included 13 reptile species from Vietnam: nine turtles, three lizards, and one crocodile. A total of 34 species were listed in appendix II, including 18 turtles, nine snakes, and seven lizards. Only two turtles were not listed in CITES appendices I or II. *Mauremys sinensis* was listed in appendix III and *Amyda ornata* was not included in any appendix (Table 3).

Similar to CITES, Decree No. 84/2021 contains appendices I and II, listing a total of 42 species. All lizards (n = 10) listed in Decree No. 84/2021 were identical to those in CITES, as were the listings of eight snakes. The ninth snake species, *Ophiophagus hannah*, however, was included in appendix I whereas internationally it was listed in appendix II. Identical national and international listings were also found for 17 turtles. Seven species were found in CITES but not in Decree No. 84/2021, two species were listed in Decree No. 84/2021 but not in CITES and three species were placed in appendix I in Decree No. 84/2021 but only in appendix II in CITES (Table 3).

Figure 3. Threatened endemic reptile species from Vietnam **A** *Cuora picturata* (IUCN: CR) **B** *Leiolepis guentherpetersi* (IUCN: EN) **C** *Boiga bourreti* (IUCN: EN) **D** *Trimeresurus truongsonensis* (IUCN: EN) (Photos: C. T. Pham (**A**) A. Rauhaus (**B**) T. Ziegler (**C, D**)).

The Vietnam Red Data Book comprises a total of 41 reptiles with 20 turtles (CR: 5, EN: 10, VU: 5), 15 snakes (CR: 2, EN: 6, VU: 7), five lizards (EN: 2, VU: 3) and one crocodile (CR: 1). Of 74 species categorized as threatened by the IUCN, 41 species (55.4%) of the IUCN listed threatened reptiles, were present in the Vietnam Red Data Book. Only six species shared the same status between the IUCN Red List and the Vietnam Red Data Book. As for 32 species with different status, 21 were evaluated as more threatened in the Vietnam Red Data Book than by the IUCN Red List. The other eleven, however, were assigned more threatened status by the IUCN Red List than in the Vietnam Red Data Book. *Cuora cyclornata* was listed in the Vietnam Red List (CR) but had not been evaluated by the IUCN. In addition, 30 of the 33 threatened endemic reptiles in the IUCN Red List were not incorporated in the Vietnam Red Data Book. Only three threatened endemic species, all belonging to the order Testudines, were listed in the Vietnam Red Data Book: *Cuora picturata*, *Mauremys annamensis* and *Rafetus swinhoei*. The latest Red Data Book was published in 2007 and while 18 threatened species were described after the publication, three snakes and nine lizards had been described before and could have been included.

In Decree No. 64/2019, consisting of species of high conservation priority, 15 reptiles, comprising two lizards, one snake, and 12 turtles, were incorporated. All of the species, except for the two lizard species, were also listed in the Vietnam Red Data Book as either CR or EN. Only four of the species, namely *Cnemaspis psychedelica*, *Cuora picturata*, *Mauremys annamensis* and *Rafetus swinhoei*, are endemic to Vietnam.

Table 3. Threat status of reptile species extant in Vietnam, including sea turtles, listed in CITES, Decree 64/2019, Decree 84/2021 or the Vietnam Red Data Book and their IUCN Red List status. Endemic: *: species is endemic to Vietnam; ** species is endemic to the Indochinese Region. *Rafetus swinhoei* is considered endemic to Vietnam because the population in China is no longer viable with the only known extant male being sterile.

Species	Endemic	IUCN Red List status	Vietnam Red Data Book	Decree 64/2019	Decree 84/2021	CITES
Crocodylia						
Crocodylus siamensis		CR	CR		IB	I
Sauria						
Cnemaspis psychedelica	*	EN		Yes	IB	I
Gekko gecko		LC	VU		IIB	II
Goniurosaurus araneus		EN			IIB	II
Goniurosaurus catbaensis	*	EN			IIB	II
Goniurosaurus huuliensis	*	CR			IIB	II
Goniurosaurus lichtenfelderi		VU			IIB	II
Goniurosaurus luii		VU			IIB	II
Leiolepis reevesii		LC	VU			
Physignathus cocincinus		VU	VU			
Shinisaurus crocodilurus vietnamensis	*	EN		Yes	IB	I
Varanus bengalensis nebulosus		NT	EN		IB	I
Varanus salvator		LC	EN		IIB	II
Serpentes						
Azemiops feae		LC	VU			
Bungarus fasciatus		LC	EN			
Coelognathus radiatus		LC	VU			
Elaphe moellendorffi		VU	VU			
Euprepiophis mandarinus		LC	VU			
Gonyosoma prasinum		LC	VU			
Malayopython reticulatus		LC	CR		IIB	II
Naja atra		VU	EN		IIB	II
Naja kaouthia		LC	EN		IIB	II
Naja siamensis		VU	EN		IIB	II
Ophiophagus hannah		VU	CR	Yes	IB	II
Oreocryptophis porphyraceus		LC	VU			
Ptyas korros		NT	EN			
Ptyas mucosa		LC	EN		IIB	II
Python bivittatus		VU			IIB	II
Python brongersmai		LC			IIB	II
Python curtus		LC			IIB	II
Subsessor bocourti		LC	VU			
Testudines						
Amyda ornata		NE			IIB	
Caretta caretta		VU	CR	Yes		I
Chelonia mydas		EN	EN	Yes		I
Cuora amboinensis		EN	VU		IIB	II

Species	Endemic	IUCN Red List status	Vietnam Red Data Book	Decree 64/2019	Decree 84/2021	CITES
Cuora bourreti	**	CR	EN	Yes	IB	I
Cuora cyclornata		NE	CR	Yes	IB	II
Cuora galbinifrons		CR	EN	Yes	IB	II
Cuora mouhotii		EN			IIB	II
Cuora picturata		CR	EN	Yes	IB	I
Cyclemys oldhami		EN			IIB	II
Cyclemys pulchristriata	**	EN			IIB	II
Dermochelys coriacea		VU	CR	Yes		I
Eretmochelys imbricata		CR	EN	Yes		I
Geoemyda spengleri		EN			IIB	II
Heosemys annandalii		CR	EN		IIB	II
Heosemys grandis		CR	VU		IIB	II
Indotestudo elongata		CR	EN			II
Lepidochelys olivacea		VU	EN	Yes		I
Malayemys subtrijuga	**	NT	VU		IIB	II
Manouria impressa		EN	VU		IIB	II
Mauremys annamensis	*	CR	CR	Yes	IB	I
Mauremys mutica		CR			IIB	II
Mauremys sinensis		CR				III
Palea steindachneri		CR	VU		IIB	II
Pelochelys cantorii		CR	EN	Yes	IB	II
Platysternon megacephalum		CR	EN		IB	I
Rafetus swinhoei	*	CR	CR	Yes	IB	II
Sacalia quadriocellata		CR			IIB	II
Siebenrockiella crassicollis		EN			IIB	II

Distribution

The diversity of the reptile fauna varied among the different subregions of Vietnam. The highest species diversity among the 13 subregions occurred in the Northwestern Uplands with 172 reptile species, followed by the Northeastern Lowlands with 166 and Northeastern Uplands and the Central Annamites with 164 species each. The highest endemic species richness was found in the Central Annamites subregion with 32 species (Fig. 4B, Suppl. material 1: table S7). Looking further at the distribution of endemic species, 89.9% (n = 143, out of total 159) were endemic to one of the three macroregions with most of them found in Southern Vietnam (59 species out of 143, 41.3%), followed by Northern Vietnam (48, 33.6%) and Central Vietnam (36, 25.2%). While 135 species (84.9%) out of total 159 endemics were recorded from just one region, 22 were documented from two regions and the remaining two from three regions. No endemic reptile species was recorded from all four regions (Uplands, Lowlands, Coasts and Islands) (Suppl. material 1: tables S8, S9, S15).

The 135 species occuring in just one region were considered regional endemics. Only four of the regional endemic reptiles (3.0%) occured in two subregions, the other 97.0% (131 species) were exclusively found in one subregion

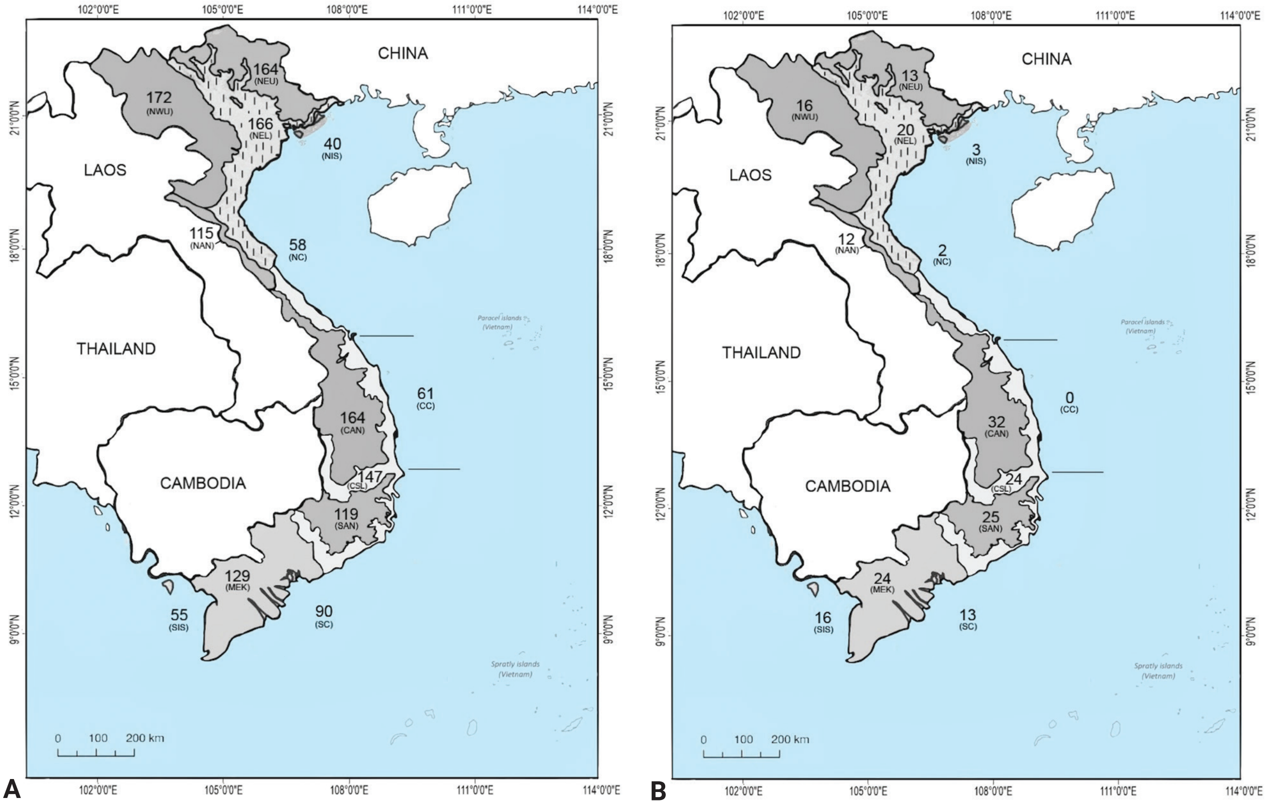


Figure 4. Map of Vietnam with its 13 subregions and number of recorded reptile species, separated by different factors **A** number of recorded reptile species per subregion among total 484 species **B** number of Vietnamese endemic reptile species per subregion among total 159 species.

only, and thus considered subregional endemics (Suppl. material 1: table S11). Approximately 74.0% of the subregional endemics (n = 131) were lizards (97 species) and the other 26.0% were snakes (34 species). None of the subregional endemics was a turtle. Of the endemic species belonging to the order Sauria (119 species), 81% (n = 97) were subregional endemics and distributed in 12 of the 13 subregions. For the order Serpentes, 92% (n = 34) of the 37 endemic species were subregional endemics, occurring in 11 subregions. Among the 13 subregions, most subregional endemics were restricted to the Central Annamites subregion, where 21 of the 131 subregional endemic species (16%) occured (Fig. 5, Suppl. material 1: table S11).

Regarding the distribution of endemic species and their IUCN Red List status, most threatened species (44.1%) were endemic to Southern Vietnam (15 out of 34 threatened species, CR: 2; EN: 2; VU: 11), further 23.5% of the threatened species (8 species, CR: 1; EN: 5; VU: 2) were endemic to Northern Vietnam and six species (CR: 3; VU: 3) to Central Vietnam. In terms of only subregional endemic species (n = 131), a considerable number (31%, 41 species) had not yet been evaluated by the IUCN and a further 27% (36 species) were assessed as DD. In percentage terms, 35% (n = 26) of all threatened species (74 species) were endemic on the subregional level (CR: 4; EN: 7; VU: 15). The subregion with most threatened subregional endemics was the Mekong Delta with seven threatened species (CR: 1; VU: 6), followed by the Southern Islands (SIS) with five threatened endemics (EN: 2; VU: 3) and the Central-Southern Lowlands (CSL) with three threatened species (CR: 1; VU: 2) (Suppl. material 1: tables S12–S14).

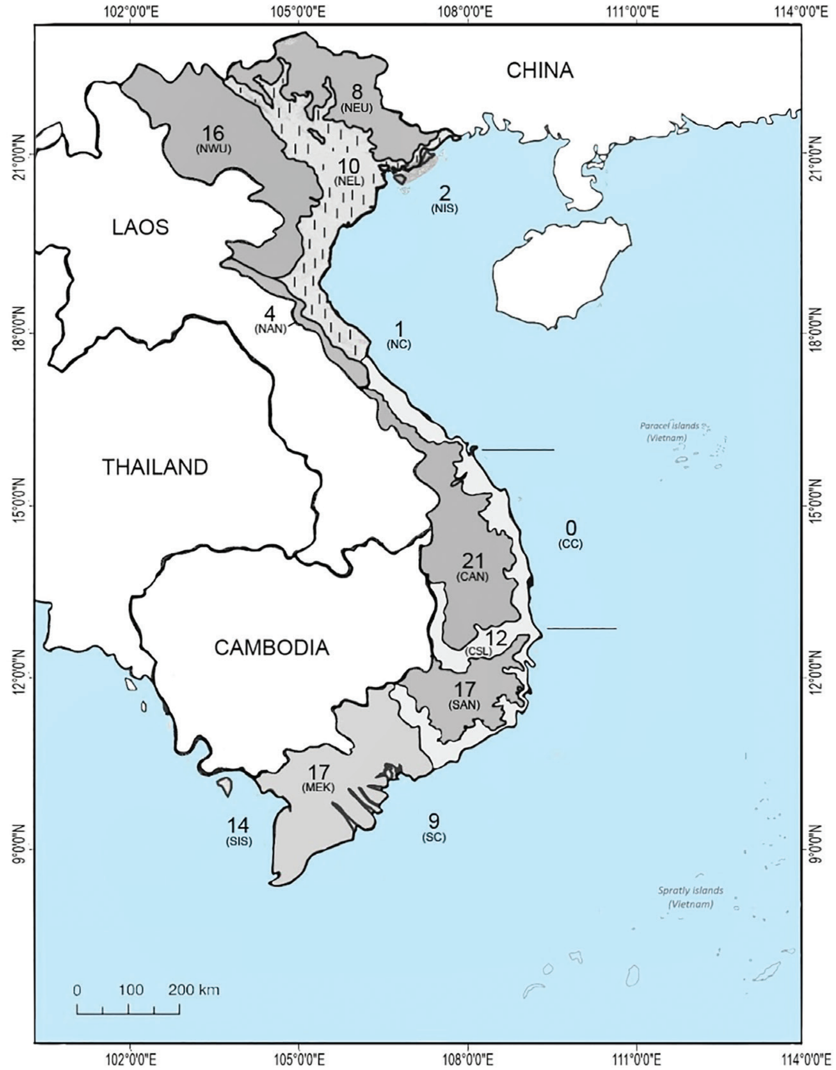


Figure 5. Map of Vietnam with its 13 subregions and number of subregional endemic reptile species (n = 131).

Further analyses showed that while 43 species of subregional endemics (n = 131) occured in multiple locations within their respective subregion, 88 species did not. These species were endemic at the local level, which means they were exclusively reported from their type localities and represented a majority of endemic species at 55.3% (n = 88) (Table 4).

Table 4. Distribution range of Vietnamese endemic reptile species, separated by size of area in which they occur.

Distribution	Description	Number of species	Percentage
Local	Type locality only	88	55%
Subregional	Multiple locations within one subregion	43	27%
Regional	Multiple locations within a region in one or two macroregions	4	3%
Macroregional	Multiple locations in two or more regions within a macroregion	13	8%
Widespread	Multiple locations in two macroregions	8	5%
Countrywide	Multiple locations in all three macroregions	3	2%
Total		159	100%

Coverage by protected areas

A total of 78 out of the 454 terrestrial and limnic reptile species included in the protected area analysis had not been reported from any protected area and are not likely to be covered. Of the species, 53 were endemic and 19 were threatened. Up to 32.1% (n = 17) of the 53 endemic species not yet found in any protected area were considered threatened (CR: 4; EN: 2; VU: 11) and another 52.8% were still listed as DD (20 species) or NE (8 species). As many as 27 of the 53 endemic species were only reported from their type locality. Another 15.1% (8 species) were subregional endemics (Figs 6, 7, Suppl. material 1: table S17).

Ex situ keeping of Vietnamese reptiles

The ZIMS analysis showed that 109 out of 484 reptile species reported to occur in Vietnam (22.5%) were held in zoos around the world. A total of 17 of the species were either endemic to Indochina (n = 7) or to Vietnam (n = 10). Thus, 6.3% of all endemic Vietnamese reptiles (10 of 159 species) were represented in zoo husbandries. According to ZIMS, the other 93.7% (n = 149) of 159 endemic reptile species were not kept in any zoo. The held endemic species consisted of seven lizards, two turtles, and one snake.

According to the IUCN Red List, 41.3% of the 109 species held were considered threatened with extinction (45 species; CR: 15; EN: 16; VU: 14) (Table 5). A further 3.7% were classified either as DD (n = 3) or NE (n = 1), 4.6% categorized as NT (n = 5) and 50.4% considered LC (n = 55) (Suppl. material 1: table S18). The 45 threatened species kept in zoos worldwide accounted for a total of 60.8% (n = 45) of all 74 threatened species extant in Vietnam (see Fig. 8 for examples). In terms of ten endemic reptile species held in zoos (n = 10), eight were categorized as threatened (CR: 3; EN: 4; VU: 1) and two as DD. As a result, 23.5% (n = 8) of all Vietnamese threatened endemic reptile species (n = 34) were held in institutions globally.

Regarding the breeding success of reptile species reported from Vietnam in zoo holdings, 49 species (45.0%) out of 109 reproduced within the last 12 months. A total of 25 of these 49 species (51.0%) were classified as threatened (CR: 10; EN: 10; VU: 5) and eight (14%) were endemic to Vietnam. Three species with most hatchlings were all evaluated as threatened but are not endemic to Vietnam, namely *Indotestudo elongata*, *Cuora amboinensis* and *Heosemys grandis*, thus their source of the breeding stock must not derive necessarily from Vietnam.

According to the ZTL, 108 reptile species extant in Vietnam were kept in European institutions. A total of 18 of these species were not included in ZIMS, the other 90 species were included in both databases (ZIMS and ZTL) and 19 species were listed in ZIMS but not in ZTL (Table 5, Suppl. material 1: table S17). As many as 40 of the 108 species (37.0%) were classified as threatened (CR: 15; EN: 14; VU: 11), four as NT (3.7%), 60 as LC (55.5%), three as DD, and one Not Evaluated (NE), *Acanthosaura murphyi*. Therefore, 54.1% (n = 40) of all 74 threatened species were represented in institutions in Europe according to the ZTL.

In terms of coordinated *ex situ* populations, four Vietnamese turtle species are both managed in AZA (Association of Zoos and Aquaria) studbooks and EAZA (European Association of Zoos and Aquaria) Ex situ programs (EEP), namely *Cuora bourreti*, *C. galbinifrons*, *C. picturata* and *Mauremys annamensis*.

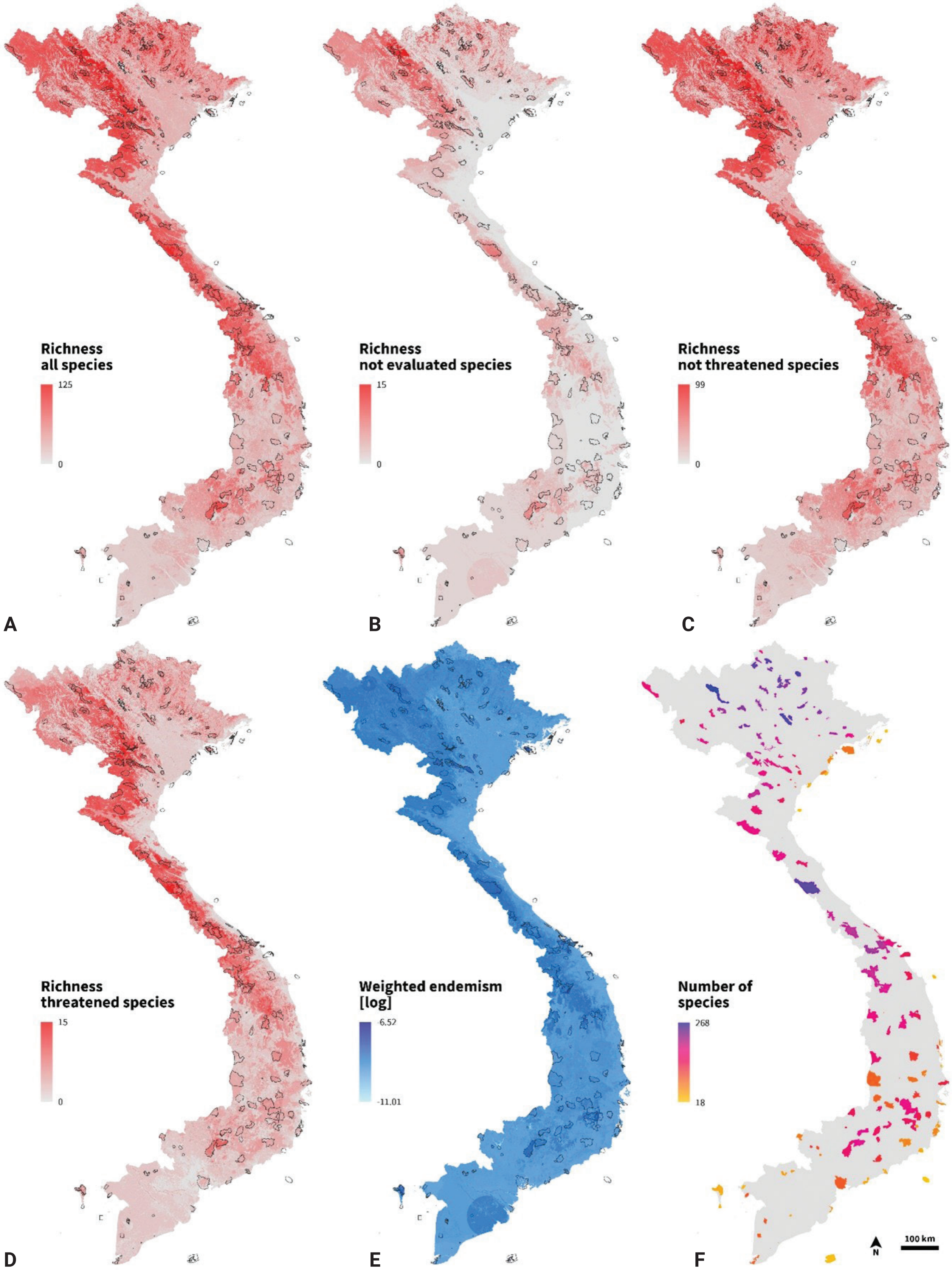


Figure 6. Species richness across the mainland of Vietnam and Vietnam's protected areas **A** all 454 terrestrial reptile species extant in Vietnam **B** all NE species **C** all not threatened species **D** all threatened species **E** weighted endemism of all reptiles **F** number of reptiles within each protected area.

Figure 7. Microendemic reptile species from Vietnam not yet recorded in any protected area **A** *Cyrtodactylus gialaiensis* **B** *Gekko truongi* **C** *Achalinus juliani* **D** *Calamaria gialaiensis* (Photos: H. Ngo (**A**) T. M. Phung (**B**) T. Ziegler (**C, D**)).

Table 5. Representation of threatened Vietnamese reptile species held according to ZIMS (n = 45) and ZTL (n = 40), including sea turtles. Species: **: species is endemic to the Indochinese Region, *: species is endemic to Vietnam. IUCN status: IUCN Red List status (IUCN 2022). Institutions: number of institutions. Individuals: number of individuals. Breeding institutions: Number of institutions which bred in the past 12 month. Hatchings: Offspring in the past 12 months.

Species	IUCN status	ZIMS				ZTL
		Institutions (regions)	Individuals	Breeding institutions	Hatchings	
Crocodylia						
Crocodylus siamensis	CR	33 (3)	268	–	–	30
Sauria						
Cnemaspis psychedelica *	EN	7 (2)	34	2	3	–
Gekko badenii *	EN	15 (2)	56	2	4	24
Goniurosaurus araneus **	EN	1 (1)	7	1	2	3
Goniurosaurus catbaensis *	EN	1 (1)	16	1	6	–
Goniurosaurus huuliensis *	CR	1 (1)	23	1	13	2
Goniurosaurus lichtenfelderi	VU	3 (2)	11	1	3	2
Goniurosaurus luii	VU	4 (1)	21	1	2	4
Physignathus cocincinus	VU	76 (3)	348	2	19	–
Shinisaurus crocodilurus vietnamensis *	EN	3 (1)	19	1	8	4
Serpentes						
Deinagkistrodon acutus	VU	3 (2)	10	1	2	8

Species	IUCN status	ZIMS				ZTL
		Institutions (regions)	Individuals	Breeding institutions	Hatchings	
Boiga bourreti	EN	–	–	–	–	1
Elaphe moellendorffi	VU	6 (3)	17	–	–	11
Elaphe taeniura	VU	26 (3)	58	–	–	42
Lycodon paucifasciatus *	VU	1 (1)	6	–	–	–
Naja atra	VU	1 (1)	1	–	–	5
Naja siamensis	VU	9 (4)	23	–	–	11
Ophiophagus hannah	VU	52 (5)	95	–	–	26
Protobothrops sieversorum	VU	1 (1)	2	–	–	1
Protobothrops trungkhanhensis	EN	1 (1)	2	–	–	1
Python bivittatus	VU	261 (6)	767	6	33	268
Testudines						
Caretta caretta	VU	26 (4)	346	–	–	42
Chelonia mydas	EN	38 (5)	977	–	–	50
Cuora amboinensis	EN	52 (4)	596	2	71	41
Cuora bourreti **	CR	15 (3)	83	3	9	6
Cuora galbinifrons	CR	24 (3)	88	2	6	8
Cuora mouhotii	EN	18 (3)	61	1	1	14
Cuora picturata *	CR	5 (3)	43	1	1	5
Cyclemys atripons	EN	2 (2)	4	–	–	–
Cyclemys oldhami	EN	3 (1)	13	–	–	3
Cyclemys pulchristriata	EN	2 (1)	10	–	–	2
Dermochelys coriacea	VU	1 (1)	2	–	–	–
Eretmochelys imbricata	CR	12 (5)	61	–	–	11
Geoemyda spengleri **	EN	41 (2)	263	2	5	15
Heosemys annandalii	CR	21 (3)	228	2	5	3
Heosemys grandis	CR	37 (3)	419	4	38	28
Indotestudo elongata	CR	53 (4)	917	9	89	32
Lepidochelys olivacea	VU	3 (3)	4	–	–	2
Manouria impressa	EN	10 (3)	25	1	2	3
Mauremys annamensis *	CR	40 (3)	224	2	6	34
Mauremys mutica	CR	17 (3)	178	–	–	9
Mauremys sinensis	CR	44 (3)	307	–	–	39
Pelochelys cantorii	CR	2 (1)	4	–	–	1
Platysternon megacephalum	CR	13 (3)	48	4	15	6
Sacalia quadriocellata	CR	12 (3)	74	1	3	6
Siebenrockiella crassicollis	EN	24 (3)	122	1	1	17

Further, there are five additional species managed only in AZA studbooks (*Cuora mouhotii*, *Geoemyda spengleri*, *Heosemys annandalii*, *Manouria impressa*, *Sacalia quadriocellata*) and four managed only in EAZA EEPs (*Cuora amboinensis*, *Mauremys mutica*, *M. sinensis*, *Siebenrockiella crassicollis*). There are three AZA studbooks for Vietnamese Squamata, namely *Shinisaurus crocodilurus*, *Malayopython reticulatus* and *Ophiophagus hannah*. Within the EAZA, there is currently only one monitoring program (Mon-T) for *Gonyosoma boulengeri*.

Figure 8. Threatened reptile species from Vietnam already in *ex situ* conservation breeding programs **A** *Mauremys annamensis* (CITES: I; IUCN: CR; Vietnam Red Data Book: CR) **B** *Cnemaspis psychedelica* (CITES: I; IUCN: EN) **C** *Gekko badenii* (IUCN: EN) **D** *Goniurosaurus huuliensis* (CITES: II; IUCN: CR) (Photos: T. Q. Nguyen (**A**) T. Ziegler (**B, D**) T. M. Phung (**C**)).

Diversity analysis

An analysis of the spatial coverage of ZIMS-registered zoos holding reptile species from or occurring in Vietnam revealed that the greatest densities are found in Europe, followed by North America. This was true for all Vietnamese reptile species, as well as when only considering endemic species (Fig. 9A, B).

Top 50 list

In the end, 55 species were included in the Top 50 list as 5 additional species had the same score (12 points). All taxa in the list were endemic to Vietnam and 80.0% (n = 44 out of 55) have only been recorded from their type localities. Subregional endemic species accounted for 14.5% (n = 8). Most of the species (65.5%) were DD (n = 27) or NE (n = 9) species and another 23.6% (n = 13) were listed as threatened with extinction (CR: 3; VU: 10). The Top Five species were all scored at 17 points and are only known from their type localities. They have no *ex situ* component and do not occur in any protected area (Table 6). Three of those species were listed as DD. The other two species, namely *Cyrtodactylus gialaiensis* and *C. nigriocularis*, were listed as CR.

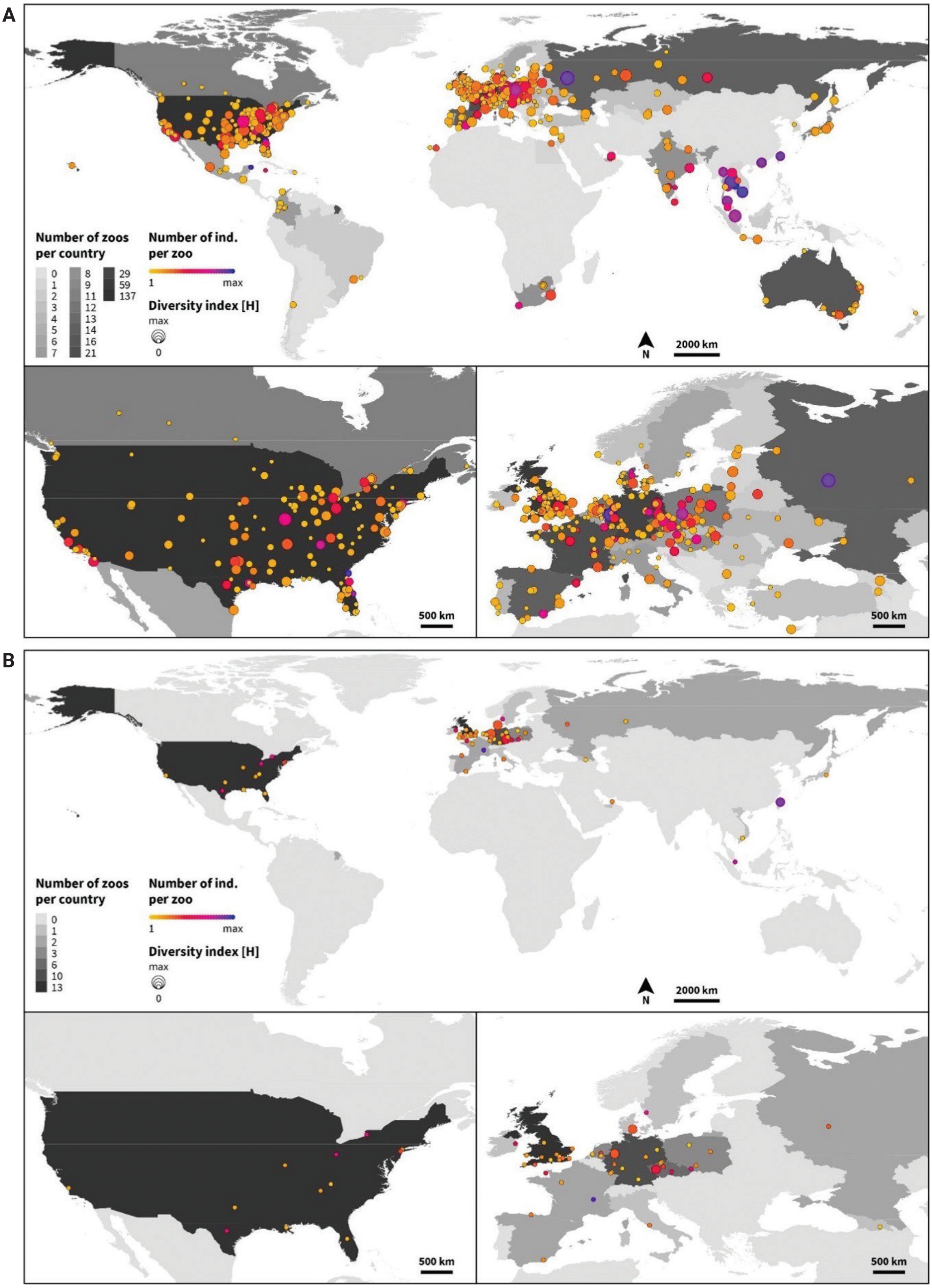


Figure 9. Geographic overview of zoo-held reptiles from or occurring in Vietnam (according to ZIMS). Countries are shaded according to the number of ZIMS members. Individual zoos are colored based on the number of individuals per zoo **A** all reptile species reported from Vietnam **B** only Vietnamese endemic reptile species.

Table 6. Top 55 list of species likely to benefit most from conservation efforts. CR, EN, VU, DD, NE: IUCN Red List Status (IUCN 2022). Type locality only: species that have received points are known exclusively from their type locality. Subregional endemic: species that have received points are endemic to one subregion. No occurrence in protected area: Species does not occur in any protected area within Vietnam. No *ex situ* populations: species that have received points are not held in zoos according to ZIMS. Not listed in national/international legislations: Species that have received points are not listed in CITES, Decree 64/2019 or Decree 84/2021. Points were given if the respective criteria were met. The highest score to be achieved was 17 points. For more detailed information about the scores see Suppl. material 1: table S19.

Species	Points
Argyrophis giadinhensis	17
Calamaria gialaiensis	17
Cyrtodactylus gialaiensis	17
Cyrtodactylus nigriocularis	17
Oligodon moricei	17
Acanthosaura brachypoda	16
Bronchocela orlovi	16
Cnemaspis aurantiacopes	16
Cyrtodactylus dati	16
Cyrtodactylus martini	16
Emoia laobaoensis	16
Eutropis darevskii	16
Gekko truongi	16
Pseudocophotis kontumensis	16
Scincella darevskii	16
Scincella rara	16
Sphenomorphus bacboensis	16
Sphenomorphus sheai	16
Ahaetulla rufusoculara	15
Calamaria sangi	15
Cyrtodactylus phumyensis	15
Cyrtodactylus thochuensis	15
Oligodon macrurus	15
Oligodon rostralis	15
Trimeresurus honsonensis	15
Cnemaspis caudanivea	14
Cnemaspis nuicamensis	14
Cyrtodactylus badenensis	14
Cyrtodactylus bichnganae	14
Cyrtodactylus grismeri	14
Cyrtodactylus huongsonensis	14
Cyrtodactylus huynhi	14
Gekko russelltraini	14
Gekko vietnamensis	14
Hemiphyllodactylus zugi	14
Achalinus juliani	13

Species	Points
Cyrtodactylus septimontium	13
Cyrtodactylus sonlaensis	13
Leiolepis guentherpetersi	13
Parafimbrios vietnamensis	13
Calamaria abramovi	12
Calamaria concolor	12
Calamaria thanhi	12
Cnemaspis tucdupensis	12
Cyrtodactylus chauquangensis	12
Cyrtodactylus cucdongensis	12
Cyrtodactylus eisenmanae	12
Cyrtodactylus hontreensis	12
Cyrtodactylus kingsadai	12
Cyrtodactylus takouensis	12
Enhydris innominata	12
Fimbrios smithi	12
Oligodon annamensis	12
Oligodon saintgironsi	12
Opisthotropis cucae	12

Discussion

As Vietnam has a very rich reptile diversity and the description rate of new species has still remained high, this study presents the current state of knowledge but it is unlikely that it represents the actual state of Vietnam's reptile fauna. Since the publication of Nguyen et al. (2009), which forms the basis for this study, 112 additional new reptile species have been described from Vietnam (Uetz et al. 2022). Especially in the last three years, the number of new species descriptions has been remarkably high. In 2019, 18 new reptile species were described, eight of which are endemic to Vietnam. In both 2020 and 2021, 10 species endemic to Vietnam were discovered. While in 2020, a total of 12 species were discovered, in 2021, there were only 11. In addition, 35 new country records have been documented since 2009 and some reptile species known from neighboring countries reported only from sites close to the border with Vietnam and thus are expected to be found from Vietnam soon. On the other hand, 32 species have been removed from the list because the populations in Vietnam belong to a different species or the species have now been synonymized with others. Another 69 species have been reassigned to different genera, which shows how much the taxonomy is still in transition and further research is required to perform taxonomic revisions and uncover cryptic diversity.

Conservation status

Given that only 86.4% (n = 418 out of 484) of the reptiles present in Vietnam have been evaluated by the IUCN Red List and not all threatened or endemic species are sufficiently protected by international and national legislation, further efforts need to be made to protect and conserve these species. An important step in that direction were two decisions at the nineteenth meeting of the Conference of the Parties (CoP) of CITES in November 2022 towards improved international protection of two reptile species occuring in Vietnam: The Green Water Dragon (*Physignathus cocincinus*) has been included in appendix II and the Indochinese Box Turtle (*Cuora galbinifrons*) has been transferred from appendix II to appendix I, both jointly proposed by Vietnam and the European Union (CITES 2022). We found that only 51.4% (n = 38 out of 74) of all threatened reptile species from Vietnam and only 3.8% (n = 6 out of 159) of all Vietnamese endemic reptile species have been included in CITES and likewise Decree 84/2021 does not list all threatened and endemic reptile species. Most notably, seven threatened turtle species have been incorporated in CITES but not in Decree 84/2021. With 96.4% (n = 27), of all 28 species listed on the IUCN Red List, listed as threatened, turtles are one of the most threatened vertebrate groups and need improved protection measures to ensure their survival.

As a study on the trade of reptiles in Vietnam has shown, *Diploderma chapaensis* and *Leiolepis guttata* have been traded in large quantities and should have been listed in the old version of the Decree (Decree 06/2019) (Janssen and Indenbaum 2019). Nonetheless, the two species were not considered in the new version (Decree 84/2021). The national legislation is apparently up to date, having been issued every two years (2019 and 2021), but many threatened and endemic species remain missing from the decrees. Consequently, re-evaluation and incorporation of additional threatened endemic species is recommended, as Vietnamese reptiles are considered to be particularly threatened by trade (Janssen and Indenbaum 2019). With regard to Decree 64/2019, only four of the 34 threatened endemic species are listed as high conservation priority, and it is recommended that the other threatened endemic species undergo further review as well. More research is also of great importance for the 69 species classified as DD as otherwise no conservation action can be undertaken and their extinction is more likely than for already assessed species (Howard and Bickford 2014; Bland et al. 2015). Furthermore, priority should also be given to the 66 species that have not yet been assessed in order to enable their assignment to a threat status; and the ones with their status being considered outdated as they have passed the 10 years validation mark. However, most of the species that have not yet been assessed have not been assessed because they have only recently been recognized and herpetologists are in the process of collecting data for IUCN assessments. The fact that endemic reptile species account for 32.9% (n = 159) of all 484 reptile species occurring in Vietnam, while representing 46.0% of all threatened reptile species (34 of 74 species), highlights that species with smaller or restricted ranges are more likely to be threatened and therefore should be protected to a greater extent (Meiri et al. 2017).

Distribution

In terms of distribution, our data only reflect the current state of scientific knowledge, but not the actual distribution of reptile species in Vietnam. This is especially evident for recently described species that have just been reported from their type locality. Further research could expand their distribution ranges. Moreover, as some Vietnamese endemic reptile species occur close to the borders of adjacent countries such as Laos, Cambodia or China, additional studies might discover new records of the taxa from these countries in the near future. For instance, *Cyrtodactylus dati*, which is found very close to the Cambodian border and is likely to occur there as well but has not yet been confirmed (Uetz et al. 2022). Therefore, some of the species listed as endemic might be in other categories in the future with new data from additional studies.

Not all 159 endemic species identified in the study are evenly distributed, but rather spread across the 13 different subregions. This pattern is in concert with previous findings on amphibians (Geissler et al. 2015). Particularly relevant are those species that are only known from their type locality or endemic to a subregion and therefore well adapted to microclimatic conditions and special habitat characteristics. With 131 species (82.4%), they represent a majority of endemic species and draw attention to the need to establish local conservation areas to safeguard the microendemic species. The Central Annamites (CAN) subregion should be a priority for increased reptile conservation efforts, as this subregion harbors most local and subregional endemics including 21 endemic species, 12 of which are classified as DD. However, the Mekong Delta (MEK) subregion and the Southern Islands (SIS) should also be prioritized in this regard, as they also have high endemic species richness and harbor most threatened species (MEK: 7; SIS: 5).

Approximately 33.5% (n = 53) of all terrestrial and limnic endemic reptile species (n = 158) have not been reported from any protected area in Vietnam and 17 of these species are considered threatened with extinction (CR: 4; EN: 2; VU: 11). Another 20 species have exclusively been reported from their type locality. For these species as well as for the 20 species considered DD and the eight species which have not been evaluated yet, their conservation status assessment should be undertaken as quickly as possible to design appropriate conservation measures in due time.

Ex situ populations

According to ZIMS, only ten of the 109 reptile species kept are endemic and 45 reptile species are considered threatened. Thus, 60.8% (n = 45 out of 74) of all threatened reptile species reported to occur in Vietnam are represented in zoos, but only eight of them are threatened endemic species. A slight majority of the reptile species held (50.5%; n = 55 out of 109) is classified as LC. On the other hand, the ZTL only lists 108 species and they differ from those reported in ZIMS. Although more LC species are listed in the ZTL (55.5%; n = 60 out of 108), one threatened species not listed in ZIMS is also included, namely *Boiga bourreti*. Despite potentially incomplete data, our analysis suggests that only a few of the reptile species present in Vietnam are kept in zoos (22.5%; n = 109 out of 484) and the number of endemic species is even smaller with only 6.3% (n = 10 out of 159) of the endemic species maintained in zoos worldwide. For the species, the number of kept individuals is limited (except for *Mauremys annamensis*). It is therefore recommended to increase the number of endemic and especially threatened endemic reptile species in zoos in order to maintain assurance *ex situ* populations of these species, so that in the event of a natural disaster, overcollection or disease outbreak, a complete extinction of these species can be prevented. However, acquiring these species presents a challenge and it is important to point out that we certainly do not recommend buying wild-caught individuals of threatened species, as this could fuel the trade and contribute to the decrease or loss of natural populations. We would rather recommend focusing on threatened species that are already kept in zoological collections, in breeding stations or at reputable breeders. It is important to invest in cooperations with partners and stations in the country to build up legal captive breeding programs. If it is necessary to aquire wild caught animals to build up a new reserve population, this should of course be in consultation with the respective authorities. There is already rising awareness on the need to shift towards threatened species in managed breeding programs within zoological collections, as was reflected by the latest decisions on recently developed regional collection plans (RCPs) within EAZA taxon advisory groups. For example, in the frame of the RCP for chelonian species, a number of EEPs was lately established amongst others for threatened Vietnamese turtle species in the genera *Cuora* and *Mauremys* (Goetz et al. 2019). Moreover, during the latest regional collection plan for lizards, it was decided to set up EEPs for the two Endangered lizard species *Cnemaspis psychedelica* and *Shinisaurus crocodilurus* as well as monitoring programs (Mon-T) for all five *Goniurosaurus* species from Vietnam (Cizelj et al. 2023).

Non-endemic species present more challenging conditions for reintroduction. If the origin of the populations is uncertain, zoos must first carry out genetic studies to assign their populations to a country of origin and to exclude genetic contamination, when reasonable, as this is the only way that successful reintroduction can take place. Since ZIMS and the ZTL do not list all institutions worldwide that keep and breed reptile species, local facilities are not included in the evaluation. However, these facilities can play a very important role in the development of conservation breeding programs, as local stations such as the Me Linh Station for Biodiversity in northern Vietnam may already keep and breed protected reptile species (Ziegler et al. 2015c, 2019c). Through international cooperation, species can thus be transferred to conservation breeding programs in zoos around the world, e.g., conservation breeding initiatives both in the country of origin and abroad were jointly built up for threatened Vietnamese Tiger gecko species (*Goniurosaurus* spp.), the Psychedelic Rock Gecko (*Cnemaspis psychedelica*), or the Vietnamese Crocodile Lizard (*Shinisaurus crocodilurus vietnamensis*), combining research and *ex situ* efforts in Vietnam and Europe (Ziegler et al. 2016, 2019c; Ngo et al. 2020; Nguyen et al. 2021; Van Schingen-Khan et al. 2022; Ziegler and Rauhaus 2022). In-country facilities have the advantage for quick and easy restocking measures, more distant facilities and zoos, respectively, are advantageous in case of disease outbreaks, natural catastrophes or political unrests.

In order to protect threatened species and meet the objectives of the One Plan Approach, *ex situ* populations must be included in global conservation planning of the respective species. The *ex situ* efforts are intended to support the *in situ* conservation efforts and, in the best case, *ex situ* populations can be reintroduced into the wild. However, since the capacity of zoos is not unlimited, not all threatened species can be protected in this manner. Breeding of species is therefore particularly recommended for species whose drivers of extinction are pollution, disease or habitat loss (Clulow et al. 2014) and in the case of Vietnam especially those which are threatened by overexploitation, as for example turtles, crocodile lizards, and psychedelic rock geckos (Van Schingen 2015; Lee et al. 2020). To determine to which level an *ex situ* component is required or beneficial for the conservation strategy of a species, the IUCN Species Survival Commission has published guidelines on the use of *ex situ* management for species conservation. In some cases *ex situ* management can be a primary part of a conservation strategy while in others it will be of secondary importance, supporting other interventions (IUCN/SSC 2014).

All recommendations should be understood as a starting point for improved reptile conservation. Since the range size of a species can play a major role in its survival (Meiri et al. 2017), the distribution range of all species should be analyzed in more detail. Meiri et al. (2017) have done such an analysis for lizards, but, for example for snakes, only little information is available. Updated distribution ranges for turtles, including Vietnamese species, have been compiled and published by the Turtle Taxonomy Working Group in 2021 (TTWG 2021), but similar to the other orders more detailed and up to date information is required as most of the turtle species extant in Vietnam (96%; n = 27) are threatened with extinction. Related to the range size, the habitats of species should also be studied in greater detail to identify habitat specialists and habitat generalists, as the specialists are often less adaptable to change and thus more vulnerable. It is therefore recommended that further conservation measures should be initiated for particularly threatened species having severely restricted distribution ranges, such as *Cyrtodactylus nigriocularis*, *Cnemaspis psychedelica*, or *Cuora picturata*. Other species used to occupy a much broader region, but their distributions have shrunk considerably due to habitat loss and overexploitation. For example, while *Mauremys annamensis* and *Cuora cyclornata* are likely extinct in the wild, *Rafetus swinhoei* is on the brink of extinction and restricted to one or a few small lakes in northern Vietnam. In April of 2023, the last known female of this species in Hanoi, Vietnam, died of unknown causes, leaving little hope to recover its population. Both *Mauremys annamensis* and *Rafetus swinhoei* have also not been recorded in any protected area and are thus of utmost importance to consider for further conservation work. Whereas ex situ programmes have been implemented in time for *Mauremys annamensis*, unfortunately, no such initiative has been established for *Rafetus swinhoei*. However, Ziegler et al. (2021) have shown that build up of conservation breeding for softshell turtles can be established, when action is taken in time.

The Top 50 list provided in the results section (Table 6) should be taken into account when planning new conservation measures, as the species listed are those currently most threatened with extinction as our study revealed. Especially the Top 5 species should be given attention, as these species would benefit most from further protection measures.

Cyrtodactylus gialaiensis (Place 3), for example, was only discovered in 2017 and up to now no obvious natural habitat has been recognized, as the species was only found in coffee plantations (Luu et al. 2017; Luu et al. 2020b). On the other hand, *Cyrtodactylus nigriocularis* (Place 4) is restricted to a few caves and under extreme pressure of human activities threatening the very small population (Nguyen et al. 2018c). This again highlights the particular need for immediate research and conservation efforts for all the species included in the Top 50 List (Table 6).

Conclusion

Conservation of reptiles in Vietnam needs to be comprehensively evaluated through extended research and prioritizing conservation measures. In particular, support is needed for the most threatened endemic species identified in this study. It is also important to focus on additional efforts to assess the status of 69 DD and 66 NE species in order to be able to protect them more effectively in the future. Successful protection of the species requires the incorporation of both *in situ* and *ex situ* conservation actions. In particular, the most threatened endemic species must be included in national and international legislations to provide them with additional support. In the implementation of the One Plan Approach, international institutions such as zoos and local facilities play a key role in offering expertise, capacity, and financial resources to support both *in situ* and *ex situ* conservation measures. Since more than half of all Vietnamese reptile species in zoos are classified as LC and only 6.3% (n = 10) of 159 Vietnamese endemic species are maintained *ex situ*, it is crucial that zoos consider shifting their focus to commit more resources for threatened endemic species both through supporting in situ projects and building up ex situ assurance colonies to be able to supply surplus individuals from breeding programs for *in situ* conservation programs, once needed.

Acknowledgements

We thank T. Pagel and C. Landsberg (Cologne Zoo), as well as S. V. Nguyen (IEBR, Hanoi) for their support of research and conservation in Vietnam. M. Flecks supported us with his graphical skills.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This research was supported by the the National Foundation for Science and Technology Development (NAFOSTED, Grant No. 106.05-2021.19) and Cologne Zoo. Cologne Zoo is partner of the World Association of Zoos and Aquariums (WAZA): Conservation Projects 07011, 07012 (Herpetodiversity Research, Amphibian and Reptilian Breeding and Rescue Stations).

Author contributions

Data analysis: Lilli Stenger, Anke Große Hovest, Cuong The Pham, Truong Quang Nguyen; conceptualization: Thomas Ziegler, Dennis Rödder, Minh Duc Le, Anna Rauhaus; methodology: Lilli Stenger, Anke Große Hovest, Anna Rauhaus, Dennis Rödder; writing, review and editing: all authors.

Author ORCIDs

Truong Quang Nguyen https://orcid.org/0000-0002-6601-0880
Cuong The Pham https://orcid.org/0000-0001-5158-4526
Minh Duc Le https://orcid.org/0000-0002-2953-2815
Dennis Rödder https://orcid.org/0000-0002-6108-1639
Thomas Ziegler https://orcid.org/0000-0002-4797-609X

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

References

Adler K (2009) Herpetological exploration, research, and conservation in Vietnam. Herpetofauna of Vietnam, Edition Chimaira, Frankfurt am Main, 33–56.

Amarasinghe AAT, Campbell P, Hallermann J, Sidik I, Supriatn J, Ineich I (2015) Two new species of the genus *Cylindrophis* Wagler, 1828 (Squamata: Cylindrophiidae) from Southeast Asia. Amphibian & Reptile Conservation 9(1): 34–51. http://hdl.handle.net/10141/612206

Bain RH, Hurley MM (2011) A biogeographic synthesis of the amphibians and reptiles of Indochina. Bulletin of the American Museum of Natural History 2011(360): 1–138. https://doi.org/10.1206/360.1

Barnosky AD, Matzke N, Tomiya S, Wogan GOU, Swartz B, Quental TB, Marshall C, McGuire JL, Lindsey EL, Maguire KC, Mersey B, Ferrer EA (2011) Has the earth's sixth mass extinction already arrived? Nature 471(7336): 51–57. https://doi.org/10.1038/nature09678

Blair ME, Le MD, Sethi G, Thach HM, Nguyen VTH, Amato G, Birchette M, Sterling EJ (2017) The importance of an interdisciplinary research approach to inform wildlife trade management in Southeast Asia. Bioscience 67(11): 995–1003. https://doi.org/10.1093/biosci/bix113

Blair ME, Le MD, Xu M (2022) Special issue: Transboundary conservation under climate change. Frontiers of Biogeography 14: e54662. https://doi.org/10.21425/F5FBG54662

Bland LM, Collen BEN, Orme CDL, Bielby JON (2015) Predicting the conservation status of data-deficient species. Conservation Biology 29(1): 250–259. https://doi.org/10.1111/cobi.12372

Böhm M, Collen B, Baillie JE, Bowles P, Chanson J, Cox N, Hammerson G, Hoffmann M, Livingstone SR, Ram M, Rhodin AGJ, Stuart SN, van Dijk PP, Young BE, Afuang LE, Aghasyan A, Garcia A, Aguilar C, Ajtic R, Akarsu F, Alencar LRV, Allison A, Ananjeva N, Anderson S, Andren C, Ariano-Sanchez D, Arrendondo JC, Auliya M, Austin CC, Avci A, Baker PJ, Barreto-Lima AF, Barrio-Amoros CL, Basu D, Bates MF, Batistella A, Bauer A, Bennett D, Böhme W, Broadley D, Brown R, Burgess J, Captain A, Carreira S, del Rosario Castaneda M, Castro F, Catenazzi A, Cedeno-Vazquez JR, Chapple DG, Cheylan M, Cisneros-Heredia DF, Cogalniceanu D, Coggerr H, Corti C, Costa GC, Couper PJ, Courtney T, Crnobrnja-Isailovic J, Crochet PA, Crother B, Cruz F, Daltry JC, Daniels RJR, Das I, de Silva A, Diesmos AC, Dirksen L, Doan TM, Dodd Jr CK, Doody JS, Dorcas ME, de Barros Filho JD, Egan VT (2013) The conservation status of the world's reptiles. Biological Conservation 157: 372–385. https://doi.org/10.1016/j.biocon.2012.07.015

Ceballos G, Ehrlich PR, Barnosky AD, Garcia A, Pringle RM, Palmer TM (2015) Accelerated modern human-induced species loss: Entering the sixth mass extinction. Science Advances 1(5): e1400253. https://doi.org/10.1126/sciadv.1400253

Ceballos G, Ehrlich PR, Dirzo R (2017) Biological annihilation via the ongoing sixth mass extinction signaled by vertebrate population losses and declines. Proceedings of the National Academy of Sciences of the United States of America 114(30): E6089–E6096. https://doi.org/10.1073/pnas.1704949114

Chamberlain S (2022) Rredlist: 'Iucn' red list client. R package version 0.7.1. https://cran.R-project.Org/package=rredlist

Chen Z, Shi S, Gao J, Vogel G, Song Z, Ding L, Dai R (2021) A new species of *Trimeresurus* (Lacépède, 1804) (Squamata: Viperidae) from Southwestern China, Vietnam, Thailand and Myanmar. Asian Herpetological Research 12(2): 167–177C. https://doi.org/10.16373/j.cnki.ahr.200084

Chichorro F, Juslén A, Cardoso P (2019) A review of the relation between species traits and extinction risk. Biological Conservation 237: 220–229. https://doi.org/10.1016/j.biocon.2019.07.001

CITES (2021a) Convention on International Trade in Wild Plants and Animals. https://cites.org/eng/app/appendices.php [Accessed on 13.03.2022]

CITES (2021b) Viet Nam. https://cites.org/eng/parties/country-profiles/vn [Accessed on 13.03.2022]

CITES (2022) Provisional list of proposals for amendment of Appendices I and II as received. https://cites.org/eng/cop/19/amendment-proposals/provisional [Accessed 14.03.2022]

Cizelj I, Aparici Plaza D, Martincová I, Hausen N [Eds] (2023) Regional Collection Plan – Sauria – for the EAZA Reptile Taxon Advisory Group - Edition One. EAZA Executive Office, Amsterdam.

Clulow J, Trudeau VL, Kouba AJ (2014) Amphibian declines in the twenty-first century: why we need assisted reproductive technologies. In: Holt WV, Brown JL, Comizzoli P (Eds) Reproductive sciences in animal conservation: Progress and Prospects. Springer, New York, 275–316. https://doi.org/10.1007/978-1-4939-0820-2_12

Conde DA, Colchero F, Gusset M, Pearce-Kelly P, Byers O, Flesness N, Browne RK, Jones OR (2013) Zoos through the lens of the IUCN Red List: A global metapopulation approach to support conservation breeding programs. PLoS ONE 8(12): e80311. https://doi.org/10.1371/journal.pone.0080311

Cowie RH, Bouchet P, Fontaine B (2022) The Sixth Mass Extinction: fact, fiction or speculation? Biological Reviews 97(2): 640–663. https://doi.org/10.1111/brv.12816

Cox N, Young BE, Bowles P, Fernandez M, Marin J, Rapacciuolo G, Bohm M, Brooks TM, Hedges SB, Hilton-Taylor C, Hoffmann M, Jenkins RKB, Tognelli MF, Alexander GJ, Allison A, Ananjeva NB, Auliya M, Avila LJ, Chapple DG, Cisneros-Heredia DF, Cogger HG, Colli GR, de Silva A, Eisemberg CC, Els J, Fong GA, Grant TD, Hitchmough RA, Iskandar DT, Kidera N, Martins M, Meiri S, Mitchell NJ, Molur S, Nogueira CC, Ortiz JC, Penner J, Rhodin AGJ, Rivas GA, Rodel MO, Roll U, Sanders KL, Santos-Barrera G, Shea GM, Spawls S, Stuart BL, Tolley KA, Trape JF, Vidal MA, Wagner P, Wallace BP, Xie Y (2022) A global reptile assessment highlights shared conservation needs of tetrapods. Nature 605(7909): 285–290. https://doi.org/10.1038/s41586-022-04664-7

Cremene C, Groza G, Rakosy L, Schileyko AA, Baur A, Erhardt A, Baur B (2005) Alterations of steppe-like grasslands in Eastern Europe: A threat to regional biodiversity hotspots. Conservation Biology 19(5): 1606–1618. https://doi.org/10.1111/j.1523-1739.2005.00084.x

Crisp MD, Laffan S, Linder HP, Monro A (2001) Endemism in the Australian flora. Journal of Biogeography 28(2): 183–198. https://doi.org/10.1046/j.1365-2699.2001.00524.x

David P, Vogel G, Pauwels OS (2008) A new species of the genus *Oligodon* Fitzinger, 1826 (Squamata: Colubridae) from southern Vietnam and Cambodia. Zootaxa 1939(1): 19–37. https://doi.org/10.11646/zootaxa.1939.1.3

Ding L, Chen Z, Suwannapoom C, Nguyen T, Poyarkov NA, Vogel G (2020) A new species of the *Pareas hamptoni* complex (Squamata Serpentes: Pareidae) from the Golden Triangle. Taprobanica 9(2): 174–193. https://doi.org/10.47605/tapro.v9i2.230

Do DT, Ngo CD, Ziegler T, Nguyen TQ (2017) First record of *Lycodon cardamomensis* Daltry & Wüster, 2002, (Squamata: Colubridae) from Vietnam. Russian Journal of Herpetology 24(2): 167–170. https://doi.org/10.30906/1026-2296-2019-24-2-167-170

Drury R (2011) Hungry for success: Urban consumer demand for wild animal products in Vietnam. Conservation & Society 9(3): 247–257. https://doi.org/10.4103/0972-4923.86995

Farkas B, Ziegler T, Pham CT, Ong AV, Fritz U (2019) A new species of *Pelodiscus* from northeastern Indochina (Testudines, Trionychidae). ZooKeys 71(824): 71–86. https://doi.org/10.3897/zookeys.824.31376

Geissler P, Nguyen TQ, Poyarkov NA, Böhme W (2011a) New records of snakes from Cat Tien National Park, Dong Nai and Lam Dong provinces, southern Vietnam. Bonn Zoological Bulletin 60(1): 9–16. https://zoologicalbulletin.de/BzB_Volumes/Volume_60_1/009_016_BzB60_1_Geissler_Peter_et_al.pdf

Geissler P, Nguyen TQ, Phung TM, Van Devender RW, Hartmann T, Farkas B, Ziegler T, Böhme W (2011b) A review of Indochinese skinks of the genus *Lygosoma* Hardwicke & Gray, 1827 (Squamata: Scincidae), with natural history notes and an identification key. Biologia 66(6): 1159–1176. https://doi.org/10.2478/s11756-011-0130-2

Geissler P, Hartmann T, Ihlow F, Rödder D, Poyarkov Jr NA, Nguyen TQ, Ziegler T, Böhme W (2015) The Lower Mekong: An insurmountable barrier for amphibians in southern Indochina? Biological Journal of the Linnaean Society 144(4): 905–914. https://doi.org/10.1111/bij.12444

Gilbert T, Gardner R, Kraaijeveld AR, Riordan P (2017) Contributions of zoos and aquariums to reintroductions: Historical reintroduction efforts in the context of changing conservation perspectives. International Zoo Yearbook 51(1): 15–31. https://doi.org/10.1111/izy.12159

Goetz M, Aparici Plaza D, Van Lint W, Fienieg E, Hausen N [Eds] (2019) Regional Collection Plan – Chelonia – for the EAZA Reptile Taxon Advisory Group –Edition One. EAZA Executive Office, Amsterdam.

Grismer LL, Quah ES (2015) The Rediscovery of *Sphenomorphus malayanus* Doria, 1888 (Squamata: Scincidae) from the Titiwangsa Mountain Range of Peninsular Malaysia and its re-description as *S. senja* sp. nov. Zootaxa 3931(1): 63–70. https://doi.org/10.11646/zootaxa.3931.1.4

Grismer LL, Wood PLJ, Quah ESH, Anuar S, Poyarkov NA, Thy N, Orlov NL, Thammachoti P, Seiha H (2019a) Integrative taxonomy of the Asian skinks *Sphenomorphus stellatus* (Boulenger, 1900) and *S. praesignis* (Boulenger, 1900) with the resurrection of *S. annamiticus* (Boettger, 1901) and the description of a new species from Cambodia. Zootaxa 4683(3): 381–411. https://doi.org/10.11646/zootaxa.4683.3.4

Grismer LL, Wood PLJ, Grismer JL, Quah ES, Thy N, Phimmachak S, Sivongxay N, Seateun S, Stuart BL, Siler CB, Mulchay DG, Anamza T, Brown RM (2019b) Geographic structure of genetic variation in the Parachute Gecko *Ptychozoon lionotum* Annandale, 1905 across Indochina and Sundaland with descriptions of three new species. Zootaxa 4638(2): 151–198. https://doi.org/10.11646/zootaxa.4638.2.1

Grismer LL, Thai P, Wood P (2020) A new endemic insular Bent-toed Gecko (Squamata: Gekkonidae: *Cyrtodctylus*) from Quang Nam Province, Central Vietnam. Zootaxa 4766(2): 389–400. https://doi.org/10.11646/zootaxa.4766.2.7

Grismer LL, Ngo HN, Qi S, Wang YY, Le MD, Ziegler T (2021a) Phylogeny and evolution of habitat preference in *Goniurosaurus* (Squamata: Eublepharidae) and their correlation with karst and granite-stream-adapted ecomorphologies in species groups from Vietnam. Vertebrate Zoology 71: 335–352. https://doi.org/10.3897/vz.71.e65969

Grismer LL, Wood JPL, Poyarkov NA, Le MD, Kraus F, Agarwal I, Grismer JL (2021b) Phylogenetic partitioning of the third-largest vertebrate genus in the world, *Cyrtodactylus* Gray, 1827 (Reptilia; Squamata; Gekkonidae) and its relevance to taxonomy and conservation. Vertebrate Zoology 71: 101–154. https://doi.org/10.3897/vertebrate-zoology.71.e59307

Gusset M (2019) Zoos and Aquariums Committing to Integrated Species Conservation. The Routledge Handbook of Animal Ethics. Routledge, 357–366. https://www.taylorfrancis.com/chapters/edit/10.4324/9781315105840-33/zoos-aquariums-committing-integrated-species-conservation-markus-gusset

Habel JC, Rasche L, Schneider UA, Engler JO, Schmid E, Rödder D, Meyer ST, Trapp N, del Diego RS, Eggermont H, Lens L, Stork NE (2019) Final countdown for biodiversity hotspots. Conservation Letters 12(6): e12668. https://doi.org/10.1111/conl.12668

Hecht VL, Pham CT, Nguyen TT, Nguyen TQ, Bonkowski M, Ziegler T (2013) First report on the herpetofauna of Tay Yen Tu nature reserve, northeastern Vietnam. Biodiversity Journal 4(4): 507–552.

Hoang NT, Ho AT, Ong VA, Thi HLL (2018) New records of *Dopasia gracilis* (Squamata: Anguidae) in bach ma national park, Thua Thien-Hue province. Studia Universitatis Moldaviae 111(1): 56–60. [Seria Ştiinţe Reale şi ale Naturii]

Holden J, Poyarkov NA (2021) A range extension for *Dryophiops rubescens* (Gray, 1835) with the first record of the species from Vietnam. Herpetology Notes 14: 795–798. https://www.biotaxa.org/hn/article/view/67227/66773

Howard SD, Bickford DP (2014) Amphibians over the edge: silent extinction risk of Data Deficient species. Diversity and distributions 20(7): 837–846. https://doi.org/10.1111/ddi.12218

IPBES (2019) Global assessment report on biodiversity and ecosystem services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. In: Brondizio ES, Settele J, Díaz S, Ngo HT (Eds) IPBES secretariat, Bonn, Germany, 1148 pp. https://doi.org/10.5281/zenodo.3831673

IUCN/SSC (2014) Guidelines on the Use of Ex Situ Management for Species Conservation. Version 2.0. IUCN Species Survival Commission, Gland, Switzerland.

IUCN (2022) Regional Red List Assessments. The IUCN Red List of Threatened Species. Version 2022-1. https://www.iucnredlist.org/about/regional [Accessed on 13.03.2022]

Janssen J, Indenbaum RA (2019) Endemic Vietnamese reptiles in commercial trade. Journal of Asia-Pacific Biodiversity 12(1): 45–48. https://doi.org/10.1016/j.japb.2018.11.010

Jung M, Dahal PR, Butchart SHM, Donald PF, De Lamo X, Lesiv M, Kapos V, Rondinini C, Visconti P (2020) A global map of terrestrial habitat types. Scientific Data 7(1): 256. https://doi.org/10.1038/s41597-020-00599-8

Krzikowski M, Nguyen TQ, Pham CT, Rödder D, Le MD, Ziegler T (2022) Assessment of the threat status of the amphibians in Vietnam – implementation of the one plan approach. Nature Conservation 49: 77–116. https://doi.org/10.3897/natureconservation.49.82145

Kurniawan N, Septiadi L, Fathoni M, Wibawa GS, Thammachoti P (2021) Out of Indochina: Confirmed specimen record and first molecular identification of *Psammophis indochinensis* Smith, 1943 (Squamata, Psammophiidae) from Bali, Indonesia. Check List 17(6): 1521–1531. https://doi.org/10.15560/17.6.1521

Le DO, Van Thong P, Bordes C, LePrince B, Ducotterd C, Zuklin T, Quang Vinh L, Julia FA, Luiselli L (2021) Characteristics of a snake community in the hilly forest of Quan Son District, northern Vietnam. Herpetological Conservation and Biology 16(3): 491–505.

Le DT, Dao AN, Pham DT, Ziegler T, Nguyen TQ (2018) New records and an updated list of snakes from Yen Bai Province, Vietnam. Herpetology Notes 11: 101–108.

Le DT, Lo NT, Tran HN, Do YT (2020) Biodiversity and composition of the herpetofauna from the Tien Hai Wetland Nature Reserve, North Vietnam. Journal of Advanced Biotechnology and Experimental Therapeutics 3(2): 116–121. https://doi.org/10.5455/jabet.2020.d115

Le MD, McCormack TE, Van Hoang H, Duong HT, Nguyen TQ, Ziegler T, Nguyen HD, Ngo HT (2020) Threats from wildlife trade: The importance of genetic data in safeguarding the endangered Four-eyed turtle (*Sacalia quadriocellata*). Nature Conservation 41: 91–111. https://doi.org/10.3897/natureconservation.41.54661

Le TA, Markowski J, Bartos M (2018) The comparative analyses of selected aspects of conservation and management of Vietnam's national parks. Nature Conservation 25: 1–30. https://doi.org/10.3897/natureconservation.25.19973

Lees AC, Pimm SL (2015) Species, extinct before we know them? Current Biology 25(5): R177–R180. https://doi.org/10.1016/j.cub.2014.12.017

Li K, Wu YY, Xu RY, Zhu F, Ren JL, Guo P, Dong BJ (2021) A new species of the *Achalinus rufescens* complex (Xenodermidae: *Achalinus*) from Fujian Province, China. Zootaxa 5026(2): 239–254. https://doi.org/10.11646/zootaxa.5026.2.5

Linh LK, Vinh LQ, Truong NQ (2019) New records of skinks (Squamata: Scincidae) from Nam Dong valuable Gymnosperm conservation area, Thanh Hoa province. Journal of Forestry Science and Technology 8: 109–116.

Luu VQ, Calame T, Nguyen TQ, Bonkowski M, Ziegler T (2015a) A new species of *Cyrtodactylus* (Squamata: Gekkonidae) from the limestone forest of Khammouane Province, central Laos. Zootaxa 4058(3): 388–402. https://doi.org/10.11646/zootaxa.4058.3.6

Luu VQ, Calame T, Nguyen TQ, Le MD, Ziegler T (2015b) Morphological and molecular review of the Gekko diversity of Laos with descriptions of three new species. Zootaxa 3986(3): 279–306. https://doi.org/10.11646/zootaxa.3986.3.2

Luu VQ, Tran DV, Nguyen TQ, Le MD, Ziegler T (2017) A new species of the *Cyrtodactylus irregularis* complex (Squamata: Gekkonidae) from Gia Lai Province, Central Highlands of Vietnam. Zootaxa 4362(3): 385–403. https://doi.org/10.11646/zootaxa.4362.3.4

Luu VQ, Ziegler T, Ha N, Le MD, Hoang TT (2019) A new species of *Lycodon* Boie, 1826 (Serpentes: Colubridae) from Thanh Hoa Province, Vietnam. Zootaxa 4586(2): 261–277. https://doi.org/10.11646/zootaxa.4586.2.3

Luu VQ, Dinh TS, Lo OV, Nguyen TQ, Ziegler T (2020a) New records and an updated list of reptiles from Ba Vi National Park, Vietnam. Bonn Zoological Bulletin 69: 1–9. https://doi.org/10.20363/BZB-2020.69.1.001

Luu VQ, Lo OV, Hoang TT, Pham TV, Le Duc O, Bordes C, Leprince B, Amori G, Luiselli L (2020b) Ecological characteristics of a recently described, critically endangered gecko species, endemic to Central Highland, Vietnam. Tropical Zoology 33(2): 53–62. https://doi.org/10.4081/tz.2020.71

Luu VQ, Ziegler T, Van Ha N, Van Lo O, Hoang TT, Ngo HT, Le MD, Tran DH, Nguyen TQ (2020c) A new species of *Achalinus* (Squamata: Xenodermidae) from Trang An Landscape Complex, Ninh Binh Province, Vietnam. Zootaxa 4877(1): 174–184. https://doi.org/10.11646/zootaxa.4877.1.8

Malcolm JR, Liu C, Neilson RP, Hansen L, Hannah L (2006) Global warming and extinctions of endemic species from biodiversity hotspots. Conservation Biology 20(2): 538–548. https://doi.org/10.1111/j.1523-1739.2006.00364.x

Mallik AK, Srikanthan AN, Pal SP, D'Souza PM, Shanker K, Ganesh SR (2020) Disentangling vines: a study of morphological crypsis and genetic divergence in vine snakes (Squamata: Colubridae: *Ahaetulla*) with the description of five new species from Peninsular India. Zootaxa 4874(1): 1–62. https://doi.org/10.11646/zootaxa.4874.1.1

McCallum ML (2015) Vertebrate biodiversity losses point to a sixth mass extinction. Biodiversity and Conservation 24(10): 2497–2519. https://doi.org/10.1007/s10531-015-0940-6

Meiri S, Bauer AM, Allison A, Castro-Herrera F, Chirio L, Colli G, Das I, Doan TM, Glaw F, Grismer LL, Hoogmoed M, Kraus F, LeBreton M, Meirte D, Ngay ZT (2017) Extinct, obscure or imaginary: The lizard species with the smallest ranges. Diversity & Distributions 24(2): 262–273. https://doi.org/10.1111/ddi.12678

Melville J, Chapple DG, Keogh JS, Sumner J, Amey A, Bowles P, Brennan IG, Couper P, Donnellan SC, Doughty P, Edwards DL, Ellis RJ, Esquerre D, Fenker J, Gardner MG, Georges A, Haines ML, Hoskin CJ, Hutchinson M, Moritz C, Nankivell J, Oliver P, Pavon-Vazquez CJ, Pepper M, Rabosky DL, Sanders K, Shea G, Singhal S, Wilmer JW, Tingley R (2021) A return-on-investment approach for prioritization of rigorous taxonomic research needed to inform responses to the biodiversity crisis. PLoS Biology 19(6): e3001210. https://doi.org/10.1371/journal.pbio.3001210

Miller AH, Davis HR, Luong AM, Do QH, Pham CT, Ziegler T, Lee JL, de Queiroz K, Reynolds G, Nguyen TQ (2020) Discovery of a New Species of Enigmatic Odd-Scaled Snake (Serpentes: Xenodermidae: *Achalinus*) from Ha Giang Province, Vietnam. Copeia 108(4): 796–808. https://doi.org/10.1643/CH2020060

Murdoch ML, Grismer LL, Wood PLJ, Neang T, Poyarkov NA, Tri NV, Nazarov RA, Aowphol A, Pauwels OSG, Nguyen HN, Grismer JL (2019) Six new species of the *Cyrtodactylus intermedius* complex (Squamata: Gekkonidae) from the Cardamom Mountains and associated highlands of Southeast Asia. Zootaxa 4554(1): 1–62. https://doi.org/10.11646/zootaxa.4554.1.1

Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GA, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403(6772): 853–858. https://doi.org/10.1038/35002501

Neang T, Henson A, Stuart BL (2020) A new species of *Cyrtodactylus* (Squamata, Gekkonidae) from Cambodia's Prey Lang Wildlife Sanctuary. ZooKeys 926: 133–158. https://doi.org/10.3897/zookeys.926.48671

Ngo HN, Nguyen TQ, Nguyen T, Barsch F, Ziegler T, van Schingen M (2016) First population assessment of the endemic insular Psychedelic Rock Gecko (*Cnemaspis psychedelica*) in southern Vietnam with implications for conservation. Amphibian & Reptile Conservation 10(2): 18–26.

Ngo HN, Nguyen TQ, Nguyen TV, van Schingen M, Ziegler T (2018) Microhabitat selection and communal nesting in the insular Psychedelic Rock Gecko, *Cnemaspis psychedelica*, in Southern Vietnam with updated information on trade. Nature Conservation 31: 1–16. https://doi.org/10.3897/natureconservation.31.28145

Ngo HN, Nguyen TQ, Phan TQ, van Schingen M, Ziegler T (2019a) A case study on trade in threatened Tiger Geckos (*Goniurosaurus*) in Vietnam including updated information on the abundance of the Endangered *G. catbaensis*. Nature Conservation 33: 1–19. https://doi.org/10.3897/natureconservation.32.33590

Ngo HN, Le Q, Nguyen Q, Le D, van Schingen M, Ziegler T (2019b) First record of the Cat Ba tiger gecko, *Goniurosaurus catbaensis*, from Ha Long Bay, Quang Ninh Province, Vietnam: Microhabitat selection, potential distribution, and threat evaluation. Amphibian & Reptile Conservation 13(2): 1–13. http://www.amphibian-reptile-conservation.org/pdfs/Volume/Vol_13_no_2/ARC_13_2_[General_Section]_1-13_e183.pdf

Ngo HT, Nguyen TT, Le MD, van Schingen-Khan M, Nguyen TQ, Rauhaus R, Vences M, Ziegler T (2020) Genetic screening of captive crocodile lizards (*Shinisaurus crocodilurus*) in Europe. Der Zoologische Garten 88: 17–30.

Ngo HN, Nguyen HQ, Phan TQ, Nguyen TQ, Gewiss LR, Rödder D, Ziegler T (2022) Modeling the environmental refugia of the endangered Lichtenfelder's Tiger Gecko (*Goniurosaurus lichtenfelderi*) towards implementation of transboundary conservation. Frontiers of Biogeography 14(1): e51167. https://doi.org/10.21425/F5FBG51167

Nguyen LT, Van HH, Nguyen TT, McCormack TE, Nguyen SN (2016) A collection of amphibians and reptiles from Bac Huong Hoa Nature Reserve, Quang Tri province, Vietnam. Paper presented at the Proceedings of the 3nd National Scientific Workshop "Amphibians and Reptiles in Vietnam.

Nguyen LT, Nguyen VDH, Nguyen SN (2018a) The southernmost distribution of the Eastern Black-bridged Leaf Turtle, *Cyclemys pulchristriata* Fritz, Gaulke & Lehr, 1997 (Reptilia, Testudines, Geoemydidae), in Ba Ria-Vung Tau Province, Vietnam. Check List 14(6): 1007–1011. https://doi.org/10.15560/14.6.1007

Nguyen LT, Do DT, Van Hoang H, Nguyen TT, McCormack TE, Nguyen TQ, Orlov NL, Nguyen VDH, Nguyen SN (2018b) A new species of the genus *Acanthosaura* Gray, 1831 (Reptilia: Agamidae) from Central Vietnam. Russian Journal of Herpetology 25(4): 259–274. https://doi.org/10.30906/1026-2296-2018-25-4-259-274

Nguyen LT, Lam NQ, Carney J, Nguyen TT, Van Hoang H, Mccormack TE, Nguyen S (2020) First record of Western Black-Bridged Leaf Turtle, *Cyclemys atripons* Iverson & McCord, 1997 (Testudines, Geoemydidae), in Vietnam. Check List 16(3): 571–577. https://doi.org/10.15560/16.3.571

Nguyen NS, Nguyen TQ, Golynsky E, Milto K (2018) *Cyrtodactylus nigriocularis*. The IUCN Red List of Threatened Species 2018: e.T104695227A104718656.

Nguyen SV, Ho CT, Nguyen TQ (2009) Herpetofauna of Vietnam. Ed. Chimaria.

Nguyen SN, Nguyen VDH, Le SH, Murphy RW (2016) A new species of kukri snake (Squamata: Colubridae: *Oligodon* Fitzinger, 1826) from Con Dao Islands, southern Vietnam. Zootaxa 4139(2): 261–273. https://doi.org/10.11646/zootaxa.4139.2.9

Nguyen SN, Nguyen LT, Nguyen VDH, Phan HT, Jiang K, Murphy RW (2017) A new species of the genus *Oligodon* Fitzinger, 1826 (Squamata: Colubridae) from Cu Lao Cham Islands, central Vietnam. Zootaxa 4286(3): 333–346. https://doi.org/10.11646/zootaxa.4286.3.2

Nguyen T, Nguyen T, Pham C, Ong A, Ziegler T (2018) New records of snakes (Squamata: Serpentes) from Hoa Binh Province, northwestern Vietnam. Bonn Zoological Bulletin 67(1): 15–24.

Nguyen T, Brakels P, Maury N, Sudavanh S, Pawangkhananant P, Idiiatullina S, Lorphengsy S, Inkhavilay K, Suwannapoom C, Poyarkov NA (2020) New herpetofaunal observations from Laos based on photo records. Amphibian & Reptile Conservation 14(2): 218–249.

Nguyen TQ, Schmitz A, Böhme W (2010) *Gekko ulikovskii* Darevsky & Orlov, 1994: A junior synonym of *Gekko badenii* Szczerbak & Nekrasova, 1994. Bonn Zoological Bulletin 57(1): 15–17

Nguyen TQ, Pham AV, Ziegler T, Ngo HT, Le MD (2017) A new species of *Cyrtodactylus* (Squamata: Gekkonidae) and the first record of *C. otai* from Son La Province, Vietnam. Zootaxa 4341(1): 25–40. https://doi.org/10.11646/zootaxa.4341.1.2

Nguyen TQ, Van Pham A, Van Tu H, Van Nguyen T, Ziegler T (2018) New records and an updated list of lizards from Son La Province, Vietnam. Herpetology Notes 11: 209–216. https://www.biotaxa.org/hn/article/download/32165/31968

Nguyen TQ, Ngo NH, Nguyen VK, Bui HM, Dang HP, Nguyen TC, Hoang VT, Ziegler T (2021) Threatened biodiversity potential of Hon Khoai Island, Southern Vietnam - Habitat of the endangered, endemic Psychedelic rock gecko. World Association of Zoos and Aquariums. https://www.waza.org/blog/threatened-biodiversity-potential-of-hon-khoai-island-southern-vietnam/

Orlov NL, Ryabov SA, Nguyen TT, Nguyen QT, Ho TC (2008) A new species of *Goniurosaurus* (Sauria: Gekkota: Eublepharidae) from north Vietnam. Russian Journal of Herpetology 15(3): 229–244.

Orlov NL, Ermakov OA, Nguyen TT, Ananjeva NB (2021) A new record of odd-scaled snake (Serpentes, Xenodermidae) from Vietnam: expanded description of *Parafimbrios vietnamensis* based on integrative taxonomy. Zookeys 1048: 79. https://doi.org/10.3897/zookeys.1048.66477

Ostrowski S, Le MD, Ngo HT, Phung TM, Nguyen TQ, Ziegler T (2021) A new *Cyrtodactylus* (Squamata: Gekkonidae) from Binh Thuan Province, southern Vietnam. European Journal of Taxonomy 731: 47–70. https://doi.org/10.5852/ejt.2021.731.1203

Pham VA, Nguyen QT (2019a) New Records of Snakes (Reptilia, Squamata, Serpentes) Distribution in Lai Chau Province, Vietnam. VNU Journal of Science: Natural Sciences and Technology 35(1): 97–103. https://doi.org/10.25073/2588-1140/vnunst.4854

Pham VA, Le DT, Nguyen SLH, Ziegler T, Nguyen TQ (2015) New provincial records of skinks (Squamata: Scincidae) from northwestern Vietnam. Biodiversity Data Journal 3: e4284. https://doi.org/10.3897/BDJ.3.e4284

Pham VA, Le MD, Ziegler T, Nguyen TQ (2019b) A new species of *Cyrtodactylus* (Squamata: Gekkonidae) from northwestern Vietnam. Zootaxa 4544(3): 360–380. https://doi.org/10.11646/zootaxa.4544.3.3

Pham VA, Ziegler T, Nguyen TQ (2020) New records and an updated checklist of snakes from Son La Province, Vietnam. Biodiversity Data Journal 8: e52779. https://doi.org/10.3897/BDJ.8.e52779

Pievani T (2014) The sixth mass extinction: Anthropocene and the human impact on biodiversity. Rendiconti Lincei. Scienze Fisiche e Naturali 25(1): 85–93. https://doi.org/10.1007/s12210-013-0258-9

Pimm SL, Russell GJ, Gittleman JL, Brooks TM (1995) The future of biodiversity. Science 269(5222): 347–350. https://doi.org/10.1126/science.269.5222.347

Poyarkov Jr NA, Nguyen TV, Vogel G (2019a) A new species of the genus *Liopeltis* Fitzinger, 1843 from Vietnam (Squamata: Colubridae). Journal of Natural History 53(27–28): 1647–1672. https://doi.org/10.1080/00222933.2019.1656784

Poyarkov Jr NA, Van Nguyen T, Orlov NL, Vogel G (2019b) A new species of the genus *Calamaria* Boie, 1827 from the highlands of the Langbian Plateau, southern Vietnam (Squamata: Colubridae). Russian Journal of Herpetology 26(6): 335–348. https://doi.org/10.30906/1026-2296-2019-26-6-335-348

Poyarkov Jr NA, Geissler P, Gorin VA, Dunayev EA, Hartmann T, Suwannapoom C (2019c) Counting stripes: Revision of the *Lipinia vittigera* complex (Reptilia, Squamata, Scincidae) with description of two new species from Indochina. Zoological Research 40(5): 358–393. https://doi.org/10.24272/j.issn.2095-8137.2019.052

Pyron RA, Burbrink FT, Colli GR, De Oca ANM, Vitt LJ, Kuczynski CA, Wiens JJ (2011) The phylogeny of advanced snakes (Colubroidea), with discovery of a new subfamily and comparison of support methods for likelihood trees. Molecular Phylogenetics and Evolution 58(2): 329–342. https://doi.org/10.1016/j.ympev.2010.11.006

Rasmussen AR, Elmberg J, Gravlund P, Ineich I (2011) Sea snakes (Serpentes: subfamilies Hydrophiinae and Laticaudinae) in Vietnam: a comprehensive checklist and an updated identification key. Zootaxa 2894(1): 1–20. https://doi.org/10.11646/zootaxa.2894.1.1

Rasmussen AR, Elmberg J, Sanders KL, Gravlund P (2012) Rediscovery of the rare sea snake *Hydrophis parviceps* Smith 1935: Identification and conservation status. Copeia 2012(2): 276–282. https://doi.org/10.1643/CH-11-116

Ren JL, Wang K, Nguyen TT, Hoang C, Zhong GH, Jiang K, Guo P, Li JT (2018) Taxonomic re-evaluation of the monotypic genus *Pararhabdophis* Bourret, 1934 (Squamata: Colubridae: Natricinae) with discovery of its type species, *P. chapaensis*, from China. Zootaxa 4486(1): 31–56. https://doi.org/10.11646/zootaxa.4486.1.2

Richmond JQ, Wostl E, Reed RN, Fisher RN (2021) Range eclipse leads to tenuous survival of a rare lizard species on a barrier atoll. Oryx 56(1): 63–72. https://doi.org/10.1017/S0030605320001404

Rull V (2022) Biodiversity crisis or sixth mass extinction. EMBO Reports. https://doi.org/10.15252/embr.202154193

Seddon PJ, Moehrenschlager A, Ewen J (2014a) Reintroducing resurrected species: Selecting DeExtinction candidates. Trends in Ecology & Evolution 29(3): 140–147. https://doi.org/10.1016/j.tree.2014.01.007

Seddon PJ, Griffiths CJ, Soorae PS, Armstrong DP (2014b) Reversing defaunation: Restoring species in a changing world. Science 345(6195): 406–412. https://doi.org/10.1126/science.1251818

Siler CD, Heitz BB, Davis DR, Freitas ES, Aowphol A, Termprayoon K, Grismer LL (2018) New supple skink, Genus *Lygosoma* (Reptilia: Squamata: Scincidae), from Indochina and redescription of *Lygosoma quadrupes*. Journal of Herpetology 52(3): 332–347. https://doi.org/10.1670/16-064

Stanford CB, Iverson JB, Rhodin AG, van Dijk PP, Mittermeier RA, Kuchling G, Berry KH, Bertolero A, Bjorndal KA, Blanck TEG, Buhlmann KA, Burke RL, Congdon JD, Diagne T, Edwards T, Eisemberg CC, Ennen JR, Forero-Medina G, Frankel M, Fritz U, Gallego-Garcia N, Georges A, Gibbons JW, Gong S, Goode EV, Shi HT, Hoang H, Hofmeyer MD, Horne BD, Hudson R, Juvik JO, Kiester RA, Koval P, Le M, Lindemann PV, Lovich JE, Luiselli L, McCormack TEM, Meyer GA, Paez VP, Platt K, Pritchard PCH, Quinn HR, Roosenburg WM, Seminoff JA, Shaffer HB, Spencer R, van Dyke JU, Vogt RC, Walde AD (2020) Turtles and tortoises are in trouble. Current Biology 30(12): R721–R735. https://doi.org/10.1016/j.cub.2020.04.088

Sterling EJ, Hurley MM, Le MD (2006) Vietnam: A Natural History. Yale University Press. https://doi.org/10.12987/9780300128215

Stolton S, Dao NT, Dudley N (2004) Categorising protected areas in Vietnam. PARKS 14(3): 23–27. www.npshistory.com/newsletters/parks/parks-1403.pdf#page=25

Sy D (2019) Assessment of species diversity and distribution of snakes (Serpentes) in Ba Vi National Park, Hanoi.

Tan NV, Loi DD, Tho DTA (2019) New Records and an Updated List of Snakes (Squamata: Serpentes) from Binh Dinh Province, Central-Southern Vietnam. Hue University Journal of Science: Natural Science 128(1B): 35–41. https://doi.org/10.26459/hueunijns.v128i1B.5203

Thao HN (2020) New distribution of *Bronchocela smaragdina* Gunther, 1864 and *Bronchocela vietnamensis* Hallermann & Orlov, 2005 in Nui Ong Nature Reserve, Vietnam. Herpetology Notes 13: 501–504. https://www.biotaxa.org/hn/article/download/57233/61896

Tollefson J (2019) Humans are driving one million species to extinction. Nature 569(7755): 171–172. https://doi.org/10.1038/d41586-019-01448-4

Tran K, Ho CT, Nguyen SV, Pham T (2007) List of Threatened Reptiles and Amphians. In: Dang NT, Tran K, Dang HH, Nguyen C, Nguyen N, Nguyen YH, Dang DT (Eds) Vietnam Red Data Book. Part I. Animals, 219–276.

Traylor-Holzer K, Leus K, Bauman K (2019) Integrated collection assessment and planning (ICAP) workshop: Helping zoos move toward the One Plan Approach. Zoo Biology 38(1): 95–105. https://doi.org/10.1002/zoo.21478

TTWG (2021) Turtle Taxonomy Working Group. Turtles of the World: Annotated Checklist and Atlas of Taxonomy, Synonymy, Distribution, and Conservation Status (9th Ed.). In: Rhodin AGJ, Iverson JB, van Dijk PP, Saumure RA, Buhlmann KA, Pritchard PCH, Mittermeier RA (Eds) Conservation Biology of Freshwater Turtles and Tortoises: A Compilation Project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group. Chelonian Research Monographs 8: 1–472.

Tung NT, Quang NH, Vinh LQ (2018) New record of bent-toed gecko (*Cyrtodactylus bobrovi* Nguyen, Le, Pham, Ngo, Hoang, Pham & Ziegler, 2015) from Cuc Phuong National Park. Journal of Forestry Science and Technology 2: 157–161.

Uetz P, Freed P, Hošek J (2022) The Reptile Database. http://www.reptile-database.org [Accessed on 13.03.2022]

Van HP, Ke QT (2014) The Web based tool for identification of amphibian and reptiles presented in three western provinces of South Eastern region, Vietnam. Nauchnii rezultat. Seriya "Fiziologya" 2: 21–32. [Научный результат. Серия "Физиология" 2: 21–32.] https://scholar.archive.org/work/ezvppjyuc5ekdapbtl7rdpmo44/access/wayback/https://cyberleninka.ru/article/n/the-web-based-tool-for-identification-of-amphibian-and-rep-tiles-presented-in-three-western-provinces-of-south-eastern-region-vietnam.pdf

Van Nguyen T, Duong LD, Duong TTA (2019) New records and updated list of snakes (Squamata: Serpentes) from Binh Dinh province, central-southern Vietnam. Hue University Journal of Science. Nature and Science 128(1B): 35–41. https://doi.org/10.26459/hueuni-jns.v128i1B.5203

Van Schingen M, Schepp U, Pham CT, Nguyen TQ, Ziegler T (2015) Last chance to see? A review of the threats to and use of the crocodile lizard. Traffic Bulletin 27: 19–26.

van Schingen-Khan M, Barthel LMF, Pham DTK, Pham CT, Nguyen TQ, Ziegler T, Bonkowski M (2022) Will climatic changes affect the Vietnamese crocodile lizard? Seasonal variation in microclimate and activity pattern of *Shinisaurus crocodilurus vietnamensis*. Amphibia-Reptilia 43(2): 155–167. https://doi.org/10.1163/15685381-bja10089

VEA (2020) Vietnam Environment Administration. http://vea.gov.vn/detail?$id=56. [Accessed on 13.03.2022]

Wahle A, Rödder D, Chapple DG, Meiri S, Rauhaus A, Ziegler T (2021) Skinks in Zoos: A global approach on distribution patterns of threatened Scincidae in zoological institutions. Global Ecology and Conservation 30: e01800. https://doi.org/10.1016/j.gecco.2021.e01800

Wang Y, Jianhuan Y, Yang LIU (2013) New Distribution Records for *Sphenomorphus tonkinensis* (Lacertilia: Scincidae) with Notes on Its Variation and Diagnostic Characters. Asian Herpetological Research 4(2): 147–150. https://doi.org/10.3724/SP.J.1245.2013.00147

Wang K, Jiang K, Wang YF, Poyarkov Jr NA, Che J, Siler CD (2018) Discovery of *Japalura chapaensis* Bourret, 1937 (Reptilia: Squamata: Agamidae) from Southeast Yunnan Province, China. Zoological Research 39(2): 105–113. https://doi.org/10.24272/j.issn.2095-8137.2017.064

Wang K, Yu ZB, Vogel G, Che J (2021) Contribution to the taxonomy of the genus *Lycodon* H. Boie in Fitzinger, 1827 (Reptilia: Squamata: Colubridae) in China, with description of two new species and resurrection and elevation of *Dinodon septentrionale chapaense* Angel, Bourret, 1933. Zoological Research 42(1): 62–86. https://doi.org/10.24272/j.issn.2095-8137.2020.286

Weaver W, Shannon CE (1949) The mathematical theory of communication. University of Illinois, Urbana, Illinois.

Ziegler T (2015a) *In situ* and *ex situ* reptile projects of the Cologne Zoo: Implications for research and conservation of South East Asia's herpetodiversity. International Zoo Yearbook 49(1): 8–21. https://doi.org/10.1111/izy.12084

Ziegler T, Nguyen TQ (2015b) Review of the genus *Sinonatrix* in Vietnam with a new country record of *Sinonatrix yunnanensis* Rao et Yang, 1998. Russian Journal of Herpetology 22(2): 84–88.

Ziegler T, Nguyen TQ (2019a) Herpetological research and conservation in Vietnam and Laos in compliance with the one plan approach. Paper presented at the Proc. 4th Nat Sci Conf Amph Rept Vietnam.

Ziegler T, Rauhaus A (2022) Vietnamese Tiger Gecko Research and Conservation Breeding Projects at the Terrarium Section of Cologne Zoo, Germany. Ratel 48(3): 5–10.

Ziegler T, Tran DTA, Nguyen TQ, Perl RGB, Wirk L, Kulisch M, Lehmann T, Rauhaus A, Nguyen TT, Le QK, Vu TN (2014) New amphibian and reptile records from Ha Giang Province, northern Vietnam. Herpetology Notes 7: 185–201.

Ziegler T, Rauhaus A, Mutschmann F, Huy PD, Pham CT, Nguyen TQ (2015c) Building up of keeping facilities and breeding projects for frogs, newts and lizards at the Me Linh Station for Biodiversity in northern Vietnam, including improvement of housing conditions for confiscated reptiles and primates. Der Zoologische Garten 85(3/4): 91–120. https://doi.org/10.1016/j.zoolgart.2015.09.001

Ziegler T, Rauhaus A, Nguyen T, Nguyen K (2015d) Südlichster Nachweis von *Gekko badenii* Szczerbak & Nekrasova, 1994. Sauria 37: 3–14. http://wildlifeatrisk.org/wp-content/uploads/2019/01/technical_report.Hon-Me-.pdf

Ziegler T, Rauhaus A, Nguyen K, Nguyen T (2016) Building of a Conservation Breeding Facility for the Psychedelic Rock Gecko (*Cnemaspis psychedelica*) in Southern Vietnam. Der Zoologische Garten 85(5): 224–239. https://doi.org/10.1016/j.zoolgart.2016.05.002

Ziegler T, Pham CT, Nguyen TQ, Wang J, Wang YY, Stuart BL (2019b) A new species of *Opisthotropis* from northern Vietnam previously misidentified as the Yellow-spotted Mountain Stream Keelback *O. maculosa* Stuart & Chuaynkern, 2007 (Squamata: Natricidae). Zootaxa 4613(3): 579–586. https://doi.org/10.11646/zootaxa.4613.3.9

Ziegler T, Van Schingen M, Rauhaus A, Dang PH, Pham DTK, Pham CT, Nguyen TQ (2019c) New insights into the habitat use and husbandry of crocodile lizards (Reptilia: Shinisauridae) including the conception of new facilities for Vietnamese crocodile lizards *Shinisaurus crocodilurus vietnamensis* in Vietnam and Germany. International Zoo Yearbook 53(1): 250–269. https://doi.org/10.1111/izy.12215

Ziegler T, Nguyen TT, Ong AV, Pham CT, Nguyen TQ (2020a) In search of the Spotted Softshell Turtle in Vietnam: An implementation of the One Plan Approach. WAZA News 2020(1): 24–27.

Ziegler T, Luu VQ, Nguyen TT, Van Ha N, Ngo HT, Le MD, Nguyen TQ (2020b) Rediscovery of Andrea's keelback, *Hebius andreae* (Ziegler & Le, 2006): First country record for Laos and phylogenetic placement. Revue Suisse de Zoologie 126(1): 61–71. https://doi.org/10.5281/zenodo.2619520

Ziegler T, Tran VA, Babb RD, Jones TR, Moler PE, Van Devender RW, Nguyen TQ (2020c) A new species of reed snake, *Calamaria* Boie, 1827 from the Central Highlands of Vietnam (Squamata: Colubridae). Revue Suisse de Zoologie 126(1): 17–26. https://doi.org/10.5281/zenodo.2619512

Ziegler T, Ziegler TN, Peusquens J, David P, Vu TN, Pham CT, Nguyen TQ, Le MD (2020d) Expanded morphological definition and molecular phylogenetic position of the Tam Dao mountain stream keelback *Opisthotropis tamdaoensis* (Squamata: Natricidae) from Vietnam. Revue Suisse de Zoologie 124(2): 377–389. https://doi.org/10.5281/zenodo.893551

ZIMS (2022) Species360 Zoological Information Management System (ZIMS). https://zims.species360.org [Accessed on 13.03.2022]

Zootierliste (2022) Electronic Database. https://zootierliste.de/?org=7 [Accessed on 13.03.2022]

Supplementary material 1

Species list, distribution, endemism, status, threats

Authors: Lilli Stenger, Anke Große Hovest, Truong Quang Nguyen, Cuong The Pham, Anna Rauhaus, Minh Duc Le, Dennis Rödder, Thomas Ziegler

Data type: docx

Link: https://doi.org/10.3897/natureconservation.53.106923.suppl1

Nature Conservation 53: 223–256 (2023)
DOI: 10.3897/natureconservation.53.106732

Research Article

An analysis of the inter-state similarity of the herpetofaunas of Mexican states

Julio A. Lemos-Espinal[1], Geoffrey R. Smith[2]

1 *Laboratorio de Ecología-UBIPRO, FES Iztacala UNAM, Avenida los Barrios 1, Los Reyes Iztacala, Tlalnepantla, edo. de México, 54090, Mexico*
2 *Department of Biology, Denison University, Granville, Ohio 43023, USA*
Corresponding author: Julio A. Lemos-Espinal (lemos@unam.mx)

Abstract

Mexico is a megadiverse country with high amphibian and reptile richness. Understanding how Mexico's herpetofauna is shared among Mexican states can contribute to developing conservation plans by figuring out which states may need to coordinate conservation actions. We generated species lists for the herpetofauna for all Mexican states, and used hierarchical clustering analyses to identify clusters of states on the basis of amphibian and reptile species separately. We also calculated pair-wise Jaccard distances for all Mexican states for amphibians, reptiles, and physiographic provinces and estimated the length of shared borders between states and the geographic (straight-line) distance between the centroids of pairs of states. We used these data to explore potential drivers of the cluster analysis results. Our cluster analysis for amphibians identified five clusters with nine subclusters, and for reptiles, resulted in four clusters with six subclusters. In general, the clusters for Mexican amphibians and reptiles have a similar composition of states. However, for amphibians, the states of Veracruz and Puebla form a cluster separate from a large cluster of northeastern Mexican states, whereas in reptiles Veracruz and Puebla cluster with northeastern Mexican states. Jaccard distances of amphibians and reptiles were highly, positively correlated. Both amphibian and reptile Jaccard distances were positively correlated with the physiographic provinces' Jaccard distance and shared border length and negatively correlated with the distance between centroids. Taken together, our results suggest that the pattern of the sharing of herpetofaunal species among Mexican states is a consequence of the states' proximity. Such a pattern is consistent with the underlying driver being the similarity of physiographic provinces (i.e., habitats and ecosystems) of these states (i.e., geographic proximity likely reflects, at least in large part, ecological similarity). Our results suggest clusters of states that should coordinate the conservation and management of their herpetofaunas. For example, clusters of states in southern Mexico share a high number of threatened amphibian species and clusters of states in northern Mexico share a high number of threatened reptile species. Oaxaca is also a state that has a unique herpetofauna and a high number of threatened species of both amphibians and reptiles.

Key words: Amphibia, hierarchical clustering, Jaccard's similarity, physiographic provinces, reptiles

Academic editor: Roger Ledo
Received: 22 May 2023
Accepted: 13 September 2023
Published: 25 September 2023

ZooBank: https://zoobank.org/633EC7FD-59E5-4F2D-B2CE-1096F32FB819

Citation: Lemos-Espinal JA, Smith GR (2023) An analysis of the inter-state similarity of the herpetofaunas of Mexican states. Nature Conservation 53: 223–256. https://doi.org/10.3897/natureconservation.53.106732

Introduction

Mexico is a megadiverse country, with high species richness for a variety of taxa (Ramamoorthy et al. 1993). In addition, Mexico is the location for several conservation hotspots (Myers et al. 2000; Lira et al. 2002; Sosa and De-nova 2012; Contreras-MacBeath et al. 2014), as well as a country with high levels of endemism for several taxa (e.g., mosses, Delgadillo 1994; mammals: Escalente et al. 2009; vascular plants, Luna-Vega et al. 2013; birds, Bertelli et al. 2017; trees, Tellez et al. 2020). Much of Mexico consists of a transition zone (i.e., the Mexican Transition Zone) between the Nearctic and Neotropical zones (Villaseñor et al. 2020), giving rise to much of its diversity.

Mexico is also an area of high amphibian and reptile richness (e.g., Flores-Villela 1993; Flores-Villela and García-Vázquez 2014; Suazo-Ortuño et al. 2023), but also an area with high extinction debt and risk for these taxa (Chen and Peng 2017). Some amphibian and reptile taxa have centers of endemism in Mexico (Flores-Villela 1993; Suazo-Ortuño et al. 2023). For example, the Sierra Madre Occidental, Sierra Madre Oriental, and the Trans-Mexican Volcanic Belt are well known for being hotspots for differentiation of various Mexican reptiles and amphibians (e.g., Cisneros-Bernal et al. 2022), creating high levels of state endemics, even within the larger ecological province.

Mexico needs conservation approaches that focus on protecting specific areas (Bolam et al. 2023). Understanding how species are shared among ecological or biogeographical provinces is of critical importance to generating such conservation or management plans. Indeed, for amphibians and reptiles, ecoregions are particularly distinct (i.e., have distinct communities) (Smith et al. 2020). However, understanding how species are shared across political entities, such as states within a country, is also important to coordinating efforts among state-level governments (see Liu et al. 2020 for a broader discussion of transborder conservation). Indeed, it has been suggested that efforts to address the factors affecting biodiversity in Mexico will need to be shifted from the federal to state or even local levels (Sarukhán et al. 2015). It is therefore important to understand how the herpetofauna are distributed and shared among political entities within Mexico (i.e., among the Mexican states).

Previous efforts to examine similarity in the herpetofauna among Mexican states have been limited to cluster analyses of the states along both sides of the United States-Mexican border (Enderson et al. 2009; Smith and Lemos-Espinal 2015) and examinations of the number of species shared between focal states and their neighboring states (e.g., Lemos-Espinal and Smith 2020a, b, c; Lemos-Espinal et al. 2020). Here we explore how amphibian and reptile species are shared among Mexican states (including Mexico City) in order to determine how conservation efforts might best be coordinated among political entities. We also examine how similarities among states may be a function of the states' proximity (distance between centroids and length of shared border) and their similarities in physiographic provinces.

Methods

Using the available literature, we collected species lists for amphibians and reptiles for all of the Mexican states, that we updated using additional literature (see Table 1). We used hierarchical clustering analyses based on Jaccard's Similarity Coefficients for Binary Data as the distance metric with single linkages methods (nearest neighbor) to generate clusters of states on the basis of amphibian and reptile species separately. We identified clusters and subclusters for amphibians and reptiles separately by visually examining the resulting cluster tree and grouping states that shared common nodes, taking into account the Jaccard distances. Subclusters were groups of states that shared nodes within a larger cluster. We used the species' lists to calculate pair-wise Jaccard distances for all Mexican states for amphibians and reptiles, separately. We also generated pairwise Jaccard distances with respect to the physiographic provinces found in each state (see Table 2). In addition, we obtained two geospatial estimates: 1) the length of shared borders between the states using the Polygon Neighbors Tool and 2) the straight-line distance between the centroids of the states using the Feature to Point Tool and Point Distance on a Lambert Conformal Conic projection in Datum WGS84 in ArcGIS 10.3.1 (Environmental Systems Research Institute, Inc, Redlands, CA). We ran non-parametric Spearman's ρ tests to examine correlations among Jaccard distance estimates for amphibians, reptiles, physiographic regions, the length of shared borders, and the distance between the centroids of the states. Cluster analyses were performed using Systat 13.2 (Systat Software Inc., San Jose, CA) and all other statistical analyses were performed using JMP 16.2 (SAS Institute, Cary, NC).

Results and discussion

Cluster analysis

Our cluster analysis for amphibians generated five clusters, with subclusters apparent in some of the clusters (Figs 1A, 2A). Cluster AI consisted of Baja California and Baja California Sur. Cluster AII includes Tabasco and Chiapas and subcluster AIIa, which includes Yucatán, Quintana Roo, and Campeche. Cluster AIII consists of Veracruz and Puebla. Cluster AIV includes Durango and four subclusters: AIVa includes Morelos and México; AIVb includes Michoacán, Jalisco, and Colima; AIVc includes Nayarit and Sinaloa; and AIVd includes Sonora and Chihuahua. Cluster AV is made up of four subclusters: AVa includes Aguascalientes and Zacatecas; AVb includes Guanajuato and Querétaro; AVc includes San Luis Potosí and Hidalgo; and AVd includes Tamaulipas, Nuevo León, and Coahuila. Tlaxcala and Mexico City connect with a larger grouping of clusters AIII, AIV, and AV. Guerrero and Oaxaca then connect to this large grouping.

The cluster analysis for reptiles resulted in four clusters, again with subclusters within some clusters (Figs 1B, 2B). Cluster RI consists of Baja California and Baja California Sur. Cluster RII includes Chiapas and Tabasco, as well as subcluster RIIa that includes Campeche, Quintana Roo, and Yucatán. Cluster RIII includes Guanajuato and two subclusters: RIIIa consists of Coahuila, Nuevo León, Tamaulipas, San Luis Potosí, Hidalgo, and Querétaro; whereas RIIIb includes Veracruz and Puebla. Cluster RIV is a large cluster containing Mexico City and Tlaxcala and three subclusters: RIVa includes Aguascalientes, Zacatecas, Durango, Chihuahua, and Sonora; RIVb includes Sinaloa, Nayarit, Jalisco, Michoacán, and Colima; and RIVc includes Morelos and México. Guerrero and Oaxaca connect to a large group that consists of clusters RIII and RIV.

Table 1. Alphabetical list of Mexican States with the literature sources used to create the species lists of amphibians and reptiles used in the cluster analyses. State names are followed by the abbreviations used in Fig. 1. Source refers to the references from which the checklist for each specific state was obtained. Updates lists references used to update the original checklist we used for each state.

State	Source	Updates
Aguascalientes (AGS)	Carbajal-Márquez and Quintero-Díaz (2016)	Cox et al. (2018);
Baja California (BC)	Grismer (2002); Hollingsworth et al. (2015)	Cox et al. (2018); Meik et al. (2018);
Baja California Sur (BCS)	Grismer (2002)	Cox et al. (2018); Meik et al. (2018);
Campeche (CAMP)	González-Sánchez et al. (2017)	Ortiz-Medina et al. (2020); Palacios-Aguilar and Flores-Villela (2020);
Chiapas (CHIS)	Johnson et al. (2015)	Hernández-Ordóñez et al. (2017); Clause et al. (2020); McCranie et al. (2020); Palacios-Aguilar and Flores-Villela (2020); Lara-Tufiño and Nieto-Montes de Oca (2021)
Chihuahua (CHIH)	Lemos-Espinal et al. (2017)	Burbrink and Guiher (2014); Montanucci (2015); Blair and Hansen (2018); Cox et al. (2018); Palacios-Aguilar and Flores-Villela (2020); Ramírez-Reyes et al. (2021b)
Coahuila (COAH)	Lemos-Espinal and Smith (2016); Lazcano et al. (2019)	Burbrink and Guiher (2014); Baeza-Tarin et al. (2018)
Colima (COL)	Lemos-Espinal et al. (2020)	Horowitz (1955); Montanucci (1979); Hillis et al. (1983); Platz (1991); Webb (2001); McCranie and Köhler (2004); Zaldivar-Riverón et al. (2004); Pérez-Ramos and Saldaña-de la Riva (2008); Lavin et al. (2014); Streicher et al. (2014); Campbell et al. (2018); Cox et al. (2018); Grünwald et al. (2018); O'Connell and Smith (2018); Ramírez-Reyes and Flores-Villela (2018); McCranie et al. (2020); Montaño-Ravalcaba et al. (2020); Palacios-Aguilar and Flores-Villela (2020); Reyes-Velasco et al. (2020a, b)
Durango (DGO)	Lemos-Espinal et al. (2018a, 2019b)	Montanucci (2015); Campbell et al. (2018); Caviedes-Solis and Nieto-Montes de Oca (2018); Campillo-García et al. (2021); Ramírez-Reyes et al. (2021b)
Guanajuato (GTO)	Leyte-Manrique et al. (2022)	
Guerrero (GRO)	Palacios-Aguilar and Flores-Villela (2018)	Ramírez-Reyes et al. (2017); Campbell et al. (2018); Caviedes-Solis and Nieto-Montes de Oca (2018); Cox et al. (2018); García-Vázquez et al. (2018); Palacios-Aguilar et al. (2018); Ramírez-Reyes and Flores-Villela (2018); Blancas-Hernández et al. (2019); Grünwald et al. (2019); Köhler et al. (2019); Kaplan et al. (2020); Palacios-Aguilar and Flores-Villela (2020); Palacios-Aguilar and Santos-Bibiano (2020); Everson et al. (2021); García-Vázquez et al. (2021); Grünwald et al. (2021a, b); Jameson et al. (2022)
Hidalgo (HGO)	Lemos-Espinal and Smith (2015); Lemos-Espinal and Dixon (2016)	Hansen et al. (2016); Badillo-Saldaña et al. (2018); Caviedes-Solis and Nieto-Montes de Oca (2018); Ramírez-Bautista et al. (2020); Valencia-Herverth et al. (2020); Bryson et al. (2021); Campillo-García et al. (2021); Tepos-Ramírez et al. (2021)
Jalisco (JAL)	Cruz-Sáenz et al. (2017)	Ramírez-Reyes et al. (2017); Campbell et al. (2018); Caviedes-Solis and Nieto-Montes de Oca (2018); Cox et al. (2018); Ramírez-Reyes and Flores-Villela (2018); Pazos-Nava et al. (2019); Ahumada-Carrillo et al. (2020); Cavazos-Camacho and Ahumada-Carrillo (2020); McCranie et al. (2020); Palacios-Aguilar and Flores-Villela (2020); Bryson et al. (2021); Campillo-García et al. (2021); Everson et al. (2021); Flores-Villela et al. (2022)
México (MEX)	Lemos-Espinal and Smith (2020d)	Campbell et al. (2018); Caviedes-Solis and Nieto-Montes de Oca (2018); Kaplan et al. (2020); Bryson et al. (2021); Campillo-García et al. (2021); Everson et al. (2021); Jameson et al. (2022)
Mexico City (CDMX)	Lemos-Espinal and Smith (2020c)	García-Alvarado (2016); Campillo-García et al. (2021); Everson et al. (2021)
Michoacán (MICH)	Alvarado-Díaz et al. (2013)	Mendoza-Hernández and Roth-Monzón (2017); Ramírez-Reyes et al. (2017); Campbell et al. (2018); Cox et al. (2018); Ramírez-Reyes and Flores-Villela (2018); McCranie et al. (2020); Palacios-Aguilar and Flores-Villela (2020); Bryson et al. (2021); Campillo-García et al. (2021); Everson et al. (2021); Hernandez et al. (2022)
Morelos (MOR)	Lemos-Espinal and Smith (2020b)	Campbell et al. (2018); Cox et al. (2018); Palacios-Aguilar and Flores-Villela (2020); Jameson et al. (2022)
Nayarit (NAY)	Woolrich-Piña et al. (2016)	Ramírez-Reyes et al. (2017, 2021a, b); Campbell et al. (2018); Cox et al. (2018); Loc-Barragán et al. (2018); Ramírez-Reyes and Flores-Villela (2018); Loc-Barragán and Woolrich-Piña (2020); McCranie et al. (2020); Palacios-Aguilar and Flores-Villela (2020); Flores-Villela et al. (2022)
Nuevo León (NL)	Lemos-Espinal et al. (2016); Nevárez de los Reyes et al. (2016)	Grünwald et al. (2018); Nevarez de los Reyes et al. (2019a, b); Campillo-García et al. (2021)

State	Source	Updates
Oaxaca (OAX)	Mata-Silva et al. (2015, 2021)	Gray et al. (2016); Parra-Olea et al. (2016); Campbell et al. (2016, 2018); Canseco-Márquez et al. (2017a, b); Ramírez-Reyes et al. (2017); Caviedes-Solis and Nieto-Montes de Oca (2018); García-Padilla et al. (2019); Mata-Silva et al. (2019); Carbajal-Márquez et al. (2020); McCranie et al. (2020); Sánchez-García et al. (2020); García-Vázquez et al. (2021); Grünwald et al. (2021a, b); Jameson et al. (2022); Nieto-Montes de Oca et al. (2022)
Puebla (PUE)	Woolrich-Piña et al. (2017)	Caviedes-Solis and Nieto-Montes de Oca (2018); Campbell et al. (2018); Cox et al. (2018); de la Torres-Loranca et al. (2020); Fernández-Badillo et al. (2020); Palacios-Aguilar and Flores-Villela (2020); Everson et al. (2021)
Querétaro (QRO)	Dixon and Lemos-Espinal (2010); Cruz-Elizalde et al. (2019)	Bryson et al. (2021); Tepos-Ramírez et al. (2021)
Quintana Roo (QR)	González-Sánchez et al. (2017)	
San Luis Potosí (SLP)	Lemos-Espinal et al. (2018b)	Guajardo Welsh et al. (2020); Palacios-Aguilar and Flores-Villela (2020); Arenas-Moreno et al. (2021); Campillo-García et al. (2021); Tepos-Ramírez et al. (2021)
Sinaloa (SIN)	Lemos-Espinal and Smith (2020a)	Campbell et al. (2018); Cox et al. (2018); Trageser and Schell (2018); Loc-Barragán et al. (2020a, b); Palacios-Aguilar and Flores-Villela (2020); Ramírez-Reyes et al. (2021b)
Sonora (SON)	Rorabaugh and Lemos-Espinal (2016); Lemos-Espinal et al. (2019a)	Cox et al. (2018); Meik et al. (2018); Barley et al. (2021); Ramírez-Reyes et al. (2021b)
Tabasco (TABA)	Barragán-Vázquez et al. (2022)	
Tamaulipas (TAM)	Farr (2015); Terán-Juárez et al. (2016)	Grünwald et al. (2018); Rautsaw et al. (2018); Sosa-Tovar et al. (2019); Campillo-García et al. (2021)
Tlaxcala (TLAX)	Fernández et al. (2006)	
Veracruz (VER)	Torres-Hernández et al. (2021)	Carbajal-Márquez et al. (2020); Schätti et al. (2020); Tepos-Ramírez et al. (2021)
Yucatán (YUC)	González-Sánchez et al. (2017)	Palacios-Aguilar and Flores-Villela (2020);
Zacatecas (ZAC)	Sigala-Rodríguez et al. (2020a, b); J.J. Sigala-Rodríguez, pers. comm.	

For the most part, the clusters for Mexican amphibians and reptiles are similar. One difference, however, is that in amphibians, Veracruz and Puebla form a cluster separate from the large cluster of the northeastern Mexican states, whereas in reptiles Veracruz and Puebla cluster with the northeastern Mexican states. Our clusters roughly correspond to the five environmental regions identified by Ochoa-Ochoa et al. (2014). The clusters we generated are generally similar to clusters generated for Mexico examining similarity in turtles in North America (Ennen et al. 2017), as well as for endemic Asteraceae, Poaceae, and Musci (Delgadillo et al. 2003). Our clusters show less similarity to the clusters for the plant families examined by Luna-Vega et al. (2013) and Lira et al. (2002), but there are still many commonalities. Much of the underlying similarity among states in our study, and the general commonalities in our cluster analysis and the clusters from the other cited studies above, likely reflects the distribution of biogeographic provinces in Mexico (e.g., Morrone et al. 2017). In other words, the environmental factors and ecosystems found in Mexican states drive the distribution of species, and those states sharing such ecosystems and habitats share more species than those that do not, and these similarities appear to generally hold for several taxa. Thus, the apparently geographic clusters we identified likely arise because of the correlation of geographic traits of states (e.g., latitude, longitude, elevation, proximity to oceans) and environmental traits of states (e.g., physiographic provinces, climate). In terms of conservation policies, such a finding suggests that policies should focus on addressing physiographic provinces rather than

Table 2. The distribution of physiographic provinces in all Mexican states. BCP = Baja California Peninsula; SP = Sonoran Plain; SMOc = Sierra Madre Occidental; SPN = Sierra and Plains of the North; SMOr = Sierra Madre Oriental; GPNA = Great Plains of North America; PCP = Pacific Coast Plain; NGCP = North Gulf Coastal Plain; CP = Central Plateau; VA = Volcanic Axis; YP = Yucatan Peninsula; SMS = Sierra Madre del Sur; SGCP = Southern Gulf Coastal Plain; SCG = Sierra of Chiapas and Guatemala; CAMR = Central American Mountain Range.

	BCP	SP	SMOc	SPN	SMOr	GPNA	PCP	NGCP	CP	VA	YP	SMS	SGCP	SCG	CAMR
Aguascalientes	0	0	1	0	0	0	0	0	1	1	0	0	0	0	0
Baja California	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Baja California Sur	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Campeche	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0
Chiapas	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
Chihuahua	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
Coahuila	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0
Colima	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
Durango	0	0	1	1	1	0	0	0	1	0	0	0	0	0	0
Guanajuato	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0
Guerrero	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
Hidalgo	0	0	0	0	1	0	0	1	0	1	0	0	0	0	0
Jalisco	0	0	1	0	0	0	0	0	1	1	0	1	0	0	0
México	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
Mexico City	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Michoacán	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
Morelos	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0
Nayarit	0	0	1	0	0	0	1	0	0	1	0	1	0	0	0
Nuevo León	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0
Oaxaca	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1
Puebla	0	0	0	0	1	0	0	1	0	1	0	1	0	0	0
Querétaro	0	0	0	0	1	0	0	0	1	1	0	0	0	0	0
Quintana Roo	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
San Luis Potosí	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0
Sinaloa	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0
Sonora	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0
Tabasco	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Tamaulipas	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0
Tlaxcala	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Veracruz	0	0	0	0	1	0	0	1	0	1	0	1	1	1	1
Yucatán	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Zacatecas	0	0	1	0	1	0	0	0	1	1	0	0	0	0	0

states per se. However, our clusters help identify the political entities that need to be involved in the discussions of the ecological entities that are the focus of conservation efforts.

For each cluster, we identified the species that are in a threatened category in the IUCN Red List (i.e., Vulnerable, Endangered, Critically Endangered) and summarized the major threats facing each species based on the IUCN Red List species accounts (IUCN 2022; see Table 3 for amphibians, Table 4 for reptiles). For amphibians, clusters AII and AIII and Oaxaca had particularly high numbers of threatened species and should be the focus of targeted conservation

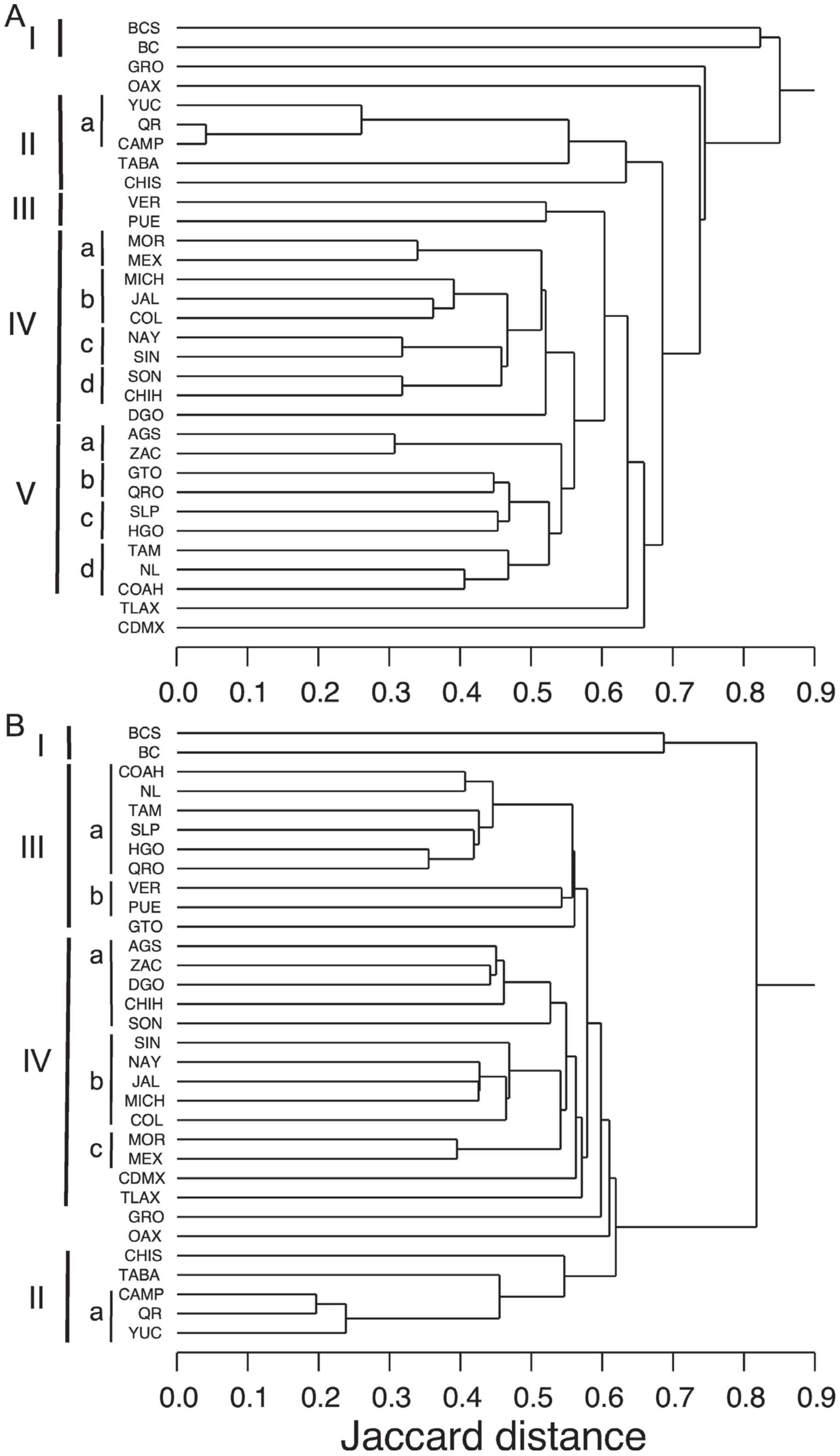


Figure 1. Cluster trees for A) amphibians and B) reptiles arising from a cluster analysis of the herpetofaunas of Mexican states. Main clusters are identified with Roman numerals and subclusters within clusters are identified with lower case letters. AGS = Aguascalientes, BC = Baja California, BCS = Baja California Sur, CAMP = Campeche, CHIS = Chiapas, CHIH = Chihuahua, COAH = Coahuila, COL = Colima, DGO = Durango, GTO = Guanajuato, GRO = Guerrero, HGO = Hidalgo, JAL = Jalisco, MEX = México, CDMX = Mexico City, MICH = Michoacán, MOR = Morelos, NAY = Nayarit, NL = Nuevo León, OAX = Oaxaca, PUE = Puebla, QRO = Querétaro, QR = Quintana Roo, SLP = San Luis Potosí, SIN = Sinaloa, SON = Sonora, TABA = Tabasco, TAM = Tamaulipas, TLAX = Tlaxcala, VER = Veracruz, YUC = Yucatán, ZAC = Zacatecas.

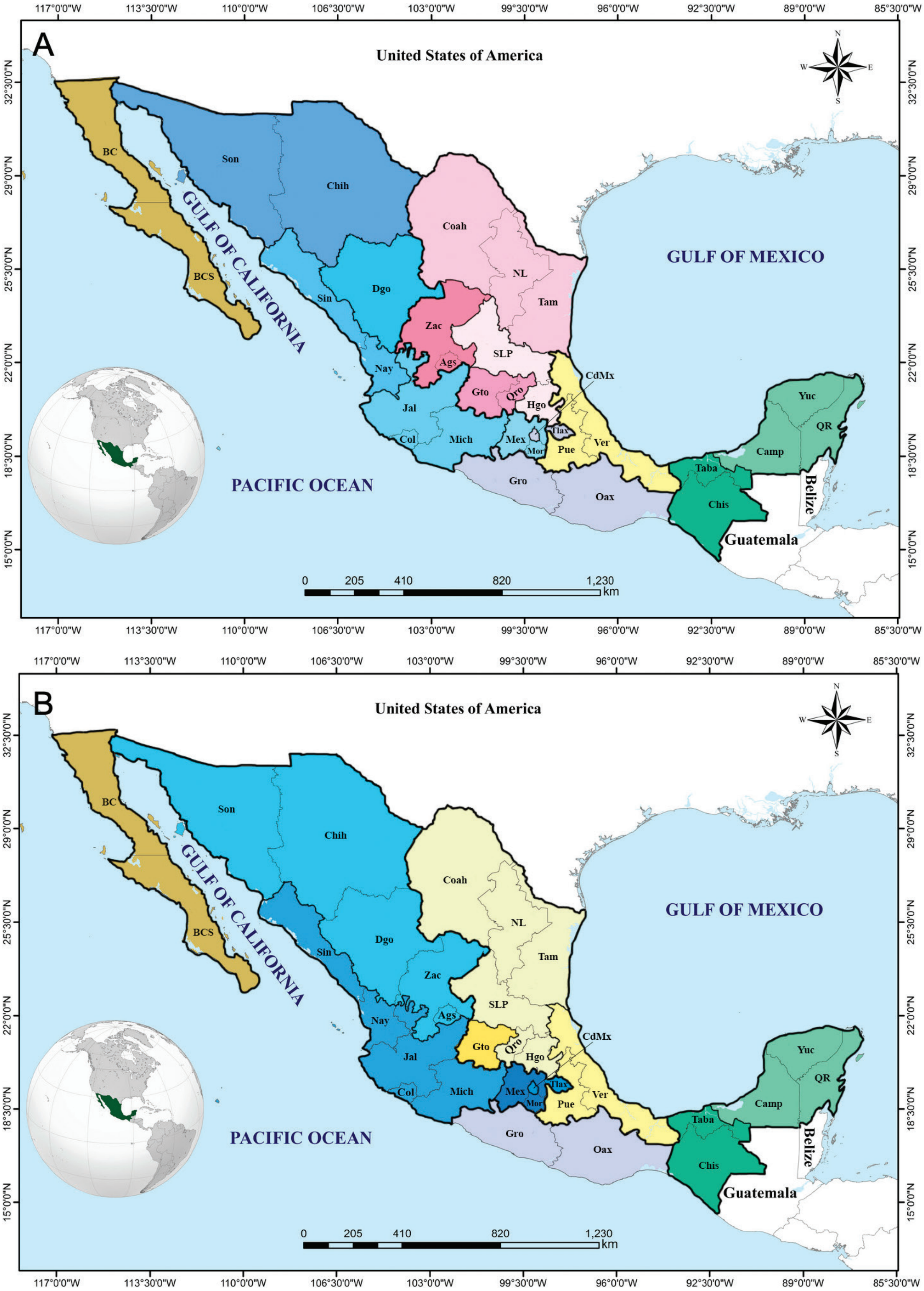


Figure 2. A map of Mexico showing the locations of the clusters of **A** amphibians and **B** reptiles identified by the cluster analyses found in Fig. 1. Different clusters are identified by color and surrounded by a thick border and subclusters within clusters are represented by different shades of the cluster color and by intermediate line thicknesses. AGS = Aguascalientes, BC = Baja California, BCS = Baja California Sur, CAMP = Campeche, CHIS = Chiapas, CHIH = Chihuahua, COAH = Coahuila, COL = Colima, DGO = Durango, GTO = Guanajuato, GRO = Guerrero, HGO = Hidalgo, JAL = Jalisco, MEX = México, CDMX = Mexico City, MICH = Michoacán, MOR = Morelos, NAY = Nayarit, NL = Nuevo León, OAX = Oaxaca, PUE = Puebla, QRO = Querétaro, QR = Quintana Roo, SLP = San Luis Potosí, SIN = Sinaloa, SON = Sonora, TABA = Tabasco, TAM = Tamaulipas, TLAX = Tlaxcala, VER = Veracruz, YUC = Yucatán, ZAC = Zacatecas.

Table 3. A list of amphibian species from each cluster that are considered in a threatened category on the IUCN Red List, as well as the primary threats to each species based on the IUCN Red List species accounts (IUCN 2022). IUCN Categories: VU = Vulnerable, EN = Endangered, CR = Critically Endangered (PE = Possibly extinct). Threats: LU = Land use (urbanization, conversion to agriculture, resource extraction, deforestation), NNS = Non-native species and disease, P = Pollution (agricultural, industrial, domestic), CC = Climate change.

	IUCN Category	**Threats**
Cluster AI VU: 0, EN: 1, CR: 0		
Anaxyrus californicus	EN	LU, NNS, CC
Cluster AII VU: 21, EN: 21, CR: 5		
Charadrahyla chaneque	VU	LU, NNS
Craugastor amniscola	VU	LU, NNS, P
Craugastor brocchi	VU	LU, NNS, P, CC
Craugastor glaucus	EN	LU, NNS
Craugastor greggi	EN	LU, NNS, P, CC
Craugastor matudai	EN	LU, NNS, P, CC
Craugastor montanus	EN	LU
Craugastor palenque	VU	LU, NNS, P, CC
Craugastor pelorus	VU	LU
Craugastor pozo	CR	LU
Craugastor stuarti	VU	LU, NNS, P
Craugastor taylori	CR	LU
Dryophytes walkeri	VU	LU, NNS, CC
Duellmanohyla chamulae	EN	LU, NNS
Exerodonta bivocata	EN	LU
Exerodonta chimalapa	EN	LU
Incilius aurarius	EN	LU, CC
Incilius tacanensis	EN	LU, NNS, CC
Incilius tutelarius	VU	LU, NNS, P, CC
Plectrohyla acanthodes	EN	LU, NNS, P, CC
Plectrohyla avia	EN	LU, NNS, P, CC
Plectrohyla hartwegi	EN	LU, NNS, P, CC
Plectrohyla ixil	VU	LU, NNS, P, CC
Plectrohyla lacertosa	EN	LU, NNS
Plectrohyla pycnochila	CR (PE)	LU, NNS
Plectrohyla sagorum	VU	LU, NNS, P
Ptychohyla macrotympanum	VU	LU, NNS
Quilticohyla zoque	EN	LU
Rana macroglossa	VU	LU, P, CC
Bolitoglossa alberchi	VU	LU, NNS
Bolitoglossa engelhardti	EN	LU, NNS, P, CC
Bolitoglossa flavimembris	EN	LU, NNS, P, CC
Bolitoglossa flaviventris	EN	LU, NNS, P
Bolitoglossa franklini	VU	LU, NNS, P, CC
Bolitoglossa hartwegi	VU	LU, NNS
Bolitoglossa mulleri	VU	LU, NNS, P
Bolitoglossa stuarti	VU	LU, NNS, P

	IUCN Category	Threats
Bolitoglossa veracrucis	EN	LU
Bradytriton silus	EN	LU, NNS, P, CC
Cryptotriton alvarezdeltoroi	EN	LU, NNS
Dendrotriton megarhinus	VU	NNS, CC
Dendrotriton xolocalcae	VU	NNS, CC
Ixalotriton niger	EN	LU
Nyctanolis pernix	VU	LU, NNS, P, CC
Pseudoeurycea brunnata	CR	LU, NNS, CC
Pseudacris goebeli	CR	LU, NNS, CC
Pseudoeurycea rex	VU	LU, NNC, CC
Cluster AIII VU: 13, EN: 28, CR: 26		
Bromeliohyla dendroscarta	EN	LU, NNS
Charadrahyla nephila	EN	LU, NNS
Charadrahyla taeniopus	VU	LU, P
Craugastor galacticorhinus	EN	LU
Craugastor megalotympanum	EN	LU
Craugastor spatulatus	EN	LU
Craugastor vulcani	EN	LU
Duellmanohyla chamulae	EN	LU, NNS
Ecnomiohyla valancifer	CR	LU
Exerodonta bivocata	EN	LU
Exerodonta xera	VU	LU
Incilius cavifrons	EN	LU
Incilius cristatus	EN	LU, P
Megastomatohyla mixomaculata	EN	LU
Megastomatohyla nubicola	CR	LU
Ptychohyla zophodes	VU	LU, NNS
Quilticohyla zoque	EN	LU
Rana chichicuahutla	CR	LU, NNS
Rana chiricahuensis	VU	LU, NNS, P, CC
Rana johni	VU	LU
Rana pueblae	CR (PE)	LU
Sarcohyla charadricola	CR (PE)	LU
Sarcohyla pachyderma	CR (PE)	LU, NNS
Sarcohyla robertsorum	VU	LU, NNS
Sarcohyla siopela	CR (PE)	LU, NNS
Tlalocohyla godmani	VU	LU, P, CC
Ambystoma altamirani	EN	LU, NNS, P
Aquiloeurycea cafetalera	VU	LU, NNS, P
Aquiloeurycea praecellens	CR (PE)	LU
Aquiloeurycea quetzalanensis	CR	LU
Bolitoglossa alberchi	VU	LU, NNS
Bolitoglossa veracrucus	EN	LU
Chiropterotriton arboreus	CR	LU
Chiropterotriton aureus	CR	LU, NNS
Chiropterotriton casasi	CR (PE)	LU, NNS
Chiropterotriton chiropterus	CR	LU, NNS

	IUCN Category	**Threats**
Chiropterotriton chondrostega	EN	LU, NNS
Chiropterotriton lavae	CR	LU
Chiropterotriton nubilus	CR	LU, NNS
Chiropterotriton orculus	VU	LU, NNS
Chiropterotriton perotensis	CR	LU, NNS
Chiropterotriton terrestris	CR	LU, NNS
Chiropterotriton totonacus	CR	LU, NNS
Isthmura gigantea	EN	LU, NNS
Isthmura naucampatepetl	CR	LU, NNS
Notophthalmus meridionalis	EN	LU, NNS, P
Parvimolge townsendi	VU	LU, NNS, P
Pseudoeurycea firscheini	EN	LU
Pseudoeurycea gadovii	VU	LU
Pseudoeurycea lineola	EN	LU, NNS
Pseudoeurycea lynchi	EN	LU
Pseudoeurycea melanomolga	EN	LU
Pseudoeurycea mixteca	VU	LU
Pseudoeurycea nigromaculata	EN	LU, NNS
Pseudoeurycea orchimelas	EN	LU
Pseudoeurycea werleri	EN	LU
Thorius dubitus	CR	LU, NNS
Thorius lunaris	CR	LU, NNS
Thorius magnipes	CR	LU, NNS
Thorius maxillabrochus	EN	LU, NNS
Thorius minydemus	EN	LU
Thorius munificus	CR	LU, NNS
Thorius narismagnus	CR	LU
Thorius pennatulus	EN	LU, NNS
Thorius schmidti	CR	LU, NNS
Thorius spilogaster	CR	LU, NNS
Thorius troglodytes	EN	LU
Cluster AIV VU: 9, EN: 15, CR: 5		
Craugastor vulcani	EN	LU
Eleutherodactylus erendirae	EN	LU
Eleutherodactylus floresvillelai	VU	LU
Eleutherodactylus grunwaldi	EN	LU
Eleutherodactylus jaliscoensis	EN	LU
Eleutherodactylus maurus	VU	LU
Eleutherodactylus nietoi	EN	LU, NNS
Eleutherodactylus rufescens	VU	LU
Eleutherodactylus teretistes	VU	LU
Eleutherodactylus wixarika	EN	LU
Incilius pisinnus	EN	LU, P
Rana chiricahuensis	VU	LU, NNS, P, CC
Rana dunni	EN	LU, P
Rana tarahumarae	VU	LU, NNS, P, CC
Rana tlaloci	CR (PE)	LU, NNS, P

	IUCN Category	Threats
Sarcohyla floresi	VU	LU, NNS
Smilisca dentata	EN	LU, P
Ambystoma altamirani	EN	LU, NNS, P
Ambystoma amblycephalum	CR	LU, NNS, P
Ambystoma andersoni	CR	LU, NNS, P
Ambystoma dumerilii	CR	LU, NNS, P
Ambystoma lermaense	EN	LU, NNS, P
Ambystoma ordinarium	EN	LU, NNS, P
Chiropterotriton orculus	VU	LU, NNS
Isthmura sierraoccidentalis	VU	LU, NNS
Pseudoeurycea altamontana	EN	LU
Pseudoeurycea longicauda	EN	LU, NNS
Pseudoeurycea robertsi	CR	LU, P
Pseudoeurycea tlilicxitl	EN	LU
Cluster AV VU: 10, EN: 9, CR: 5		
Bromeliohyla dendroscarta	EN	LU, NNS
Charadrahyla taeniopus	VU	LU, P
Rana chiricahuensis	VU	LU, NNS, P, CC
Rana johni	VU	LU
Sarcohyla charadricola	CR (PE)	LU
Sarcohyla robertsorum	VU	LU, NNS
Smilisca dentata	EN	LU, P
Tlalocohyla godmani	VU	LU, P, CC
Aquiloeurycea galeanae	VU	LU
Chiropterotriton arboreus	CR	LU
Chiropterotriton chico	VU	NNS
Chiropterotriton chiropterus	CR	LU, NNS
Chiropterotriton chondrostega	EN	LU, NNS
Chiropterotriton cieloensis	VU	NNS, CC
Chiropterotriton cracens	VU	NNS, CC
Chiropterotriton dimidiatus	VU	NNS
Chiropterotriton magnipes	EN	LU, NNS
Chiropterotriton miquihuanus	EN	LU, NNS
Chiropterotriton mosaueri	CR	LU
Chiropterotriton multidentatus	EN	LU, NNS
Chiropterotriton terrestris	CR	LU, NNS
Isthmura gigantea	EN	LU, NNS
Notophthalmus meridionalis	EN	LU, NNS, P
Pseudoeurycea altamontana	EN	LU
Guerrero VU: 7, EN: 12, CR: 9		
Craugastor guerreroensis	EN	LU
Craugastor saltator	EN	LU, NNS
Craugastor uno	VU	LU
Charadrahyla pinorum	VU	LU, NNS
Charadrahyla trux	EN	LU, NNS, P
Dryophytes arboricola	VU	LU
Exerodonta melanomma	VU	LU

	IUCN Category	Threats
Incilius cycladen	VU	LU, NNS, P
Incilius gemmifer	EN	LU
Quilticohyla erythromma	VU	LU, NNS
Rana omiltemana	EN	LU, NNS
Sarcohyla chryses	EN	LU, NNS
Sarcohyla floresi	VU	LU, NNS
Sarcohyla mykter	EN	LU, NNS
Sarcohyla thorectes	EN	LU, NNS
Sarcohyla toyota	CR	LU, NNS
Ambystoma altamirani	EN	LU, NNS, P
Isthmura maxima	EN	LU
Pseudoeurycea ahuitzotl	CR	LU, NNS
Pseudoeurycea amuzga	EN	LU, NNS
Pseudoeurycea kuautli	CR	LU
Pseudoeurycea mixcoatl	CR	LU, NNS
Pseudoeurycea tenchalli	CR	LU, NNS
Pseudoeurycea teotepec	CR (PE)	LU, NNS
Pseudoeurycea tlahcuiloh	CR	LU, NNS
Thorius grandis	CR	LU, NNS
Thorius infernalis	CR	LU, NNS
Thorius omiltemi	EN	LU, NNS
Mexico City VU: 1, EN: 4, CR: 2		
Eleutherodactylus grandis	EN	LU, P
Rana tlaloci	CR (PE)	LU, NNS, P
Ambystoma altamirani	EN	LU, NNS, P
Ambystoma mexicanum	CR	LU, NNS, P
Chiropterotriton orculus	VU	LU, NNS
Pseudoeurycea altamontana	EN	LU
Pseudoeurycea tlilicxitl	EN	LU
Oaxaca VU: 15, EN: 36, CR: 28		
Bromeliohyla dendroscarta	EN	LU, NNS
Charadrahyla altipotens	EN	LU
Charadrahyla chaneque	VU	LU, NNS
Charadrahyla esperancensis	VU	LU, NNS
Charadrahyla nephila	EN	LU, NNS
Charadrahyla pinorum	VU	LU, NNS
Charadrahyla sakbah	EN	LU, P
Craugastor spatulatus	EN	LU
Craugastor uno	VU	LU
Duellmanohyla chamulae	EN	LU, NNS
Ecnomiohyla echinata	CR (PE)	LU, NNS
Exerodonta chimalapa	EN	LU
Exerodonta melanomma	VU	LU
Exerodonta xera	VU	LU
Incilius cycladen	VU	LU, NNS, P
Incilius gemmifer	EN	LU
Incilius spiculatus	EN	LU

	IUCN Category	Threats
Incilius tutelarius	VU	LU, NNS, P, CC
Megastomatohyla mixe	CR	LU
Megastomatohyla pellita	CR	LU, NNS
Plectrohyla hartwegi	EN	LU, NNS, P, CC
Ptychohyla zophodes	VU	LU, NNS
Quilticohyla acrochorda	CR	LU, NNS, P
Quilticohyla zoque	EN	LU
Sarcohyla ameibothalame	EN	LU, NNS
Sarcohyla calvicollina	CR (PE)	LU, NNS
Sarcohyla cembra	EN	LU, NNS
Sarcohyla crassa	CR	LU, NNS
Sarcohyla cyanomma	CR (PE)	LU, NNS
Sarcohyla cyclada	VU	LU, NNS
Sarcohyla hazelae	VU	LU, P
Sarcohyla labeculata	EN	LU, NNS
Sarcohyla labedactyla	CR	LU, NNS
Sarcohyla pentheter	VU	LU, NNS
Sarcohyla psarosema	CR (PE)	LU, NNS
Sarcohyla sabrina	CR (PE)	LU, NNS
Sarcohyla siopela	CR (PE)	LU, NNS
Sarcohyla thorectes	EN	LU, NNS
Bolitoglossa alberchi	VU	LU, NNS
Bolitoglossa macrinii	EN	LU
Bolitoglossa oaxacensis	EN	LU
Bolitoglossa riletti	EN	LU
Bolitoglossa veracrucus	EN	LU
Bolitoglossa zapoteca	EN	LU, NNS
Isthmura boneti	EN	LU, NNS
Isthmura maxima	EN	LU
Ixalotriton niger	EN	LU
Ixalotriton parvus	CR	LU
Pseudoeurycea anitae	CR (PE)	LU, NNS
Pseudoeurycea aquatica	CR	LU, NNS
Pseudoeurycea aurantia	CR	LU
Pseudoeurycea cochranae	VU	LU
Pseudoeurycea conanti	EN	LU
Pseudoeurycea juarezi	EN	LU, NNS
Pseudoeurycea mixteca	VU	LU
Pseudoeurycea mystax	EN	LU, NNS
Pseudoeurycea obesa	CR	LU
Pseudoeurycea orchileucos	EN	LU, NNS
Pseudoeurycea papenfussi	EN	LU, NNS, CC
Pseudoeurycea ruficauda	EN	LU
Pseudoeurycea saltator	CR	LU
Pseudoeurycea smithi	CR	LU, NNS
Pseudoeurycea unguidentis	CR (PE)	LU, NNS
Pseudoeurycea werleri	EN	LU

	IUCN Category	Threats
Thorius arboreus	CR	LU, NNS
Thorius aureus	CR	LU, NNS, CC
Thorius boreas	EN	LU, NNS, CC
Thorius insperatus	CR	LU, NNS
Thorius longicaudus	CR (PE)	LU, NNS
Thorius macdougalli	EN	LU, NNS
Thorius maxillabrochus	EN	LU, NNS
Thorius minutissimus	CR	LU, NNS
Thorius narisovalis	EN	LU, NNS
Thorius papaloae	CR	LU, NNS
Thorius pinicola	EN	LU, NNS
Thorius pulmonaris	CR	LU, NNS
Thorius schmidti	CR	LU, NNS
Thorius smithi	CR	LU, NNS
Thorius tlaxiacus	EN	LU, NNS
Tlaxcala VU: 2, EN: 1, CR: 0		
Sarcohyla robertsorum	VU	LU, NNS
Isthmura gigantea	EN	LU, NNS
Pseudoeurycea gadovii	VU	LU

Table 4. A list of reptile species from each cluster that are considered in a threatened category on the IUCN Red List, as well as the primary threats to each species based on the IUCN Red List species accounts (IUCN 2022). IUCN Categories: VU = Vulnerable, EN = Endangered, CR = Critically Endangered (PE = Possibly extinct). Threats: LU = Land use (urbanization, conversion to agriculture, resource extraction, deforestation), NNS = Non-native species and disease, P = Pollution (agricultural, industrial, domestic), CC = Climate change, EXP = Exploitation, PER = Persecution, N/A = No threat listed.

	IUCN Category	Threats
Cluster RI VU: 9, EN: 3, CR: 2		
Anniella geronimensis	EN	LU
Aspidoscelis catalinensis	VU	NNS
Aspidoscelis labialis	VU	LU
Sauromalus hispidus	EN	LU, NNS, CC
Sauromalus klauberi	VU	NNS, CC
Uta encantadae	VU	N/A
Uta lowei	VU	N/A
Uta tumidarostra	VU	N/A
Crotalus catalinensis	CR	LU, NNS, EXP
Caretta caretta	VU	LU, NNS, P, CC
Chelonia mydas	EN	LU, EXP
Dermochelys coriacea	VU	LU, P, CC, EXP
Eretmochelys imbricata	CR	LU, P, CC, EXP
Lepidochelys olivacea	VU	LU, P, CC, EXP
Cluster RII VU:10, EN: 6, CR: 3		
Crocodylus acutus	VU	LU, NNS, CC, EXP
Abronia matudai	EN	LU
Anolis barkeri	VU	LU

	IUCN Category	Threats
Anolis hobartsmithi	EN	LU
Anolis pygmaeus	EN	LU
Heloderma alvarezi	VU	LU, CC, PER
Lepidophyma lipetzi	EN	LU
Bothriechis aurifer	VU	LU, EXP
Bothriechis rowleyi	VU	LU, EXP
Cachryx defensor	VU	LU, EXP
Leptophis modestus	VU	LU
Rhadinella posadasi	EN	LU
Caretta caretta	VU	LU, NNS, P, CC
Chelonia mydas	EN	LU, EXP
Chelydra rossignonii	VU	LU, EXP
Dermatochelys mawii	CR	LU, P, EXP
Dermochelys coriacea	VU	LU, P, CC, EXP
Eretmochelys imbricata	CR	LU, P, CC, EXP
Lepidochelys kempii	CR	LU, EXP
Cluster RIII VU: 18, EN: 22, CR: 5		
Abronia chiszari	EN	LU
Abronia graminea	EN	LU, EXP
Abronia taeniata	VU	LU, EXP
Anolis barkeri	VU	LU
Anolis naufragus	VU	LU
Crotaphytus antiquus	EN	LU
Crotaphytus reticulatus	VU	LU, NNS
Gerrhonotus parvus	EN	LU
Lepidophyma gaigeae	VU	N/A
Lepidophyma micropholis	VU	N/A
Ophisaurus ceroni	EN	LU
Sceloporus chaneyi	EN	LU
Sceloporus cyanostictus	EN	LU
Sceloporus exsul	CR	LU
Sceloporus goldmani	EN	LU
Sceloporus maculosus	VU	LU
Sceloporus megalepidurus	VU	LU
Sceloporus oberon	VU	LU
Uma exsul	EN	LU
Xenosaurus grandis	VU	LU
Xenosaurus newmanorum	EN	LU, PER
Xenosaurus platyceps	EN	LU, NNS
Adelophis copei	VU	LU
Chersodromus rubriventris	EN	LU
Ficimia hardyi	EN	LU
Mixcoatlus melanurus	EN	LU, PER
Ophryacus undulatus	VU	LU
Rhadinaea marcellae	EN	LU
Rhadinaea montana	EN	LU
Storeria hidalgoensis	VU	LU

	IUCN Category	Threats
Tantilla shawi	EN	LU
Thamnophis melanogaster	EN	P
Thamnophis mendax	EN	LU
Thamnophis scaliger	VU	LU
Caretta caretta	VU	LU, NNS, P, CC
Chelonia mydas	EN	LU, EXP
Chelydra rossignonii	VU	LU, EXP
Dermatemys mawii	CR	LU, EXP
Dermochelys coriacea	VU	LU, P, CC, EXP
Eretmochelys imbricata	CR	LU, P, CC, EXP
Gopherus flavomarginatus	CR	LU, EXP
Lepidochelys kempii	CR	LU, EXP
Terrapene coahuila	EN	LU
Trachemys gaigeae	VU	LU, P, CC, EXP
Trachemys taylori	EN	LU, NNS
Cluster RIV VU: 19, EN: 9, CR: 3		
Crocodylus acutus	VU	LU, P, CC, EXP
Abronia deppii	EN	LU
Aspidoscelis martyris	VU	N/A
Barisia herrerae	EN	LU, PER
Barisia rudicollis	EN	LU, PER
Ctenosaura clarki	VU	LU
Ctenosaura conspicuosa	VU	NNS, CC
Ctenosaura nolascensis	VU	NNS, CC, EXP
Sauromalus varius	VU	NNS, CC
Sceloporus goldmani	EN	LU
Sceloporus maculosus	VU	LU
Sceloporus megalepidurus	VU	LU
Urosaurus auriculatus	EN	NNS
Urosaurus clarionensis	VU	NNS
Uta palmeri	VU	N/A
Adelophis copei	VU	LU
Crotalus pusillus	EN	LU
Crotalus stejneger	VU	LU
Masticophis anthonyi	CR	NNS
Thamnophis melanogaster	EN	P
Thamnophis scaliger	VU	LU
Caretta caretta	VU	LU, NNS, P, CC
Chelonia mydas	EN	LU, EXP
Dermochelys coriacea	VU	LU, P, CC, EXP
Eretmochelys imbricata	CR	LU, P, CC, EXP
Gopherus evgoodei	VU	LU, NNS, CC
Gopherus flavomarginatus	CR	LU, EXP
Lepidochelys olivacea	VU	LU, P, CC, EXP
Terrapene coahuila	EN	LU
Trachemys gaigeae	VU	LU, P, CC, EXP
Trachemys yaquia	VU	LU, P, CC, EXP

	IUCN Category	Threats
Guerrero VU: 6, EN: 4, CR: 1		
Crocodylus acutus	VU	LU, P, CC, EXP
Abronia deppii	EN	LU
Abronia martindelcampoi	EN	LU
Abronia mixteca	VU	LU, EXP, PER
Ctenosaura clarki	VU	LU
Mixcoatlus barbouri	EN	LU
Ophryacus undulatus	VU	LU
Chelonia mydas	EN	LU, EXP
Dermochelys coriacea	VU	LU, P, CC, EXP
Eretmochelys imbricata	CR	LU, P, CC, EXP
Lepidochelys olivacea	VU	LU, P, CC, EXP
Oaxaca VU: 14, EN: 7, CR: 3		
Crocodylus acutus	VU	LU, P, CC, EXP
Abronia fuscolabialis	EN	LU
Abronia graminea	EN	LU, EXP
Abronia juarezi	EN	LU
Abronia mixteca	VU	LU, EXP, PER
Abronia oaxacae	VU	LU
Anolis pygmaeus	EN	LU
Ctenosaura oaxacana	CR	LU, EXP
Heloderma alvarezi	VU	LU, CC, PER
Sceloporus megalepidurus	VU	LU
Xenosaurus grandis	VU	LU
Bothriechis rowleyi		LU, EXP
Exiliboa placata	VU	LU
Micrurus ephippifer	VU	LU
Mixcoatlus melanurus	EN	LU, PER
Ophryacus undulatus	VU	LU
Tantilla flavilineata	EN	LU
Tantalophis discolor	VU	LU
Chelonia mydas	EN	LU, EXP
Chelydra rossignonii	VU	LU, EXP
Dermatemys mawii	CR	LU, P, EXP
Dermochelys coriacea	VU	LU, P, CC, EXP
Eretmochelys imbricata	CR	LU, P, CC, EXP
Lepidochelys olivacea	VU	LU, P, CC, EXP

efforts. Unsurprisingly, the main threat to amphibians in these clusters, and indeed all clusters, is changes in anthropogenic land use, including conversion to agriculture, urbanization, and resource extraction. This result emphasizes the need for conservation and management policies that prevent habitat loss and fragmentation by human activity and that seek to restore lost or degraded habitats. Another common threat in these clusters is the specter of non-native species and diseases, primarily the potential for *Batrachochytrium dendrobatidis* and *B. salamandrivorans* to cause amphibian declines. In addition, introduction of non-native species appears to affect some species. The monitoring of emerging diseases and policies to prevent the spread or introduction of non-native species, especially fish, should be pursued. Finally, climate change is listed as a threat to species in these clusters. The increased frequency of drought is of particular concern for many of the amphibian species in these clusters. Efforts to ensure water flow or sufficient aquatic habitats for amphibians are needed. Pollution, ranging from agricultural run-off, industrial contamination, mine or drilling waste, and domestic waste, is also another key threat for several species. Policies should be considered that minimize the input of pollutants into the aquatic or terrestrial habitats of amphibians.

For reptiles, cluster RIII has the highest number of threatened species, followed by cluster RIV and Oaxaca. As with amphibians, anthropogenic land use change in its many forms is ubiquitous as a threat to reptiles in each cluster. Again, efforts to minimize habitat loss and fragmentation are desperately needed, as are efforts to restore or reclaim habitats. For many species of reptiles, harvesting for the pet trade or for food, as well as persecution by humans, is another major threat. Establishment and enforcement of laws regulating the harvesting or killing of reptiles should be considered. In addition, education of local residents about the value and harmlessness of many of the persecuted species of reptiles could help to reduce persecution. Climate change, particularly through its effects on changing or shifting habitats, is also a potential threat to several species of reptiles, as is pollution. In addition, introduced species (e.g., rats, cats, and dogs) are important threats to island species in cluster RI.

When we consider the clusters of states that have high numbers of threatened amphibians and reptiles it is interesting to note that the states included in those clusters do not greatly overlap. For amphibians, the clusters with high numbers of threatened species are in southern Mexico whereas those for reptiles are in northern Mexico. However, Oaxaca has a high number of threatened species of both amphibians and reptiles, suggesting it needs to be a high priority of conservation efforts (see also Mata-Silva et al. 2015, 2021). The difference in the apparent distribution of high numbers of threatened species of amphibians and reptiles, as well as the secondary threats for each group (i.e., beyond anthropogenic land use change), suggested that each taxonomic group may need to be considered separately rather than as a single herpetofauna as conservation efforts are prioritized.

Similarities

The Jaccard distances of amphibians and reptiles were highly, positively correlated (Fig. 3; n = 32, Spearman's $\rho = 0.90$, $P < 0.0001$). Amphibian Jaccard distances were positively correlated with the physiographic province Jaccard distance (Fig. 4A; n = 32, Spearman's $\rho = 0.684$, $P < 0.0001$) and shared border length (Fig. 4B; n = 32, Spearman's $\rho = 0.498$, $P < 0.0001$), and negatively correlated with the distance between centroids (Fig. 4C; n = 32, Spearman's $\rho = -0.716$, $P < 0.0001$). Reptile Jaccard distances were positively correlated with physiographic province Jaccard distances (Fig. 4D; n = 32, Spearman's $\rho = 0.76$, $P < 0.0001$) and shared border length (Fig. 4E; n = 32, Spearman's $\rho = 0.536$, $P < 0.0001$), and negatively correlated with distance between centroids (Fig. 4F; n = 32, Spearman's $\rho = -0{,}779$, $P < 0.0001$). The length of the shared border and the Jaccard distance for physiographic provinces were

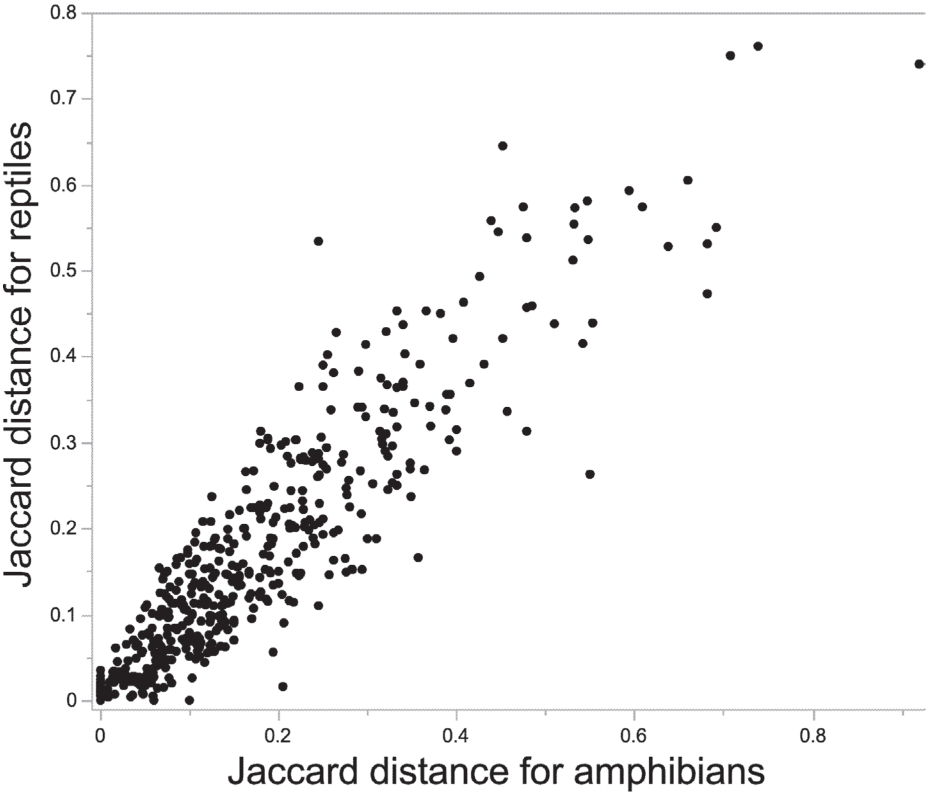


Figure 3. The correlation between the Jaccard distance of amphibians and reptiles among all pairs of Mexican states.

positively correlated (Fig. 5A; n = 32, Spearman's ρ = 0.543, P < 0.0001). The distance between centroids and the Jaccard distance for physiographic regions were negatively correlated (Fig. 5B; n = 32, Spearman's ρ = -0.778, P < 0.0001).

Taken together, our results suggest that many of the similarities and differences in the herpetofauna among Mexican states is a consequence of their proximity, at least in terms of the distance between the states. Such correlations are likely the result of such proximity on the similarity of physiographic provinces (i.e., habitats and ecosystems) of these states. These results are consistent with previous studies on similarities among communities or states of other taxa in Mexico and other geographic areas. For example, similarity of state-level floras and faunas typically decreases with the geographic distance between states (e.g., Qian and Ricklefs 2006). In addition, beta diversity of European reptiles increases with geographic distance between communities (Keil et al. 2012). In Mexico, similarity of flora among states is explained in good part by the proximity of the states, with neighboring states often sharing endemics and other species (Delgadillo et al. 2003).

The positive correlation between shared border length and Jaccard distances for reptiles, amphibians, and physiographic provinces deserves exploration. This result is, on the surface, counterintuitive: states that share a longer border would be expected to share more species or physiographic provinces (i.e., a negative correlation between Jaccard distances and shared border lengths). However, we used a shared border metric that is the absolute length of a shared border. Thus, a low value for shared border length could indicate a variety of things. First, a value of zero for shared border length clearly indi-

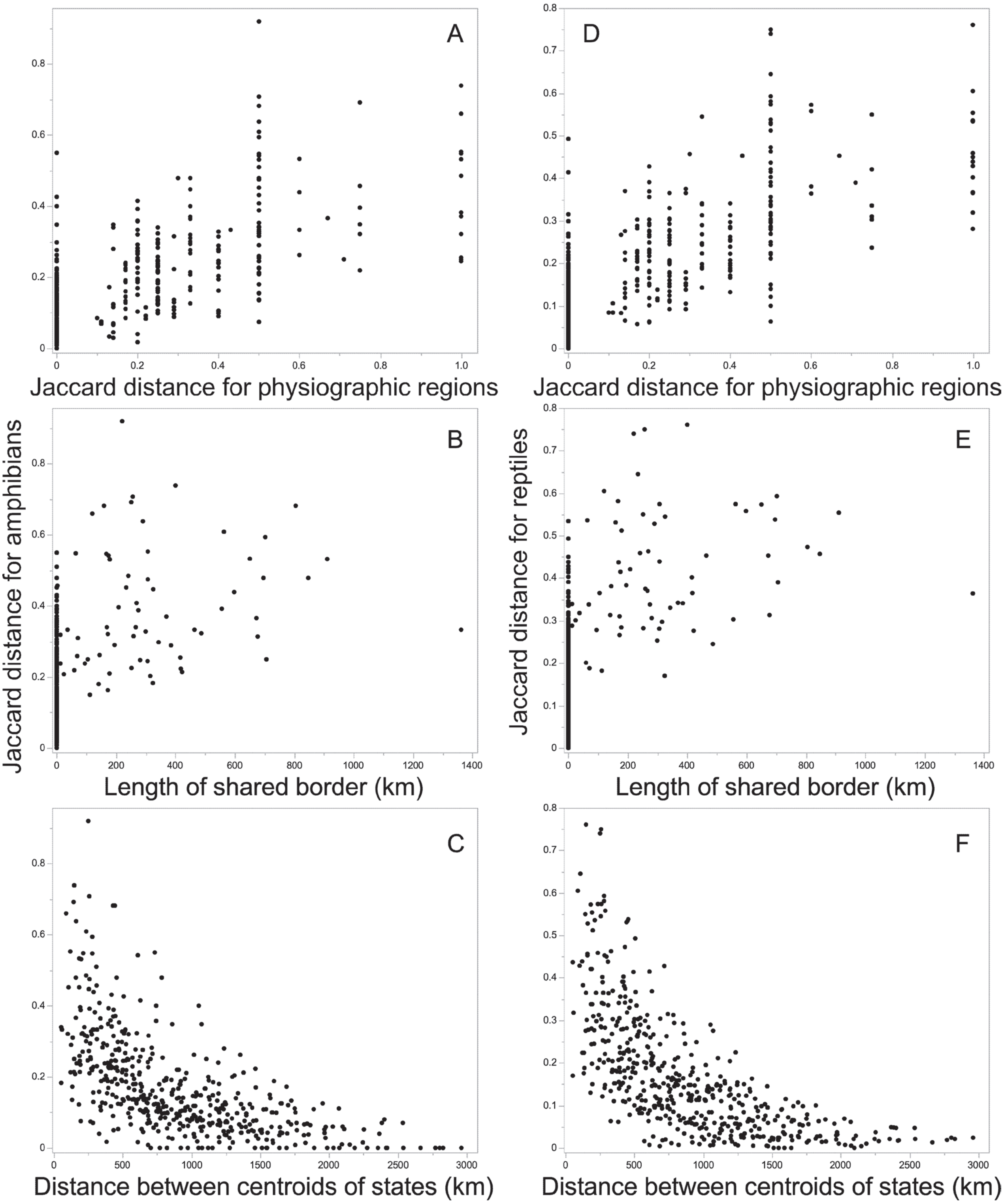


Figure 4. The relationships between Jaccard distances for physiographic regions, length of shared border, and distance between centroids of all pairs of Mexican states and the Jaccard distances of amphibians (**A, B, C** respectively) and reptiles (**D, E, F** respectively).

cates the states do not touch at all. Second, two small states that share much of their border would still have a relatively low value for shared border length (e.g., Mexico City, México, and Morelos; Aguascalientes and Zacatecas). Third, two states may be very close geographically but have either no shared border

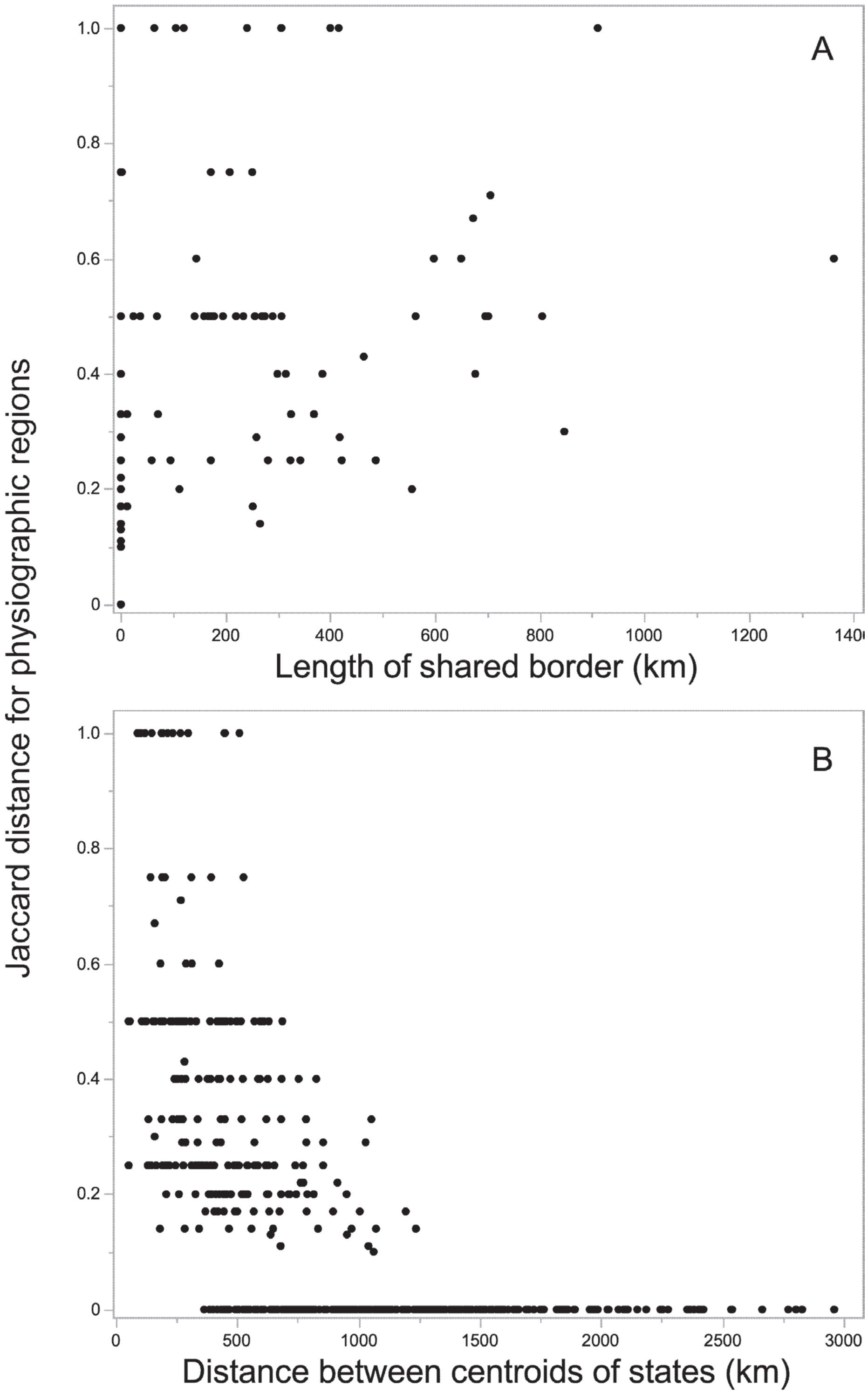


Figure 5. The relationship of length of shared border and distance between centroids with the Jaccard distance of physiographic provinces among all pairs of Mexican states.

(e.g., Tlaxcala and Mexico City, Chiapas and Campeche) or a very short, shared border (e.g., Baja California and Baja California Sur, Zacatecas and Nayarit, Nayarit and Sinaloa). It may be more useful to look more carefully at Figs 4B and 4E. Visually, it looks as if there is only a weak correlation between the absolute length of a shared border and Jaccard distance. Thus, when considering states that should work together for conservation, focus should be on other aspects, such as shared physiographic provinces or species rather than simply a shared border.

Conclusions

Political borders can have significant effects on the abundance and diversity of populations and communities due to differing policies across the border (e.g., Shanas et al. 2006; Opermanis et al. 2012). It therefore should be a priority for states sharing species to work together for conservation rather than each state working in isolation (see also Dertien et al. 2020). Our results hopefully suggest clusters of states that would do well to work together to coordinate the conservation and management of their herpetofaunas (i.e., states in the same clusters or subclusters; see Fig. 2). This is especially true given the nature of the major threats to the Mexican herpetofauna are the type of threats that span across potential borders and would benefit from concerted efforts and elevated communication, including loss of habitats, pollution, harvesting and pet trade, invasive species, and climate change (Suazo-Ortuño et al. 2023).

Acknowledgments

We are grateful to A. Núñez Merchand from the National Commission for the Understanding and Use of Biodiversity (CONABIO) for kindly providing the Mexican political map and generating the centroid distances and shared border lengths. We are grateful to J. Sigala-Rodríguez for allowing us access to an unpublished list of amphibians and reptiles of Zacatecas.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This study was made possible through the generous support provided by the Dirección General de Asuntos del Personal Académico – Programa de Apoyos para la Superación del Personal Académico de la UNAM (DGAPA-PASPA) through the scholarship assigned to JLE for his sabbatical year at the University of Colorado, Boulder and by the Dirección General de Asunto del Personal Académico – Programa de Apoyo a Proyectos de Investigación e Innovación Tecnológicas (DGAPA-PAPIIT) through the project IN202021.

Author contributions

Conceptualization: GRS, JLE. Data curation: GRS, JLE. Formal analysis: JLE, GRS. Funding acquisition: GRS.

Author ORCIDs

Julio A. Lemos-Espinal https://orcid.org/0000-0003-3952-9852
Geoffrey R. Smith https://orcid.org/0000-0001-7115-649X

Data availability

All of the data that support the findings of this study are available in the main text.

References

Ahumada-Carrillo IT, Grünwald CI, Cuellar MAL, Jones JM (2020) Some records of amphibians and reptiles from the Wixarica Region in the State of Jalisco, Mexico. Herpetological Review 51: 277–281.

Alvarado-Díaz J, Suazo-Ortuño I, Wilson LD, Medina-Aguilar O (2013) Patterns of physiographic distribution and conservation status of the herpetofauna of Michoacan, Mexico. Amphibian & Reptile Conservation 7: 128–170. http://amphibian-reptile-conservation.org/pdfs/Volume/Vol_7_no_1/Special_Mexico_Issue_ARC_7_1_128-170_e71_high_res.pdf

Arenas-Moreno DM, Muñoz-Nolasco FJ, Bautista del Moral A, Rodríguez-Miranda LA, Dominguez-Guerrero SF, Méndez de la Cruz FR (2021) A new species of *Lepidophyma* (Squamata: Xantusiidae) from San Luis Potosí, México, with notes on its physiological ecology. Zootaxa 4949(1): 115–130. https://doi.org/10.11646/zootaxa.4949.1.6

Badillo-Saldaña LM, Lara-Tufiño JD, Ramírez-Bautista A, Campbell JA (2018) New state record and potential distribution of the snake *Sibon nebulatus* (Dipsadidae) from Mexico. Western North American Naturalist 78(2): 242–246. https://doi.org/10.3398/064.078.0201

Baeza-Tarin F, Hernandez T, Giovaneto L, Trevino AE, Lazcano D, Graham SP (2018) Geographic distribution: *Lampropeltis gentilis*. Herpetological Review 49: 505.

Barley AJ, Reeder TW, Nieto-Montes de Oca A, Cole CJ, Thomson RC (2021) A new diploid parthenogenetic whiptail lizard from Sonora, Mexico is the 'missing link' in the evolutionary transition to polyploidy. American Naturalist 198(2): 295–309. https://doi.org/10.1086/715056

Barragán-Vázquez M del R, Ríos-Rodas L, Fucsko LA, Porras LW, Mata-Silva V, Rocha A, DeSantis DL, García-Padilla E, Johnson JD, Wilson LD (2022) The herpetofauna of Tabasco, Mexico: Composition, distribution, and conservation status. Amphibian & Reptile Conservation 16: 1–61. http://amphibian-reptile-conservation.org/pdfs/Volume/Vol_16_no_2/ARC_16_2_[General_Section]_1-61_e315.pdf

Bertelli S, Szumik C, Goloboff PA, Giannini NP, Navarro-Sigüenza AG, Peterson AT, Cracraft J (2017) Mexican land birds reveal complexity in fine-scale patterns of endemism. Journal of Biogeography 44(8): 1836–1846. https://doi.org/10.1111/jbi.12987

Blair RC, Hansen RW (2018) Geographic distribution: *Lampropeltis alterna*. Herpetological Review 49: 718.

Blancas-Hernández JC, Palacios-Aguilar R, Santos-Bibiano R (2019) *Tropidodipsas sartori* (Cope, 1863) (Squamata: Dipsadidae): an addition to the snake fauna from Guerrero, Mexico. Herpetozoa (Wien) 32: 91–93. https://doi.org/10.3897/herpetozoa.32.e35911

Bolam FC, Ahumada J, Akçakaja HR, Brooks TM, Elliott W, Hoban S, Mair L, Mallon D, McGowan PJK, Raimondo D, Rodríguez JP, Roe D, Seddon MB, Shen X, Stuart SN, Watson JEM, Butchart SHM (2023) Over half of threatened species require targeted recovery actions to avert human-induced extinction. Frontiers in Ecology and the Environment 21(2): 64–70. https://doi.org/10.1002/fee.2537

Bryson Jr RW, Grummer JA, Connors EM, Tirpak J, McCormack JE, Klicka J (2021) Cryptic diversity across the Trans-Mexican Volcanic Belt of Mexico in the montane bunchgrass lizard *Sceloporus subniger* (Squamata: Phrynosomatidae). Zootaxa 4963(2): 335–353. https://doi.org/10.11646/zootaxa.4963.2.5

Burbrink FT, Guiher TJ (2014) Considering gene flow when using coalescent methods to delimit lineages of North American pitvipers of the genus *Agkistrodon*. Zoological Journal of the Linnean Society 173(2): 505–526. https://doi.org/10.1111/zoj.12211

Campbell JA, Solano-Zavaleta I, Flores-Villela O, Caviedes-Solis IW, Frost DR (2016) A new species of *Abronia* (Squamata: Anguidae) from the Sierra Madre del Sur of Oaxaca, Mexico. Journal of Herpetology 50(1): 149–156. https://doi.org/10.1670/14-162

Campbell JA, Brodie Jr ED, Caviedes-Solis IW, Nieto-Montes de Oca A, Luja VH, Flores-Villela O, García-Vázquez UO, Sacher GC, Wostl E, Smith EN (2018) Systematics of the frogs allocated to *Sarcohyla bistincta* sensu lato (Cope, 1877), with description of a new species from western Mexico. Zootaxa 4422(3): 366–384. https://doi.org/10.11646/zootaxa.4422.3.3

Campillo-García G, Flores-Villela O, Butler BO, Velasco Vinasco JA, Ramírez Corona F (2021) Hidden diversity within a polytypic species: The enigmatic *Sceloporus torquatus* Wiegmann, 1828 (Reptilia, Squamata, Phrynosomatidae). Vertebrate Zoology 71: 781–798. https://doi.org/10.3897/vz.71.e71995

Canseco-Márquez L, Aguilar-López JL, Luría-Manzano R, Pineda E, Caviedes-Solís IW (2017a) A new species of treefrog of the genus *Ptychohyla* (Anura: Hylidae) from southern Mexico. Zootaxa 4317(2): 279–290. https://doi.org/10.11646/zootaxa.4317.2.5

Canseco-Márquez L, Ramírez-González CG, González-Bernal E (2017b) Discovery of another new species of *Charadrahyla* (Anura; Hylidae) from the cloud forest of northern Oaxaca, México. Zootaxa 4329(1): 64–72. https://doi.org/10.11646/zootaxa.4329.1.2

Carbajal-Márquez RA, Quintero-Díaz GE (2016) The herpetofauna of Aguascalientes, Mexico. Revista Mexicana de Herpetologie 2: 1–30. http://sociedadherpetologica-mexicana.org.mx/wp-content/uploads/2016/08/RMHVol2No1-Articulos.pdf

Carbajal-Márquez RA, Cedeño-Vázquez JR, Martínez-Arce A, Neri-Castro E, Machkour-M'Rabet SC (2020) Accessing cryptic diversity in Neotropical rattlesnakes (Serpentes: Viperidae: *Crotalus*) with the description of two new species. Zootaxa 4729(4): 451–481. https://doi.org/10.11646/zootaxa.4729.4.1

Cavazos-Camacho C, Ahumada-Carrillo IT (2020) Geographic distribution: *Pituophis lineatocollis*. Herpetological Review 51: 275.

Caviedes-Solis IW, Nieto-Montes de Oca A (2018) A multilocus phylogeny of the genus *Sarcohyla* (Anura: Hylidae), and an investigation of species boundaries using statistical species delimitation. Molecular Phylogenetics and Evolution 118: 184–193. https://doi.org/10.1016/j.ympev.2017.09.010

Chen Y, Peng S (2017) Evidence and mapping of extinction debts for global forest-dwelling reptiles, amphibians and mammals. Scientific Reports 7(1): 44305. https://doi.org/10.1038/srep44305

Cisneros-Bernal AY, Rodríguez-Gómez F, Flores-Villela O, Fujita MK, Velasco JA, Fernández JA (2022) Phylogeography supports lineage divergence for an endemic rattlesnake (*Crotalus ravus*) of the Neotropical montane forest in the Trans-Mexican Volcanic Belt. Biological Journal of the Linnean Society. Linnean Society of London 137(3): 496–512. https://doi.org/10.1093/biolinnean/blac066

Clause AG, Luna-Reyes R, Nieto-Montes de Oca A (2020) A new species of *Abronia* (Squamata: Anguidae) from a protected area in Chiapas, Mexico. Herpetologica 76(3): 330–343. https://doi.org/10.1655/Herpetologica-D-19-00047

Contreras-MacBeath T, Brito Rodríguez M, Sorani V, Godlspink C, McGregor Reid G (2014) Richness and endemism of the freshwater fishes of Mexico. Journal of Threatened Taxa 6(2): 5421–5433. https://doi.org/10.11609/JoTT.o3633.5421-33

Cox CL, Davis Rubosky AR, Holmes IA, Reyes-Velasco J, Roelke CE, Smith EN, Flores-Villela O, McGuire JA, Campbell JA (2018) Synopsis and taxonomic revision of three genera in the snake tribe Sonorini. Journal of Natural History 52(13–16): 945–988. https://doi.org/10.1080/00222933.2018.1449912

Cruz-Elizalde R, Ramírez-Bautista A, Hernández-Salinas U, Berriozabal-Islas C, Wilson LD (2019) An updated checklist of the herpetofauna of Querétaro, Mexico: Species richness, diversity, and conservation status. Zootaxa 4638(2): 273–290. https://doi.org/10.11646/zootaxa.4638.2.7

Cruz-Sáenz D, Muñoz-Nolasco FJ, Mata-Silva V, Johnson JD, García-Padilla E, Wilson LD (2017) The herpetofauna of Jalisco, Mexico: Composition, distribution, and conservation. Mesoamerican Herpetology 4: 23–118. https://mesoamericanherpetology.com/uploads/3/4/7/9/34798824/mh_4-1_cruz-saenz_et_al.pdf

De la Torres-Loranca MA, Martínez-Fuentes RG, Canseco-Marquez L, García-Vázquez UO (2020) New records of amphibians and reptiles from Sierra de Zongolica, Veracruz and Puebla, Mexico. Herpetological Review 51: 550–553.

Delgadillo MC (1994) Endemism in the Neotropical moss flora. Biotropica 26(1): 12–16. https://doi.org/10.2307/2389105

Delgadillo MC, Villaseñor Ríos JL, Aranda PD (2003) Endemism in the Mexican flora: A comparative study in three plant groups. Annals of the Missouri Botanical Garden 90(1): 25–34. https://doi.org/10.2307/3298523

Dertien JS, Self S, Ross BE, Barrett K, Baldwin RF (2020) The relationship between biodiversity and wetland cover varies across regions of the conterminous United States. PLoS ONE 15(5): e0232052. https://doi.org/10.1371/journal.pone.0232052

Dixon JR, Lemos-Espinal JA (2010) Anfibios y Reptiles del Estado de Querétaro, México/ Amphibians and Reptiles of the State of Querétaro, Mexico. CONABIO, Mexico, 428 pp.

Enderson EF, Quijada-Mascarenas A, Turner DS, Rosen PC, Bezy RL (2009) The herpetofauna of Sonora, Mexico, with comparisons to adjoining states. Check List 5(3): 632–672. https://doi.org/10.15560/5.3.632

Ennen JR, Matamoros WA, Agha M, Lovich JE, Sweat SC, Hoagstrom CW (2017) Hierarchical, quantitative biogeographic processes for all North American turtles and their contribution to the biogeography of turtles and the continent. Herpetological Monograph 31(1): 114–140. https://doi.org/10.1655/HERPMONOGRAPHS-D-16-00013

Escalente T, Szumik C, Morrone JJ (2009) Areas of endemism of Mexican mammals: Reanalysis applying the optimality criterion. Biological Journal of the Linnean Society, Linnean Society of London 98(2): 468–478. https://doi.org/10.1111/j.1095-8312.2009.01293.x

Everson KM, Gray LN, Jones AG, Lawrence NM, Foley ME, Sovacool KL, Kratovil JD, Hotaling S, Hime PM, Storfer A, Parra-Olea G, Percino-Daniel R, Aguilar-Miguel X, O'Neill EM, Zambrano L, Shaffer HB, Weisrock DW (2021) Geography is more important than life history in the recent diversification of the tiger salamander complex. Proceedings of the National Academy of Sciences of the United States of America 18(17): 1–10. https://doi.org/10.1073/pnas.2014719118

Farr W (2015) Herpetofauna of Tamaulipas. In: Lemos-Espinal JA (Ed.) Amphibians and Reptiles of the US – Mexico Border States / Anfibios y Reptiles de los Estados de la Frontera México – Estados Unidos. Texas A&M University Press, College Station, 101–121.

Fernández JA, Sánchez O, Flores-Villela O (2006) Anfibios y reptiles del Estado de Tlaxcala. Publicaciones de la Sociedad Herpetológica Mexicana 3: 224240.

Fernández-Badillo L, Morales-Capellán N, González-Bonilla GT, Canseco-Márquez L, Hernández-Silva DA (2020) On the critically endangered Cofre de Perote salamander (*Isthmura naucampatepetl*): Discovery of a new population in Puebla, Mexico, and update of its known distribution. Amphibian & Reptile Conservation 14: 200–205. http://amphibian-reptile-conservation.org/pdfs/Volume/Vol_14_no_3/ARC_14_3_[General_Section]_200-205_e267.pdf

Flores-Villela O (1993) Herpetofauna of Mexico: Distribution and endemism. In: Ramamoorthy TJ, Bye R, Lot A, Fa J (Eds) Biological Diversity of Mexico: Origins and Distribution. Oxford University Press, New York, 253–280.

Flores-Villela O, García-Vázquez UO (2014) Biodiversidad de reptiles en México. Revista Mexicana de Biodiversidad 85: S467–S475. https://doi.org/10.7550/rmb.43236

Flores-Villela O, Smith EN, Campillo-García G, Martínez-Méndez N, Campbell JA (2022) A new species of *Sceloporus* of the *torquatus* group (Reptilia: Phrynosomatidae) from west Mexico. Zootaxa 5134(2): 286–296. https://doi.org/10.11646/zootaxa.5134.2.7

García-Alvarado F (2016) Geographic distribution: *Hemidactylus frenatus*. Herpetological Review 47: 423.

García-Padilla E, Figiel JJ, Wilson LD, Mata-Silva V (2019) Geographical distribution: *Bolitoglossa mexicana*. Herpetological Review 50: 744.

García-Vázquez UO, Pavón-Vázquez GJ, Blancas-Hernández JC, Blancas-Calva E, Centenero-Alcalá E (2018) A new rare species of the *Rhadinaea decorata* group from the Sierra Madre del Sur of Guerrero, Mexico (Squamata, Colubridae). ZooKeys 780: 137–154. https://doi.org/10.3897/zookeys.780.25593

García-Vázquez UO, Pavón-Vázquez CJ, Feria-Ortiz M, Nieto-Montes de Oca A (2021) A new species of Blue-tailed Skink (Scincidae: *Plestiodon*) from the Sierra Madre del Sur, Mexico. Herpetologica 77(1): 85–93. https://doi.org/10.1655/0018-0831-77.1.85

González-Sánchez VH, Johnson JD, García-Padilla E, Mata-Silva V, DeSantis DL, Wilson LD (2017) The herpetofauna of the Mexican Yucatan Peninsula: Composition, distribution, and conservation. Mesoamerican Herpetology 4: 264–380.

Gray L, Meza-Lázaro R, Poe S, Nieto-Montes de Oca A (2016) A new species of semiaquatic *Anolis* (Squamata: Dactyloidae) from Oaxaca and Veracruz, Mexico. The Herpetological Journal 26: 253–262.

Grismer LL (2002) Amphibians and Reptiles of Baja California, including its Pacific Islands and the Islands of the Sea of Cortés. University of California Press, Berkeley, 413 pp. https://doi.org/10.1525/california/9780520224179.001.0001

Grünwald CI, Reyes-Velasco J, Franz-Chávez H, Morales-Flores K, Ahumada-Carrillo I, Jones JM, Boissinot S (2018) Six new species of *Eleutherodactylus* (Anura: Eleutherodactylidae: subgenus *Syrrhophus*) from Mexico, with a discussion of their systematic relationships and the validity of related species. Mesoamerican Herpetology 5: 7–81.

Grünwald CI, Franz-Chávez H, Morales-Flores KI, Ahumada-Carrillo IT, Jones JM (2019) A rare new treefrog of the genus *Sarcohyla* (Anura: Hylidae) from Guerrero, Mexico. Zootaxa 4712(3): 345–364. https://doi.org/10.11646/zootaxa.4712.3.2

Grünwald CI, Reyes-Velasco J, Franz-Chávez H, Morales-Flores K, Ahumada-Carrillo IT, Rodríguez CM, Jones JM (2021a) Two new species of *Eleutherodactylus* (Anura: Eleutherodactylidae) from southern Mexico, with comments on the taxonomy of related species and their advertisement calls. Amphibian & Reptile Conservation 15: 1–35. http://amphibian-reptile-conservation.org/pdfs/Volume/Vol_15_no_1/ARC_15_1_[Taxonomy_Section]_1-35_e272.pdf

Grünwald CI, Toribio-Jiménez S, Montaño-Ruvalcaba C, Franz-Chávez H, Peñaloza-Montaño MA, Barrera-Nava EY, Jones JM, Rodríguez CM, Hughes IM, Strickland JL, Reyes-Velasco J (2021b) Two new species of snail-eating snakes of the genus *Tropidodipsas* (Serpentes, Dipsadidae) from southern Mexico, with notes on related species. Herpetozoa (Wien) 34: 233–257. https://doi.org/10.3897/herpetozoa.34.e69176

Guajardo Welsh R, Lazcano D, Bryson RW (2020) Geographic distribution: *Lampropeltis leonis*. Herpetological Review 51: 80.

Hansen RW, Fernández-Badillo L, Ramírez-Bautista A, Avalos-Torales O (2016) Geographic distribution: *Lampropeltis mexicana*. Herpetological Review 47: 262–263.

Hernandez A, Dufresnes C, Raffaëlli J, Jelsch E, Dubey S, Santiago-Pérez AL, Rosas-Espinoza VC, Berea Nuñez P (2022) Hope in the dark: Discovery of a population related to the presumably extinct mice-endemic Blunt-headed Salamander (*Ambystoma amblycephalum*). Neotropical Biodiversity 8(1): 35–44. https://doi.org/10.1080/23766808.2022.2029323

Hernández-Ordóñez O, Cervantes-López MdJ, González-Hernández A, Andresen E, Reynoso VH (2017) First record of the limestone rainfrog *Craugastor psephosypharus* (Amphibia: Anura: Craugastoridae) in Mexico. Revista Mexicana de Biodiversidad 88(1): 260–264. https://doi.org/10.1016/j.rmb.2017.01.005

Hillis DM, Frost JS, Wright DA (1983) Phylogeny and biogeography of the *Rana pipiens* complex: A biochemical evaluation. Systematic Biology 32(2): 132–143. https://doi.org/10.1093/sysbio/32.2.132

Hollingsworth BD, Mahrdt CR, Grismer LL, Lovich RE (2015) Herpetofauna of Baja California. In: Lemos-Espinal JA (Ed.) Amphibians and Reptiles of the US – Mexico Border States / Anfibios y Reptiles de los Estados de la Frontera México – Estados Unidos. Texas A&M University Press, College Station, 15–33.

Horowitz SB (1955) An arrangement of the subspecies of the horned toad, *Phrynosoma orbiculare* (Iguanidae). American Midland Naturalist 54(1): 204–218. https://doi.org/10.2307/2422188

IUCN (2022) The IUCN Red List of Threatened Species. Version 2022-2. https://www.iucnredlist.org [Accessed on 6 August 2023]

Jameson TJM, Streicher JW, Manuelli L, Head JJ, Smith EN (2022) Miniaturization in direct-developing frogs from Mexico with the description of six new species. Herpetological Monograph 36(1): 1–48. https://doi.org/10.1655/0733-1347-36.1.1

Johnson JD, Mata-Silva V, García-Padilla E, Wilson LD (2015) The herpetofauna of Chiapas, Mexico: Composition, physiographic distribution, and conservation status. Mesoamerican Herpetology 2: 272–329.

Kaplan M, Heimes P, Aguilar R (2020) A new species of *Sarcohyla* (Anura: Hylidae: Hylini) from the Sierra Madre del Sur of Guerrero and Estado de México, México. Zootaxa 4743(3): 382–390. https://doi.org/10.11646/zootaxa.4743.3.5

Keil P, Schweiger O, Kühn I, Kunin WE, Kuussaari M, Settele J, Henle K, Brotons L, Pe’er G, Lengyel S, Moustakas A, Steinicke H, Storch D (2012) Patterns of beta diversity in Europe: The role of climate, land cover and distance across scales. Journal of Biogeography 39(8): 1473–1486. https://doi.org/10.1111/j.1365-2699.2012.02701.x

Köhler G, Petersen CBP, Méndez de la Cruz FR (2019) A new species of anole from the Sierra Madre del Sur in Guerrero, Mexico (Reptilia, Squamata, Dactyloidae: *Norops*). Vertebrate Zoology 69: 145–160. https://doi.org/10.26049/VZ69-2-2019-02

Lara-Tufiño JD, Nieto-Montes de Oca A (2021) A new species of night lizard of the genus *Lepidophyma* (Xantusiidae) from southern Mexico. Herpetologica 77(4): 320–334. https://doi.org/10.1655/Herpetologica-D-21-00019.1

Lavin PA, Lazcano D, Gadsden H (2014) Anfibios y reptiles exóticos y traslocados invasores. In: Mendoza R, Koleff P (Eds) Especies Acuáticas Invasoras en México. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México, 435–441.

Lazcano D, Nevárez-de los Reyes M, García-Padilla E, Johnson JD, Mata-Silva V, DeSantis DL, Wilson LD (2019) The herpetofauna of Coahuila, Mexico: Composition, distribution, and conservation status. Amphibian & Reptile Conservation 13: 31–94. http://amphibian-reptile-conservation.org/pdfs/Volume/Vol_13_no_2/ARC_13_2_[General_Section]_31-94_e189ab.pdf

Lemos-Espinal JA, Dixon JR (2016) Anfibios y Reptiles de Hidalgo / Amphibians and Reptiles of Hidalgo. CONABIO, México, 763 pp. https://doi.org/10.15560/11.3.1642

Lemos-Espinal JA, Smith GR (2015) Amphibians and reptiles of the state of Hidalgo, Mexico. Check List 11(3): 1642. https://doi.org/10.15560/11.3.1642

Lemos-Espinal JA, Smith GR (2016) Amphibians and reptiles of the state of Coahuila, Mexico, with comparisons with adjoining states. ZooKeys 593: 117–137. https://doi.org/10.3897/zookeys.593.8484

Lemos-Espinal JA, Smith GR (2020a) A checklist of the amphibians and reptiles of Sinaloa, Mexico with a conservation status summary and comparisons with neighboring states. ZooKeys 931: 85–114. https://doi.org/10.3897/zookeys.931.50922

Lemos-Espinal JA, Smith GR (2020b) A conservation checklist of the herpetofauna of Morelos, with comparisons with adjoining states. ZooKeys 941: 121–144. https://doi.org/10.3897/zookeys.941.52011

Lemos-Espinal JA, Smith GR (2020c) A conservation checklist of the amphibians and reptiles of Mexico City, with comparisons with adjoining states. ZooKeys 951: 109–131. https://doi.org/10.3897/zookeys.951.52578

Lemos-Espinal JA, Smith GR (2020d) A conservation checklist of the amphibians and reptiles of the State of Mexico, Mexico with comparisons with adjoining states. ZooKeys 953: 137–159. https://doi.org/10.3897/zookeys.953.50881

Lemos-Espinal JA, Smith GR, Cruz A (2016) Amphibians and reptiles of the state of Nuevo Léon, Mexico. ZooKeys 594: 123–141. https://doi.org/10.3897/zookeys.594.8289

Lemos-Espinal, J.A. Smith GR, Woolrich-Piña GA, Cruz A (2017) Amphibians and reptiles of the state of Chihuahua, Mexico, with comparisons with adjoining states. ZooKeys 658: 105–130. https://doi.org/10.3897/zookeys.658.10665

Lemos-Espinal JA, Smith GR, Gadsden-Esparza H, Valdez-Lares R, Woolrich-Piña GA (2018a) Amphibians and reptiles of the state of Durango, Mexico, with comparisons with adjoining states. ZooKeys 748: 65–87. https://doi.org/10.3897/zookeys.748.22768

Lemos-Espinal JA, Smith GR, Woolrich-Piña GA (2018b) Amphibians and reptiles of the state of San Luis Potosí, Mexico, with comparisons with adjoining states. ZooKeys 753: 83–106. https://doi.org/10.3897/zookeys.753.21094

Lemos-Espinal JA, Smith GR, Rorabaugh JC (2019a) A conservation checklist of the amphibians and reptiles of Sonora, Mexico, with updated species lists. ZooKeys 829: 131–160. https://doi.org/10.3897/zookeys.829.32146

Lemos Espinal JA, Smith GR, Valdez-Lares R (2019b) Amphibians and Reptiles of Durango, México. ECO-Herpetological Publishing and Distribution, Rodeo, 414 pp.

Lemos-Espinal JA, Smith GR, Pierce LJS, Painter CW (2020) The amphibians and reptiles of Colima, Mexico, with a summary of their conservation status. ZooKeys 927: 99–125. https://doi.org/10.3897/zookeys.927.50064

Leyte-Manrique A, Mata-Silva V, Báez-Montes Ó, Fucsko LA, DeSantis DL, García-Padilla E, Rocha A, Johnson JD, Porras LW, Wilson LD (2022) The herpetofauna of Guanajuato, Mexico: Composition, distribution, and conservation status. Amphibian & Reptile

Conservation 16(2): 133–180. http://amphibian-reptile-conservation.org/pdfs/Volume/Vol_16_no_2/ARC_16_2_[General_Section]_133-180_e321.pdf

Lira R, Villaseñor JL, Ortíz E (2002) A proposal for the conservation of the family Cucurbitaceae in Mexico. Biodiversity and Conservation 11(10): 1699–1720. https://doi.org/10.1023/A:1020303905416

Liu J, Yong DL, Choi C-Y, Gibson L (2020) Transboundary frontiers: An emerging priority for biodiversity conservation. Trends in Ecology & Evolution 35(8): 679–690. https://doi.org/10.1016/j.tree.2020.03.004

Loc-Barragán JA, Lazcano D, Woolrich-Piña GA (2018) Notes on the herpetofauna of Nayarit, Mexico 2: Amphibians and reptiles of the municipality of Compostela. Bulletin of the Chicago Herpetological Society 53: 205–212.

Loc-Barragán JA, Woolrich-Piña GA (2020) *Sceloporus bulleri*. Mexico: Nayarit. Herpetological Review 51: 778.

Loc-Barragán JA, Franz-Chávez H, Grünwald AJ, Grünwald CI (2020a) Geographic distribution: *Eleutherodactylus palidus*. Herpetological Review 51: 532.

Loc-Barragán JA, Reyes-Velasco J, Woolrich-Piña G, Grünwald C, De Anaya M, Rangel-Mendoza J, López-Luna M (2020b) A new species of mud turtle of genus *Kinosternon* (Testudines: Kinosternidae) from the Pacific Coastal Plain of north western Mexico. Zootaxa 4885(4): 509–529. https://doi.org/10.11646/zootaxa.4885.4.3

Luna-Vega I, Espinosa D, Rivas G, Contreras-Medina R (2013) Geographical patterns and determinants of species richness in Mexico across selected families of vascular plants: Implications for conservation. Systematics and Biodiversity 11(2): 237–256. https://doi.org/10.1080/14772000.2013.797517

Mata-Silva V, Johnson JD, Wilson LD, García-Padilla E (2015) The herpetofauna of Oaxaca, Mexico: Composition, physiographic distribution, and conservation status. Mesoamerican Herpetology 2: 6–62.

Mata-Silva V, Rocha A, Ramírez-Bautista A, Berriozabal-Islas C, Wilson LD (2019) A new species of forest snake of the genus *Rhadinaea* from tropical montane rainforest in the Sierra Madre del Sur of Oaxaca, Mexico (Squamata, Dipsadidae). ZooKeys 813: 55–65. https://doi.org/10.3897/zookeys.813.29617

Mata-Silva V, García-Padilla E, Rocha A, De Santis DL, Johnson JD, Ramírez-Bautista A, Wilson LD (2021) A reexamination of the herpetofauana of Oaxaca, Mexico: Composition, update, physiographic distribution, and conservation commentary. Zootaxa 4996(2): 201–252. https://doi.org/10.11646/zootaxa.4996.2.1

McCranie JR, Köhler G (2004) Laemanctus longipes. Catalogue of American Amphibians and Reptiles 795: 1–4.

McCranie JR, Matthews AJ, Hedges B (2020) A morphological and molecular revision of lizards of the genus *Marisora* Hedges & Conn (Squamata: Mabuyidae) from Central America and Mexico, with descriptions of four new species. Zootaxa 4763(3): 301–353. https://doi.org/10.11646/zootaxa.4763.3.1

Meik JM, Schaack S, Flores-Villela O, Streicher JW (2018) Integrative taxonomy at the nexus of population divergence and speciation in insular speckled rattlesnakes. Journal of Natural History 52(13–16): 989–1016. https://doi.org/10.1080/00222933.2018.1429689

Mendoza-Hernández AA, Roth-Monzón AJ (2017) Geographic distribution: *Trachemys ornata*. Herpetological Review 48: 387–388.

Montaño-Ravalcaba C, Reyes-Velasco J, Grünwald CI, Jones JM (2020) Geographic distribution: *Geophis pyburni*. Herpetological Review 51: 545.

Montanucci RR (1979) Notes on systematics of horned lizards allied to *Phrynosoma orbiculare* (Lacertilia: Iguanidae). Herpetologica 35: 116–124. https://www.jstor.org/stable/3891775

Montanucci RR (2015) A taxonomic revision of the *Phrynosoma douglassii* species complex (Squamata: Phrynosomatidae). Zootaxa 4015: 1–177. https://doi.org/10.11646/zootaxa.4015.1.1

Morrone JJ, Escalante T, Rodríguez-Tapia G (2017) Mexican biogeographic provinces: Map and shapefiles. Zootaxa 4277(2): 277–279. https://doi.org/10.11646/zootaxa.4277.2.8

Myers N, Mittermeier RA, Mittermeier CG, de Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403(6772): 853–858. https://doi.org/10.1038/35002501

Nevárez de los Reyes M, Lazcano D, García-Padilla E, Mata-Silva V, Johnson JD, Wilson LD (2016) The herpetofauna of Nuevo León, Mexico: Composition, distribution, and conservation. Mesoamerican Herpetology 3: 558–638.

Nevarez de los Reyes M, Lazcano D, Wilson LD (2019a) Geographic distribution: *Gerrhonotus ophiurus*. Herpetological Review 50: 328.

Nevarez de los Reyes M, Lazcano D, Centenero-Alcalá E, Eryan-Sánchez-Morales N, Serra-Ortíz MA, Wilson LD (2019b) Geographic distribution: *Boa imperator*. Herpetological Review 50: 528.

Nieto-Montes de Oca A, Castresana-Villanueva N, Canseco-Márquez L, Campbell JA (2022) A new species of *Xenosaurus* (Squamata: Xenosauridae) from the Sierra de Juárez of Oaxaca, Mexico. Herpetologica 78: 40–50. https://doi.org/10.1655/Herpetologica-D-21-00041.1

O'Connell KA, Smith EN (2018) The effect of missing data on coalescent species delimitation and a taxonomic revision of whipsnakes (Colubridae: *Masticophis*). Molecular Phylogenetics and Evolution 127: 356–366. https://doi.org/10.1016/j.ympev.2018.03.018

Ochoa-Ochoa LM, Munguía M, Lira-Noriega A, Sánchez-Cordero V, Flores-Villela O, Navarro-Sigüenza A, Rodríguez P (2014) Spatial scale and β-diversity of terrestrial vertebrates in Mexico. Revista Mexicana de Biodiversidad 85(3): 918–930. https://doi.org/10.7550/rmb.38737

Opermanis O, MacSharry B, Aunins A, Sipkova Z (2012) Connectedness and connectivity of the Natura 2000 network of protected areas across country borders in the European Union. Biological Conservation 153: 227–238. https://doi.org/10.1016/j.biocon.2012.04.031

Ortiz-Medina JA, Cabrera-Ceri DI, Nahuat-Cervera PE, Chablé-Santos JB (2020) New distributional records from the herpetofauna of Campeche and Yucatán, Mexico. Herpetological Review 51: 83–87.

Palacios-Aguilar R, Flores-Villela O (2018) An updated checklist of the herpetofauna from Guerrero, Mexico. Zootaxa 4422(1): 1–24. https://doi.org/10.11646/zootaxa.4422.1.1

Palacios-Aguilar R, Flores-Villela O (2020) Taxonomic revision and comments on two groups of the genus *Coniophanes* (Squamata: Dipsadidae). Vertebrate Zoology 70: 111–124. https://doi.org/10.26049/VZ70-2-2020-02

Palacios-Aguilar R, Santos-Bibiano R (2020) A new species of direct-developing frog of the genus *Eleutherodactylus* (Anura: Eleutherodactylidae) from the Pacific lowlands of Guerrero, Mexico. Zootaxa 4750(2): 250–260. https://doi.org/10.11646/zootaxa.4750.2.8

Palacios-Aguilar R, Santos-Bibiano R, Flores-Villela O (2018) A new species of *Lepidophyma* (Squamata: Xantusiidae) from the Pacific lowlands of Guerrero, Mexico. Journal of Herpetology 52(3): 327–331. https://doi.org/10.1670/17-061

Parra-Olea G, Rovito SM, García-París M, Maisano JA, Wake DB, Hanken J (2016) Biology of tiny animals: three new species of minute salamanders (Plethodontidae: *Thorius*) from Oaxaca, Mexico. PeerJ 4: e2694. https://doi.org/10.7717/peerj.2694

Pazos-Nava FN, Álvaro-Montego RI, Capul-Magaña FG, de Quevedo-Machain RG, Flores-Guerro US, Velasco JA, Escobedo-Galván AH (2019) First verified record of *Anolis sagrei* Cocteau in Duméril and Bibron, 1837 from the central Pacific Coast of Mexico. BioInvasions Records 8(3): 568–574. https://doi.org/10.3391/bir.2019.8.3.12

Pérez-Ramos E, Saldaña de La Riva L (2008) Morphological revision of lizards of the *formosus* group, genus *Sceloporus* (Squamata: Sauria) of southern México, with description of a new species. Bulletin of the Maryland Herpetological Society 44: 77–97.

Platz JE (1991) *Rana berlandieri* Baird, Rio Grande Leopard Frog. Catalogue of American Amphibians and Reptiles 508: 1–4.

Qian H, Ricklefs RE (2006) The role of exotic species in homogeneizing North American flora. Ecology Letters 9(12): 1293–1298. https://doi.org/10.1111/j.1461-0248.2006.00982.x

Ramamoorthy TP, Bye R, Lot A, Fa J [Eds] (1993) Biological Diversity of Mexico: Origins and Distribution. Oxford University Press, Oxford, 856 pp.

Ramírez-Bautista A, Hernández-Salinas U, Cruz-Elizalde R, Berriozabal-Islas C, Moreno-Lara I, De Santis DL, Johnson JD, García-Padilla E, Mata-Silva V, Wilson LD (2020) The herpetofauna of Hidalgo, Mexico: Composition, distribution, and conservation status. Amphibian & Reptile Conservation 14: 63–118. http://amphibian-reptile-conservation.org/pdfs/Volume/Vol_14_no_1/ARC_14_1_[General_Section]_63-118_e224.pdf

Ramírez-Reyes T, Flores-Villela O (2018) Taxonomic changes and description of two new species from the *Phyllodactylus lanei* complex (Gekkota: Phyllodactylidae) in Mexico. Zootaxa 4407(2): 151–190. https://doi.org/10.11646/zootaxa.4407.2.1

Ramírez-Reyes T, Piñero D, Flores-Villela O, Vázquez-Domínguez E (2017) Molecular systematic, species delimitation and diversification patterns of the *Phyllodactylus lanei* complex (Gekkota: Phyllodactylidae) in Mexico. Molecular Phylogenetics and Evolution 115: 82–94. https://doi.org/10.1016/j.ympev.2017.07.008

Ramírez-Reyes T, Barraza-Soltero IK, Nolasco-Luna JR, Flores-Villela O, Escobedo-Galván AH (2021a) A new species of leaf-toed gecko (Phyllodactylidae, *Phyllodactylus*) from María Cleofas Island, Nayarit, Mexico. ZooKeys 1024: 117–136. https://doi.org/10.3897/zookeys.1024.60473

Ramírez-Reyes T, Flores-Villela O, Piñero D, Lathrop A, Murphy RW (2021b) Genomic assessment of the *Phyllodactylus tuberculosus* complex (Reptilia: Phyllodactylidae) in America. Zoologica Scripta 50(5): 529–542. https://doi.org/10.1111/zsc.12492

Rautsaw RM, Holding ML, Strickland JL, Castañeda Gaytán JJ, García González FC, Castañeda Gaytán JG, Borja Jiménez JM, Parkinson CL (2018) Geographic distribution: *Hypsiglena tanzeri*. Herpetological Review 49: 287.

Reyes-Velasco J, Adams RH, Boissinot S, Parkinson CL, Campbell JA, Castoe TA, Smith EN (2020a) Genome-wide SNPs clarify lineage diversity confused by coloration in coralsnakes of the *Micrurus diastema* species complex (Serpentes: Elapidae). Molecular Phylogenetics and Evolution 147: 106770. https://doi.org/10.1016/j.ympev.2020.106770

Reyes-Velasco JR, Grünwald CI, Jones JM, Ahumada Carrillo IT (2020b) Una revisión de la herpetofauna de Colima, México. Revista Latinoamerica de Herpetologia 3(2): 61–82. https://doi.org/10.22201/fc.25942158e.2020.2.164

Rorabaugh JC, Lemos-Espinal JA (2016) A Field Guide to the Amphibians and Reptiles of Sonora, Mexico. ECO Herpetological Publishing and Distribution, Rodeo, 688 pp.

Sánchez-García F, García-Padilla E, Wilson LD, Mata-Silva V (2020) *Gerrhonotus ophiurus*. Mexico: Oaxaca. Herpetological Review 51: 776.

Sarukhán J, Urquiza-Haas T, Koleff P, Carabias J, Dirzo R, Ezcurra E, Cerdeira-Estrada S, Suberón J (2015) Strategic actions to value, conserve, and restore the natural capital of megadiversity countries: The case of Mexico. Bioscience 65(2): 164–173. https://doi.org/10.1093/biosci/biu195

Schätti B, Heimes P, Tillack F, Kucharzewski C, Torres-Pérez Coeto J (2020) *Pituophis deppei* (Duméril, Bibron & Duméril, 1854) or a reassessment of Mexican bullsnakes (Reptilia: Squamata: Colubridae). Vertebrate Zoology 70: 483–545. https://doi.org/10.26049/VZ70-4-2020-01

Shanas U, Galyun YA, Alshamlih M, Cnaani J, Guscio D(U), Khoury F, Mittler S, Nassar K, Shapira I, Simon D, Sulltan H, Topel E, Ziv Y (2006) Reptile diversity and rodent community structure across a political border. Biological Conservation 132: 292–299. https://doi.org/10.1016/j.biocon.2006.04.021

Sigala-Rodríguez JJ, Quintero-Díaz GE, Ahumada-Carrillo IT, Carrillo-Lara DE (2020a) Anfibios. In: La Biodiversidad en Zacatecas. Estudio de Estado. CONABIO, Mexico, 229–234.

Sigala-Rodríguez JJ, Quintero-Díaz GE, Ahumada-Carrillo IT, Carbajal-Márquez RA, Enríquez ED, Vacio de la Torre ME (2020b) Reptiles. In: La biodiversidad en Zacatecas. Estudio de Estado. CONABIO, Mexico, 235–240.

Smith GR, Lemos-Espinal JA (2015) Herpetofaunal diversity of the United States-Mexico border. In: Lemos-Espinal JA (Ed.) Amphibians and Reptiles of the US-Mexico Border States. Texas A&M University Press, College Station, 196–205.

Smith JR, Hendershot JN, Nova N, Daily GC (2020) The biogeography of ecoregions: Descriptive power across regions and taxa. Journal of Biogeography 47(7): 1413–1426. https://doi.org/10.1111/jbi.13871

Sosa V, De-nova JA (2012) Endemic angiosperm lineages in Mexico: Hotspots for conservation. Acta Botánica Mexicana 100: 293–315. https://www.scielo.org.mx/scielo.php?pid=S0187-71512012000300010&script=sci_arttext&tlng=en

Sosa-Tovar GH, García-Padilla E, Wilson LD, Mata-Silva V (2019) Geographic distribution: *Hemidactylus mabouia*. Herpetological Review 50: 746.

Streicher JW, Devitt TJ, Goldberg CS, Malone JH, Blackmon H, Fujita MK (2014) Diversification and asymmetrical gene flow across time and space: Lineage sorting and hybridization in polytypic barking frogs. Molecular Ecology 23(13): 3273–3291. https://doi.org/10.1111/mec.12814

Suazo-Ortuño I, Ramírez-Bautista A, Alvarado-Díaz J (2023) Amphibians and reptiles of Mexico: Diversity and conservation. In: Jones RW, Ornelas-García CP, Pineda-López R, Álvarez F (Eds) Mexican Fauna in the Anthropocene. Springer Nature Switzerland, Cham, 105–127. https://doi.org/10.1007/978-3-031-17277-9_6

Tellez O, Mattana E, Diazgranados M, Kühn N, Castillo-Lorenzo E, Lira R, Montes-Leyva L, Rodriguez I, Flores Ortiz CL, Way M, Dávila P, Ulian T (2020) Native trees of Mexico: Diversity, distribution, uses and conservation. PeerJ 8: e9898. https://doi.org/10.7717/peerj.9898

Tepos-Ramírez M, Flores-Villela O, Velasco JA, Pedraza Lara C, García Rubio OR, Jadin RC (2021) Molecular phylogenetics and morphometrics reveal a new endemic jumping pitviper (Serpentes: Viperidae: *Metlapilcoatlus*) from the Sierra Madre Oriental of Mexico. Journal of Herpetology 55(2): 181–191. https://doi.org/10.1670/20-028

Terán-Juàrez SA, García-Padilla E, Mata-Silva V, Johnson JD, Wilson LD (2016) The herpetofauna of Tamaulipas, Mexico: Composition, distribution, and conservation status. Mesoamerican Herpetology 3: 43–113.

Torres-Hernández LA, Ramírez-Bautista A, Cruz-Elizalde R, Hernández-Salinas U, Berriozabal-Islas C, De Santis DL, Johnson JD, Rocha A, García-Padilla E, Mata-Silva V, Fucsko LA, Wilson LD (2021) The herpetofauna of Veracruz, Mexico: Composition, distribution, and conservation status. Amphibian & Reptile Conservation 15: 72–155. http://amphibian-reptile-conservation.org/pdfs/Volume/Vol_15_no_2/ARC_15_2_[General_Section]_72-155_e285.pdf

Trageser SJ, Schell RA (2018) Geographic distribution: *Marisora brachypoda*. Herpetological Review 49: 504.

Valencia-Herverth R, Fernández-Badillo L, Valencia-Herverth J (2020) Geographic distribution: *Terrapene mexicana*. Herpetological Review 51: 537–538.

Villaseñor JL, Ortiz E, Delgadillo-Moya C, Juárez D (2020) The breadth of the Mexican Transition Zone as defined by its flowering plant genetic flora. PLoS ONE 15(6): e0235267. https://doi.org/10.1371/journal.pone.0235267

Webb RG (2001) Frogs of the *Rana tarahumarae* group in western Mexico. In: Johnson JD, Webb RG, Flores-Villela O (Eds) Mesoamerican Herpetology: Systematics, Zoogeography and Conservation. University of Texas at El Paso, El Paso, 20–43.

Woolrich-Piña GA, Ponce Campos P, Loc-Barragán J, Ramírez-Silva JP, Mata-Silva V, Johnson JD, García-Padilla E, Wilson LD (2016) The herpetofauna of Nayarit, Mexico: Composition, distribution, and conservation. Mesoamerican Herpetology 3: 376–448.

Woolrich-Piña GA, García-Padilla E, DeSantis DL, Johnson JD, Mata-Silva V, Wilson LD (2017) The herpetofauna of Puebla, Mexico: Composition, distribution, and conservation status. Mesoamerican Herpetology 4: 791–884.

Zaldivar-Riverón A, León-Regagnon V, Nieto-Montes de Oca A (2004) Phylogeny of the Mexican coastal leopard frogs of the *Rana berlandieri* group based on mtDNA sequences. Molecular Phylogenetics and Evolution 30(1): 38–49. https://doi.org/10.1016/S1055-7903(03)00141-6

Nature Conservation

Nature Conservation 53: 257–278 (2023)
DOI: 10.3897/natureconservation.53.106260

Research Article

Hydrological regime and forest development have indirect effects on soil fauna feeding activity in Central European hardwood floodplain forests

Nicole Scheunemann[1], David J. Russell[1]

1 *Senckenberg Museum of Natural History Görlitz, Department of Soil Zoology, Am Museum 1, 02826 Görlitz, Germany*
Corresponding author: Nicole Scheunemann (nicole.scheunemann@senckenberg.de)

This article is part of:
Restoration of Wetlands
Edited by Mathias Scholz, Szabolcs Lengyel, Agustín Sánchez-Arcilla, Joanna Staneva, Vicente Gracia and Mindert de Vries

Academic editor: Szabolcs Lengyel
Received: 12 May 2023
Accepted: 2 September 2023
Published: 29 September 2023

ZooBank: https://zoobank.org/9F696E9F-3225-4C78-AAF2-EABFC5CB0C0E

Citation: Scheunemann N, Russell DJ (2023) Hydrological regime and forest development have indirect effects on soil fauna feeding activity in Central European hardwood floodplain forests. Nature Conservation 53: 257–278. https://doi.org/10.3897/natureconservation.53.106260

Abstract

Soil fauna act as regulators of decomposition processes via their feeding activity, thereby playing an important role in regulating carbon cycling and sequestration. Hardwood floodplain forests are critically endangered habitats, but strongly contribute to carbon sequestration in Central Europe. In the present study, within a floodplain forest-development programme, we investigated the feeding activity of soil fauna via the Bait Lamina test in hardwood floodplain forests of the middle Elbe River in Germany in sites with different hydrological regimes and forest-development stages, with neighbouring grassland sites for comparison.

While statistically significant differences in overall feeding activity between general hydrological regimes or forest development stages were not found, decreases in feeding activity with soil depth were strongly modulated by these factors, indicating more unfavourable conditions for soil fauna at increasing soil depth due to, e.g., anoxic conditions in floodplains of tributaries or low soil moisture content below the shallow rooting zone of grasslands. Registered effects of soil texture on soil fauna feeding activity were dependent on forest-tree density, and combined effects indicate that soil-fauna feeding activity varies with soil temperature during spring, but with soil moisture in early autumn.

In conclusion, our results highlight the importance of the current abiotic conditions on soil-fauna feeding activities in floodplain forests, i.e. soil temperature, moisture and ground water level. Hydrological regime and forest development have a strong impact on the effect of these conditions, indirectly affecting soil fauna feeding activity and highlighting the multifactorial influence on soil fauna functional activity to be considered in floodplain-forest restoration programs.

Key words: Bait Lamina, drivers, soil, soil depth, soil moisture, soil texture

Introduction

Central European hardwood floodplain forests are endangered ecosystems that are characterized by high biodiversity (Tockner et al. 2002) and carbon sequestration ability (Giese et al. 2003; Heger et al. 2021). Inundation events occurring more or less regularly shape these ecosystems and their species communities (Ward et al. 1999; Russell and Griegel 2006; Glaeser and Wulf

2009). However, the area covered by hardwood floodplain forests has declined considerably over the last centuries due to, on the one hand, river management for floodwater protection (e.g., river regulation, dyke building) and, on the other hand, deforestation and agricultural use (Koenzen 2009, 2021).

Along the Middle Elbe River of Germany, efforts are being undertaken to restore natural hardwood floodplain forests, primarily by re-establishing natural oak forests, among other measures (Damm 2013). Thereby, effects of forest restoration on biodiversity and, especially, important ecosystem services are being evaluated. Important factors influencing restoration activities are the hydrological river regime of specific restoration sites as well as different forms of forest re-establishment (hereafter called forest development stages). The influence of soil type and other soil parameters on forest establishment and the associated impacts on biodiversity and ecosystem services are of special importance in these studies.

Floodplains are hotspots of carbon sequestration (Walling et al. 2006; Kiss et al. 2015; Shupe et al. 2022), an important ecosystem service in light of global climate change. Thereby, soils are the basis of important carbon-cycle processes such as degradation of organic matter, mineralisation to inorganic compounds as well as carbon sequestration (e.g., Comerford et al. 2013; Adhikari and Hartemink 2016; Saccá et al. 2017). Soil mesofauna (i.e. soil fauna of 0.2–2 mm body size such as Collembola and Enchytraeids) generally act as regulators of these processes, i.e. by influencing the activity of soil microorganisms via their feeding activity (Bardgett 2005; Crowther et al. 2011; Filser et al. 2016). A high feeding activity of soil fauna is usually accompanied by heightened activity of microorganisms (i.e., growth, enzyme production, nutrient translocation; Tordoff et al. 2011) and positive effects on carbon cycling (Geissen and Brümmer 1999; Römbke 2014; Zhang et al. 2015).

The Bait Lamina test is a well-established and frequently used method for estimating feeding activity of soil mesofauna (Sulkava et al. 1996; Helling et al. 1998; Gongalsky et al. 2008) by providing food bait, the loss of which being an indicator of faunal feeding activity (von Törne 1990; Kratz 1998). The method provides insight into the activity patterns of soil fauna along a depth gradient covering the uppermost 8 centimetres of soil (von Törne 1990; Römbke 2014). Soil fauna activity is not necessarily connected to soil fauna densities or species richness, as even large numbers of, e.g., Collembola might be rather inactive depending on the temporal habitat conditions (Gongalsky et al. 2004). Therefore, mesofauna densities are not a proxy for feeding activities, but rather feeding activity of the existing fauna appears to be regulated to a large extent by prevailing abiotic factors. Several field studies investigated the differences in soil fauna feeding activity between seasons, some suggesting that high temperatures result in highest activities in summer in spite of lower soil moisture (Gongalsky et al. 2008; Rozen et al. 2010; Krishna and Mohan 2017), while others highlight the importance of soil moisture as the main driver of soil-fauna feeding activity (Simpson et al. 2012). However, to our knowledge, the effects of other soil properties on soil fauna feeding activity are widely unknown (Geissen et al. 2007; van Gestel et al. 2009; Birkhofer et al. 2011; Klimek et al. 2015), although their effect on the soil ecosystem itself might be tremendous (Bardgett 2005; Frouz 2018).

In the present study, we investigated soil fauna feeding activity in German hardwood floodplain forests along the middle Elbe River. Corresponding to the studied factors in the forest-restoration programme, we compared forest sites with different hydrological regime, i.e. in the active Elbe floodplain affected by sporadic inundation events, in the seepage water zone behind the Elbe dykes that were historically flooded but currently are only affected by high groundwater levels, and nearby tributaries usually independent from the main Elbe hydrological regime. Further, within the active floodplain, we assessed differences in the soil-faunal feeding activities between forests of different age and tree density (reflecting forest-restoration methods) and compared them to neighbouring agriculturally managed grasslands as the starting point of renewed forest restoration (referred to as forest development stages in the following). We also considered various soil properties, vegetation and soil fauna in the analyses to assess potential drivers of soil fauna feeding activity in the floodplain forests and their potential effects within forest development stages or hydrological regimes.

We hypothesized that soil fauna feeding activity in the studied hardwood floodplain forests is affected (1) by hydrological regime with lower activity rates in potentially water saturated active floodplain soils as compared to drained soils behind the dykes, and (2) by forest development stage with activity rates increasing from grasslands to young and further to old forests. In addition, we hypothesize that (3) differing environmental conditions within these gradients will influence the overall gradient effects on soil fauna feeding activities.

Methods

Study site and sampling design

The study area is part of the UNESCO Middle Elbe Biosphere Reserve in northern Germany, and covers ca. 100 km of the Elbe River. The area is characterized by a Central European temperate climate with a mean annual temperature of 9.3 °C and mean annual precipitation of 615 mm (measured at the Lenzen weather station, 53.08°N, 11.48°E). The Elbe floodplain is an anthropogenically altered landscape with a history of dyking, deforestation and agricultural use, with the studied hardwood floodplain forests representing small remnants of the historically extended floodplain forest ecosystem. The arboral vegetation in the study sites is typical for central European hardwood floodplain forests and is characterized by oak (*Quercus robur*) and elm (*Ulmus laevis*); typical understory vegetation is hawthorn (*Crataegus monogyna*). For further information on the study area see Shupe et al. (2021).

In the research programme in which the current study took place, two gradients of ecological habitats are being investigated: a gradient of hydrological regimes and a forest development gradient. For the hydrological regimes, nine forest sites in the study area were investigated (Fig. 1), of which three were located in the active floodplain (i.e. the Elbe floodplain area in front of a dyke flooded during sporadic inundation events, usually after snowmelt in early spring; although none took place during the study period), three in the seepage water zone (i.e. the zone behind the Elbe dykes currently affected by occasional high groundwater levels), and three in the floodplains of tributaries independent from the active Elbe flooding regime. For the forest development gradient, a total of 16 sites of the re-forestation programme were investigated, all of which are located in the active floodplain (Fig. 1). Of these sites four represented dense older forest stands (mean tree age 80–200 years with multi-layered canopy cover and a rich shrub layer), four were in sparse older forest stands (mean tree age 80–200 years with a less developed canopy and shrub layer), and four established in young forest plantations (mean tree age 17–26 years with a strong grass-dominated herbaceous layer). Additionally, four sites were located in nearby agricultural grasslands and served as a control, since these represented the starting point of reforestation. Old dense, old sparse, young forests and grassland are referred to as forest development stages in the following.

Environmental data on vegetation (e.g., cover and species number of vegetation layers, litter cover and thickness, leaf area index) were collected by botanical project partners (see Suppl. material 1. Mean tree age was estimated by measuring annual tree rings of four dominant oak trees per forest site (Shupe et al. 2021). Data on soil properties (e.g., pH, texture, C and N content) of individual forest sites, as well as precipitation data were obtained from soil-science project partners (Vásconez Navas et al. 2023, see Suppl. material 1). The average number of flooding days per year as a 25-year-mean (1990–2016) was estimated for the individual active floodplain forest sites using a digital model developed for the Elbe river by Weber and Hatz (2020) and Weber and Rosenzweig (2020). Density and species number of soil fauna as used as explanatory variables were obtained from 10 MacFadyen soil cores per forest site (Scheunemann et al., in prep.).

Soil temperature and moisture were assessed at the starting day of each Bait Lamina test at 10 measurement points per forest site. Soil temperature at 10 cm depth was measured using a digital soil thermometer, while soil moisture was calculated from soil cores up to 5 cm depth taken adjacent to the soil thermometer. These soil cores had been used for extraction of soil fauna by heat (data not presented, Scheunemann et al., in prep.). The fresh soil cores were weighed, then dried during animal extraction over 10 days at maximally 55 °C and weighed again. The gravimetric water content was calculated as follows:

$$\%_{H2O} = ((\text{fresh weight} - \text{dry weight}) / \text{fresh weight}) * 100)$$

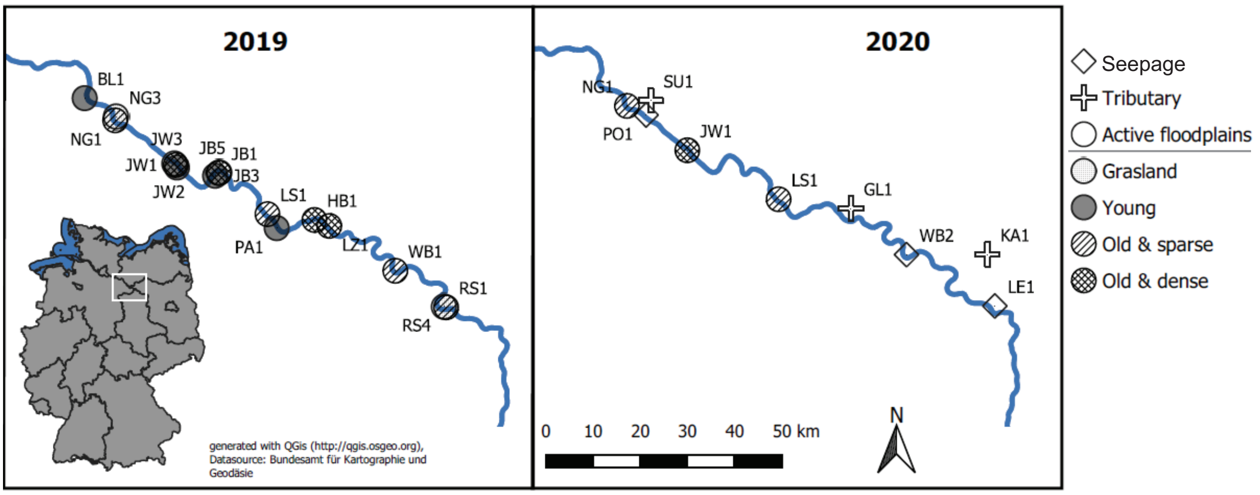


Figure 1. Map of sampling sites along the Elbe river investigated in 2019 (forest development gradient, left) and 2020 (hydrological regimes, right).

Calibration with additional drying at 105 °C for two days revealed essentially no difference to the method above. Mean values of soil temperature and moisture measurements per forest site and season were then used as explanatory variables in the statistical analyses.

Bait lamina test

Feeding activity of soil mesofauna was assessed by the Bait Lamina test according to the ISO standardisation 18311:2016 (ISO 18311 2018). Test strips (12 × 0.5 × 0.1 cm) are made of PVC and contain a row of 16 drilled holes of 1 mm diameter at a distance of 5 mm from each other. The holes were filled with a mixture of powdered cellulose (70%), wheat bran (27%; < 500 µm particle size) and activated charcoal (3%) as standard bait for soil invertebrates. Filled Bait Lamina strips were obtained from terra protecta GmbH, Berlin, Germany.

We measured soil fauna feeding activity along the forest development gradient in 2019 and in the hydrological regimes in 2020 (see below for detailed sampling dates). The Bait Lamina tests were carried out at the forest sites in three plots at each site during the average periods of highest soil-faunal activity (and avoiding summer dry periods) in late spring and early autumn to assess seasonal differences. Distance between plots within a forest site was a minimum of 10 m to avoid autocorrelation (Irmler 1998), distance between the two closest sites was minimally 300 m (between forest and comparative grassland sites) and maximally 10 km (between active floodplain and tributary sites; Fig. 1). Test strips were placed vertically in the soil such that the bait holes were located at soil depths between 1 and 8 cm to allow evaluation of bait-material disappearance in the respective soil depths. In each plot of every site, 16 test strips were placed in the soil in a 4 × 4 grid with 10 cm distance between single strips. Test strips were exposed to the soil for four weeks. In the forest-development gradient, the experiment started in spring between April 29 and May 3 2019 and finished on May 27/28 2019, and again started in early autumn between September 23 and 26 2019 and finished on October 21/22 2019. In the hydrological regimes, the experiment started in spring on May 11/12 2020 and finished on June 8/9 2020, while in early autumn it started on September 22/23 2020 and finished on October 20/21 2020.

After removal from the field, test strips of each plot were collected together, placed in a separate plastic bag and frozen at -20 °C to stop microbial decomposition of the bait material until further processing. In the lab, attached soil was carefully removed from defrosted strips and the strips visually inspected for empty bait holes by holding against a diapositive slide viewer as a light source. As stipulated by ISO 18311:2016, data was collected as “actively fed on” if light could be observed to shine through a bait hole, and as “not fed on” with bait holes through which no light emitted (even if small feeding traces were found at the sides). Every individual hole in each test strip was inspected in this way, resulting in a data set consisting of binary data representing the feeding activity at each Bait Lamina position (= soil depth). Strips that had been disturbed (chewed on or removed from soil by wild animals) during the four-week field exposure were excluded from further analyses. From all 16 bait laminae of one plot the percentage of bait holes that had been fed on was calculated for every soil depth. Further, the results of the three plots per forest site were averaged to avoid pseudoreplication.

Statistical analyses

A number of numerical environmental parameters (i.e. soil properties, vegetation and soil fauna parameters; see Suppl. material 1) were available for all forest sites. In a first model-building step, these were analysed for collinearity, which was performed separately for both datasets (forest development gradient 2019 and hydrological regime 2020), since these partially consisted of different forest sites. Collinearity of numerical parameters was investigated by principal component analysis using the prcomp() function in the “vegan” package (Oksanen et al. 2020) after standardization to zero mean and unit variance using the decostand() function. To achieve dimension reduction, we used the resulting site scores of the first three principle components (PC; all that explained more than 10% of variance) instead of the original 42 site parameters as independent variables in the subsequent analyses. Inspection of the scores of individual environmental factors showed that, in the 2020 data set (hydrological regime), PC1 represented the hydrological regime (i.e., number of flooding days per year), PC2 represented soil parameters and PC3 represented vegetation parameters as well as soil pH (Suppl. material 2). In contrast, for the 2019 data set (forest development), the first PC mainly reflected vegetation parameters (e.g. species numbers of shrubs and trees, leaf area index, litter cover), PC2 reflected soil parameters (e.g., total C and N content, pH, soil moisture) and PC3 reflected soil fauna density and further vegetation parameters (Suppl. material 3). Further, we built a correlation matrix using the corrplot() function in the “corrplot” package in R studio (Wei and Simko 2021, not presented). The correlation matrices were inspected for correlation indices, and factors with correlation indices > 0.5 or <-0.5 were considered to be autocorrelated. We calculated correlation indices using Pearson’s as well as Spearman’s correlation coefficient, which both showed similar results. As expected after the PCA, we found correlations within vegetation parameters, soil properties and soil fauna parameters, supporting the reduction of the measured environmental data to the respective PCs.

As categorical variables we used hydrological regime (active floodplain, seepage water zone, tributary) or forest development (old dense, old sparse, young forest, grassland), as well as soil texture estimated a priori by visual inspection in the field (the categories sandy and loamy corresponded well with sand content measured in the lab). In case we found high correlation of a categorical variable with the respective PC (GVIF value > 5 after using the vif() function of the “car” package; Fox and Weisberg 2019), we excluded the respective PC from further analyses and only retained the categorical variable. In this way, in the hydrological regime data set PC1 was replaced by the categorical “hydrology” variable, and in the forest development data set the categorical variable “forest development” replaced PC1.

We then applied linear mixed effects models (lmer() in the “lme4” package (Bates et al. 2015)) to the bait-lamina data sets of the mean percentage of “fed upon” baits at a respective soil depth as dependent variable and site identity and season as random terms. The null models only included soil depth and the two random terms as independent variables, while hydrology (or forest development), soil texture, the respective PCs as well as all possible two-fold interactions of these parameters were added consecutively to the model (forward selection) by hand. Based on Akaike’s Information Criterion (AIC) and anova() functions in the “stats” package (implemented in R), after every step models were compared and only the better model was kept. In case of the forest development data, this procedure resulted in forest development being not included in the model as a single term. Stats::anova() comparing the model with and without forest development as single term (next to soil depth and the two random terms) resulted in no significant difference (p = 0.46) between these models. Forest development did not have a significant effect on feeding activity either (car::Anova showed forest development p = 0.54 for the model containing forest development), therefore we kept the simpler model. In a later step of model selection, however, interactions of forest development with other factors were included in the model as they significantly improved the model. The significance level of variables and interactions was estimated via Type III sums-of-squares Wald Chi-square test using the Anova() function in the "car" package. The final models were as follows:

for hydrological regime:

"Feeding activity" ~ Soil_depth + Hydrology + Texture + PC2 + PC3 +
Hydrology : Soil_depth + Hydrology : Texture + Hydrology : Season +
Soil_depth : Season + Texture : Season + (1|SiteID) + (1|Season);

and for forest development:

"Feeding activity" ~ Soil_depth + Soil_depth : Forest_development
+ Soil_depth : PC2 + Soil_depth : Season + Soil_depth : Texture +
Forest_development : Texture + (1|SiteID) + (1|Season).

Pairwise comparisons of factors were calculated using the emmeans() function in the "emmeans" package (Lenth 2022). Since soil depth was treated as a numerical instead of factorial variable, pairwise comparisons including soil depth were performed using the lstrends() function in the "emmeans" package.

Furthermore, the average depth of faunal activity was evaluated using the Depth index (DI) (Gongalsky et al. 2004), calculated as:

$$DI = ((\sum n_i d_i) / N),$$

with DI being the mean depth of feeding activity, n_i the number of all pierced bait holes at depth i, d_i the respective soil depth [in mm] and N being the total number of pierced bait holes over all soil depths and test strips of one plot. Separate linear mixed effects models (lmer()) for the hydrological regime and forest development data set were built with DI as the response variable using the methods described above.

The final models for effects on the Depth Index were as follows:

for hydrological regime:

Depth Index ~ Hydrology + Hydrology : Season + (1|SiteID) + (1|Season);

and for forest development:

Depth Index ~ Forest development + Texture + Forest_development : Texture +
Forest_development : Season + (1|SiteID) + (1|Season).

All statistical analyses were performed using R statistical software, version 4.1.2 (2021-11-01, "Bird Hippie", R Core Team (2021), with R studio version 1.4.1717. Graphical visualizations were created using the "ggplot2" package (Wickham 2016).

Results

Ecological site gradients

The hydrological regime was related to a number of abiotic site parameters (Table 1; Suppl. material 1: table S1A). Briefly, average number of flooding days per year was 5.2 in the active floodplain, but 0 in the seepage water zone and tributary floodplains. Leaf area index, i.e. density of the forest, increased with distance to the main river, while pH and average sand content of soil decreased. Within the active floodplain, the four forest development stages were rather similar in their environmental factors. However, they differed in leaf area index with old dense and young forests having higher canopy cover (high leaf area index) than old sparse forests (Table 2; Suppl. material 1: table S1B). Comprehensive information on ecological parameters of single sampling sites is given in Suppl. material 1.

Feeding activities in different hydrological regimes

The hydrological regime did not directly affect overall feeding activities ($p = 0.11$), and soil texture, soil parameters (represented by PC2) and vegetation parameters (represented by PC3) alone also did not significantly affect feeding activity. In contrast, soil fauna feeding activity decreased with soil depth in all hydrological regimes ($p < 0.001$, Table 3), and general feeding activity rates were higher in autumn than in spring (only included as random term in the model). Feeding activities increased from spring to autumn to a higher extent at shallow than at

Table 1. Site parameters in sampling sites according to hydrological regime (sampled 2020); Values represent means ± standard deviation.

Hydrology (2020)	**Active floodplain**		**Seepage water zone**		**Tributary**	
	spring | autumn		**spring | autumn**		**spring | autumn**	
Soil temperature [°C]	9.57 ± 0.24	15.0 ± 0.71	10.59 ± 1.06	14.37 ± 0.28	10.39 ± 1.21	13.90 ± 0.25
Soil moisture [%H_2O]	15.24 ± 3.99	9.95 ± 1.97	18.61 ± 3.93	17.77 ± 5.6	25.23 ± 3.98	17.79 ± 4.18
pH (at 10 cm soil depth)	4.93 ± 0.17		4.13 ± 0.62		3.82 ± 0.11	
Leaf area index	2.21 ± 0.46		3.22 ± 0.61		4.83 ± 0.24	

Table 2. Site parameters in sampling sites according to forest development (sampled 2019); Values represent means ± standard deviation.

Forest development stage (2019)	**Grassland**		**Young forest**		**Old dense forest**		**Old sparse forest**	
	spring | autumn		**spring | autumn**		**spring | autumn**		**spring | autumn**	
Soil temperature [°C]	13.10 ± 1.10	14.86 ± 1.36	11.29 ± 0.81	14.36 ± 0.28	11.88 ± 1.04	14.22 ± 0.35	11.81 ± 0.80	14.44 ± 0.99
Soil moisture [%H_2O]	24.69 ± 5.51	14.44 ± 2.26	20.36 ± 3.20	10.87 ± 5.02	24.89 ± 4.66	17.43 ± 5.59	20.33 ± 1.54	15.17 ± 0.95
pH (at 10 cm soil depth)	5.05 ± 0.25		5.15 ± 0.26		5.15 ± 0.46		5.24 ± 0.19	
Leaf area index	0		2.74 ± 0.33		2.97 ± 0.43		2.00 ± 0.30	

larger soil depths, resulting in a stronger decrease in feeding activity with soil depth in autumn (soil depth x season: $p < 0.0001$, Fig. 2). However, the decrease in feeding activity with soil depth was also affected by hydrology, as reflected in a stronger depth decrease in tributary sites than in the active floodplain or seepage water zone (interaction soil depth x hydrology: $p < 0.0001$; pairwise comparisons $p = 0.0001$ for tributary *vs*. seepage water zone, $p = 0.0004$ for tributary *vs*. active floodplain, but $p = 0.88$ for active *vs*. seepage water zone). Activity rates varied according to the season in sites of different soil textures, with sites on sandy soils showing higher activities than those on loamy soils in spring, but not in autumn (interaction soil texture x season: $p < 0.0001$). The interaction of soil texture and hydrology was not included in the model because all tributary sites were of loamy soil, unbalancing the experimental design in this respect. For comparison of soil fauna feeding activity of single sites see Suppl. material 4.

The Depth Index, i.e. average soil depth of soil fauna feeding activity, was significantly affected by hydrological regime ($p = 0.001$) with maximum average activity being at larger depths in the seepage water zone compared to tributary sites. In active floodplain sites, the Depth Index was similar to that of the seep-

Table 3. Analysis of Deviance, results for hydrological regime dataset (**A**) and forest developmental stage dataset (**B**), separated into general feeding activity and Depth.

	Chisq	Df	Pr (>Chisq)
A) Hydrological regime			
Feeding activity			
Soil depth	**414.8**	**1**	**<0.0001 *****
Hydrology	**4.4**	**2**	**0.11**
Soil texture	**0.2**	**1**	**0.65**
PC2 (soil parameters)	**0.02**	**1**	**0.86**
PC3 (vegetation & soil pH)	**3.4**	**1**	**0.07**
Soil depth: Hydrology	**22.7**	**2**	**< 0.0001 *****
Hydrology: Season	**47.3**	**3**	**< 0.0001 *****
Soil depth: Season	**28.5**	**1**	**< 0.0001 *****
Texture: Season	**19.7**	**1**	**< 0.0001 *****
Depth index			
Hydrology	**13.6**	**2**	**0.0011 ****
Hydrology: Season	**5.8**	**3**	**0.12**
B) Forest development			
Feeding activity			
Soil depth	**39.9**	**1**	**<0.0001 *****
Soil depth: Forest development	**10.6**	**3**	**0.0130 ***
Soil depth: PC2 (Soil parameters)	**2.3**	**1**	**0.1304**
Soil depth: Season	**28.1**	**1**	**<0.0001 *****
Soil depth: Soil texture	**13.8**	**1**	**0.0002**
Forest development: Season	**36.1**	**4**	**<0.0001 *****
Forest development: Soil texture	**39.4**	**3**	**<0.0001 *****
Depth index			
Forest development	**23.3**	**3**	**<0.0001 *****
Soil texture	**6.4**	**1**	**0.0113 ***
Forest development: Soil texture	**10.8**	**3**	**0.0126 ***
Forest development: Season	**18.4**	**4**	**0.0010 ***

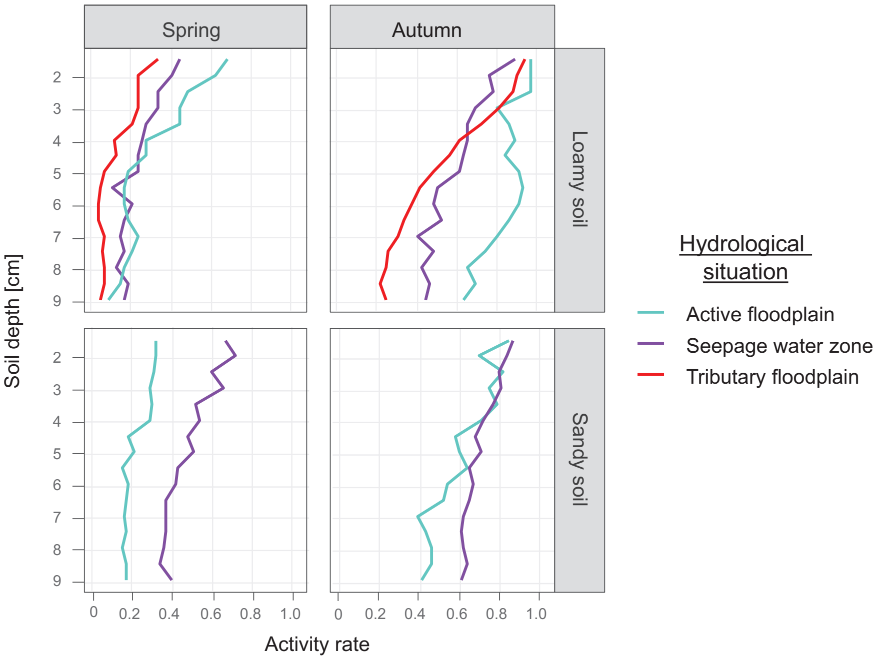


Figure 2. Feeding activities in different hydrological situations separated by season and soil texture.

age water zone, but the difference to tributary sites was not significant (Fig. 3, Table 3A). Neither season nor soil texture nor other environmental variables (PCs) modified this effect.

Feeding activities in the forest development stages

Within the active floodplain, forest developmental stage, soil texture, PC2 and PC3 were not included in the final model as single factors (only as interaction terms) due to higher AIC values in models containing these variables. Therefore, the effect of these variables individually was considered to be non-significant. As in the hydrological gradient, soil fauna feeding activity decreased with soil depth in all forest development stages ($p < 0.0001$, Table 3B). Further, the decrease in feeding activity with soil depth was more pronounced during spring than autumn (soil depth x season interaction $p < 0.0001$), and in sandy compared to loamy soils ($p < 0.0001$ for spring *vs*. autumn and $p = 0.0004$ for loamy *vs*. sandy, Fig. 4). General feeding activity rates were higher in autumn than in spring (only included as random term in the model), but significant differences between forest developmental stages were only observed in spring (forest developments x season interaction $p < 0.0001$) with lower feeding activity rates in grasslands than in old dense and young forests in spring.

Further, in grasslands on sandy soil, feeding activity was lower than in dense old and young forests on sandy soil ($p = 0.01$), while on loamy soil the differences between forest developmental stages were not significant. In addition, in grasslands on sandy soils feeding activity rates decreased stronger with soil depth than in young forests (interaction soil depth x forest development: $p = 0.01$; comparison young *vs*. grassland across depth with $p = 0.029$; all other comparisons $p > 0.05$). For soil fauna feeding activity in the individual sampling sites, see Suppl. material 5.

Forest development significantly affected Depth Index, i.e. maximum average soil-fauna activity occurred at a larger average soil depth in young forests than

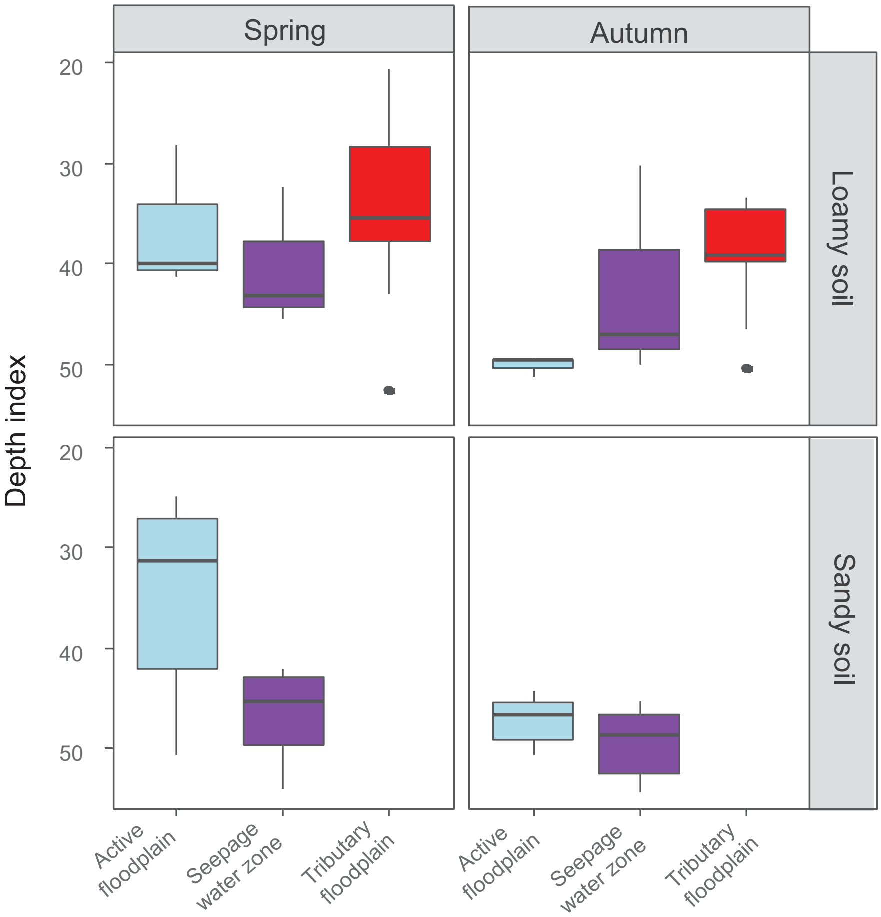


Figure 3. Depth index after Gongalsky et al. (2004) for hydrological situations according to season and soil texture; all investigated tributary sites were of loamy soil texture, therefore no value for sandy tributaries. Note the inverted y-axis for better imaginability of feeding depth.

in grasslands (p = 0.04), while average soil depth of the activities in old dense and old sparse forests were between these values (Table 3B, Fig. 5). Soil texture affected Depth Index, resulting in maximum average soil-fauna feeding activity at greater soil depths in loamy compared to sandy soils. The effect of forest development on the Depth Index was modified by season and soil type. While in spring the mean Depth Index was lower in grasslands than old dense and young forests (but not old sparse forests), no difference was found in autumn. While no significant difference was found between forest development stages on loamy soils, the mean Depth Index was significantly lower in grasslands compared to young, old dense and old sparse forests on sandy soils (p = 0.03).

Discussion

General effects

The feeding-activities observed here for the most part conform to other studies, confirming the suitability of the Bait-Lamina test in the current study, although adjustment of exposure time would have been necessary during autumn 2019 (see below). Soil-fauna feeding activity significantly decreased with soil depth and increased from spring to autumn in both gradients, similar to findings of

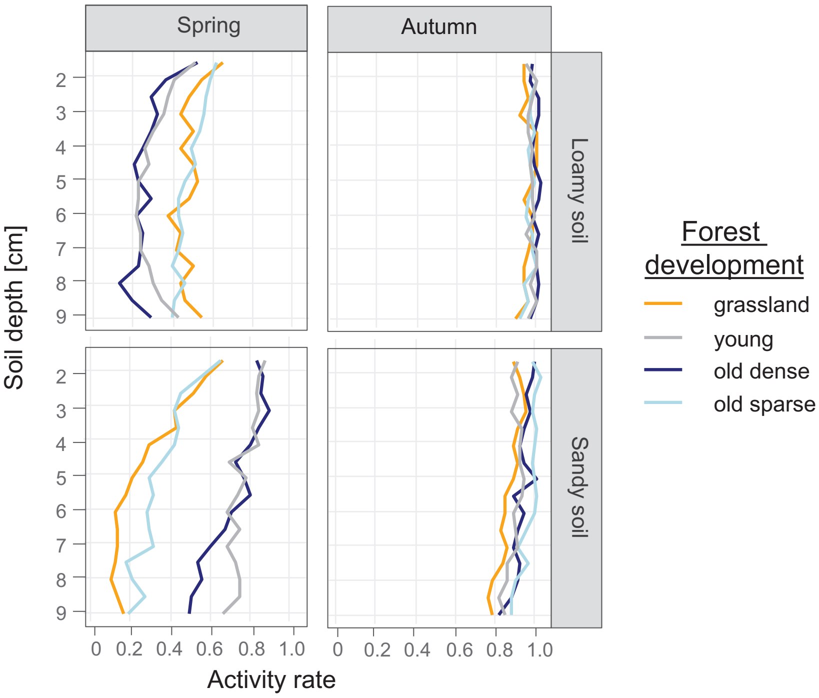


Figure 4. Feeding activities in different forest development stages separated by season and soil texture.

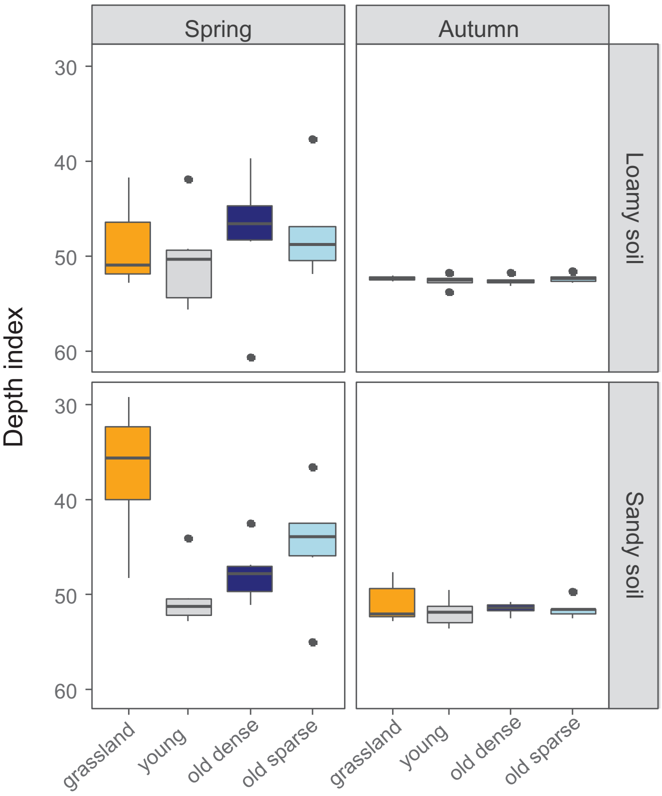


Figure 5. Depth index after Gongalsky et al. (2004) for different forest development stages according to season and soil texture; Note the inverted y-axis for better visualisation of feeding depth.

earlier studies (e.g., Geissen et al. 2007; Rozen et al. 2010). This was expected, since decreasing soil fauna density with soil depth results in lower measured activity in deeper soil (Birkhofer et al. 2011). Soil fauna feeding activity has also been observed to increase with temperature (Gongalsky et al. 2004; Rozen et al. 2010; Simpson et al. 2012; Klimek et al. 2015), suggesting potential higher activity in early autumn and at sun-exposed soil surfaces.

Nonetheless, in the forest development gradient, feeding activities in autumn were close to 100% in all forest and grassland sites (see Fig. 4), indicating that the ISO-recommended exposition time of 4 weeks during autumn was too long to reveal differences in feeding activity. In the hydrological gradient, on the other hand, four weeks exposition was appropriate in early autumn, since the measured activities remained below 90%, suggesting that the slightly lower temperatures in this sampling year reduced overall activity (Suppl. material 1 for comparison of soil temperatures). Interestingly, soil fauna density and species richness did not significantly influence observed feeding activities, confirming previous reports that such community parameters cannot serve as proxies for feeding activity (see Introduction).

Hydrological regime

Besides frequency of inundation, the hydrological regime was strongly associated with further environmental factors, separating (1) forests of low leaf-area index, i.e. sparse canopy cover, on soils of neutral pH in the active floodplain from (2) very dense forests on strongly acidic soils in the tributary floodplains and (3) forests in the seepage water zone with intermediate canopy cover and soil pH. The factor "hydrological regime" therefore represented a number of environmental factors and was expected to significantly affect soil fauna feeding activity as well. However, a general overall effect was not confirmed and no significant differences in average feeding activity were found between hydrological regimes. Soil fauna feeding activity was characterized by high variability between different sites of the same hydrological regime, indicating that further factors besides general inundation type (and associated environmental conditions) affected soil fauna activities in our study. In addition, since no severe flooding event in the Elbe floodplain had occurred for 6 years prior to our study, we suggest that no effect of direct inundation persisted in the active floodplain sites, which would be in line with findings by Russell (2008) showing changes in the soil fauna community structure persisting only for several months after an inundation event.

Although overall feeding activity of soil fauna did not differ between hydrological regimes, the decrease in activity with soil depth was significantly stronger in tributary sites than sites of the seepage water zone or active floodplain, in particular in autumn. This indicates that soil-fauna activity was prevented deeper in the soils of the tributary sites, but not in the other hydrological regimes (cf. Rozen et al. 2010). High ground-water levels and resulting anoxia in deeper soil layers are a plausible explanation for restricted faunal vertical movement – and thus their low activity – in these sites. It is remarkable that anoxia by high ground water level had occurred mainly in the floodplain of tributaries, but not in the active floodplain, which contradicts our expectations. We therefore assume that the stagnant water in sites behind the dyke is much more negatively affecting soil fauna feeding activities than the running (flood) water in the active floodplain. In addition, anoxic conditions reduce mobility of mobile nutrients and increase toxicity of aluminium (Geissen and Brümmer 1999; Geissen et al. 2007), thereby potentially reducing soil fauna density and activity. In our study, hydromorphic soil characteristics indicated that anoxic conditions – due to a high groundwater table – occurred at rather shallow soil depths in sites of tributaries as well as seepage water zone (at 35 and 40 cm, respectively, compared to > 100 cm in the active floodplain soils; Vásconez Navas et al. 2023). Surprisingly, a reduction of soil fauna activity in deeper soil only occurred in tributary floodplain sites but not the seepage water zone, indicating at least temporal differences in soil water saturation despite occasional anoxic conditions in both hydrological regimes. All investigated tributaries are regulated for agricultural purposes (pers. comm. A. Gröngröft). We therefore assumed a complete independence of water levels between the Elbe River and its tributaries with anoxic conditions by high groundwater table probably persisting throughout the summer in the tributary floodplain soils. In sites of the active floodplain and seepage water zone, on the contrary, low water levels of the Elbe River in summer resulted in lower groundwater tables, allowing soil fauna to migrate deeper. Consequently, our results indicate a higher impact of recent hydrological effects (i.e. high groundwater tables) than of long-term inundation effects.

Soil texture alone apparently did not affect soil fauna feeding activity in our study, but the absence of sandy tributary sites hampered a comparison of soil-texture effects between all hydrological regimes. In the seepage water zone higher soil fauna activities were observed in sandy compared to loamy soils. This indicates better abiotic conditions for the soil fauna in sandy soils in the seepage water zone with probably better aeration and warming of the soil surface, in spite of the lower water holding capacity and therefore lower mean soil moisture of sandy soils (Bardgett 2005). However, this effect was restricted to the seepage water zone, since activity rates were similar in sandy and loamy soils in sites of the active floodplain, suggesting that in the active floodplain different parameters might affect soil fauna activity. Furthermore, when comparing activity rates in sites of the seepage water zone to those of the active floodplain, the positive effect of sandy soil becomes only visible during spring. During early autumn, on the contrary, activity rates were similar in the active floodplain and sandy seepage water zone sites, but there was a negative effect of loamy soil in sites of the seepage water zone. Our sampling campaigns took place in years of extreme drought in Europe, leading to very dry soils in the investigated sites. In particular, in the seepage water zone sites with loamy soil, very low soil water content was observed, and in addition loamy soils became very dry and hard during summer. Therefore, the positive impact of increasing temperature on soil fauna during early autumn probably was limited by a decrease in soil moisture, as soil fauna is known to avoid dry conditions by retreating to deeper soil horizons or inactive stages (Gongalsky et al. 2008; Thakur et al. 2018). In conclusion, abiotic soil conditions seemed to affect soil fauna activity in particular in the seepage water zone, indicating a limitation of soil fauna feeding activity by soil temperature in spring, but by soil moisture in autumn.

Forest development gradient

Since in the present study all sites of the forest development gradient were located in the active floodplain, they represented a habitat diversification in this specific hydrological regime. However, environmental and vegetation parameters varied mainly between grassland sites on the one hand and forest sites on the other hand.

This was expected to be reflected in the observed soil fauna activity. Earlier studies did not find differences in soil fauna feeding activity between central European coniferous and deciduous forests of different ages (Geissen et al. 2007; Klimek et al. 2015), but between grasslands and forest sites in general (Hamel et al. 2007). In our study, forest development also did not directly affect soil fauna feeding activity and we found neither a significant difference in overall feeding activity between grasslands and forests, nor between forests of different age or density. Instead, we found that forest development modulated the effect of soil depth, i.e., a steeper decrease of activity with soil depth was observed in grasslands than in young forests. Differences in soil fauna feeding activity between grasslands and young forests only occurred at > 2.5 cm soil depth, and DI values suggested that in young forests mean soil fauna activity occurred at a greater soil depth as compared to grasslands. We therefore conclude that in grasslands soil fauna activity was restricted to the soil surface by environmental factors, probably by rapidly declining soil moisture with soil depth due to intense water uptake of grass roots (pers. obs.). On the other hand, in forests (in particular young forest plantations) soil moisture was likely appropriate for soil fauna feeding activity at least to 9 cm depth.

Soil texture also did not affect soil fauna feeding activity directly in this gradient and, due to very high general feeding activities in autumn, significant differences between soil fauna activities in different forests or grassland sites were only observed in the spring sampling. In old dense and young forests, feeding activity was higher in sandy compared to loamy sites. The most probable explanation for this result is the lower exposition of the soil to sunlight in denser forests. Old dense and young forests exhibited lower soil temperatures (mean difference of ~0.5 °C) and higher moisture (mean difference of ~5%) in loamy sites compared to sandy sites, while in open habitats (sparse forests and grasslands) these differences were less pronounced. This indicates that in shady habitats, sandy soils provided better habitat conditions for soil fauna than loamy soils due to faster warming (before development of canopy cover during spring) and reduced potential anoxia, while the close canopy cover prevented desiccation of the soil surface (Thakur et al. 2018). On the contrary, in grasslands and old sparse forests the effect of soil texture was restricted to deeper soil with a marked decrease in soil fauna feeding activity in sandy but not in loamy soils. This was surprising since the higher porosity of sandy soil, resulting in higher oxygen content in deeper soil, was expected to increase soil fauna activity in deeper soil (cf. Hassink et al. 1993). Soil moisture and temperature were similar in loamy and sandy soils in open habitats, indicating that the causes of similar feeding activity rates at the soil surface, but differences in deeper soil, might not lie in exposure to sunlight. Instead, differences most probably were related to site-specific effects e.g. pH or groundwater level, that resulted in strong differences in feeding activity rates between sites of individual forest developmental stages (Suppl. material 5).

Conclusion

In the present study we found soil fauna feeding activity to be much more affected by current than average (concluded from hydromorphic soil characters) groundwater levels. This indicates that soil fauna activity rates fluctuate with time and react to variable conditions in soil moisture and/or soil temperature within short time. Further, varying conditions within the floodplain, depending mainly on vegetation cover, shape environmental conditions. The soil fauna feeding activity in floodplain hardwood forests seems to be limited by soil temperature during spring, but by soil moisture during summer with hydrological regime and forest density affecting soil fauna feeding activity only indirectly via influencing vegetation cover and therefore soil temperature and moisture.

Our study made a first attempt to assess the effects of environmental conditions on soil fauna activity in hardwood floodplain forest-restoration sites, showing that a combination of various site conditions is highly influential for faunal activity. Future, more specific studies are needed to understand the mechanisms of these effects and investigate the contribution of soil fauna to ecosystem services such as decomposition and carbon cycling in floodplain habitats, as well as to understand the detailed relationships between soil fauna density and diversity, environmental conditions, and feeding activity. Our results indicate dense forests on sandy soil, located in the active floodplain or seepage water zone, having the highest potential value for the ecosystem service of organic-matter decomposition. From a nature conservational perspective, these could be preferred sites for floodplain forest restoration in current grassland.

Acknowledgements

We thank Raphael Weniger, Sebastian Moll, Bernd Dobner and Kamil Grabczewski for help during the sampling campaigns, and Dr. Jens Oldeland for statistical consultation. Thanks also go to MediAN project partners for providing environmental data used for statistical analyses: soil environmental data was collected by Lizeth Vásconez Navas and Adrian Heger, tree inventory data by Heather Shupe, all from Hamburg University. Botanical environmental data was collected by Timo Hartmann from Helmholtz Centre for Environmental Sciences in Leipzig.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This study was part of the MediAN project, funded by the German Federal Ministry for Education and Research, funding number 01LC1601A.

Author contributions

Conceptualization: DJR. Data curation: NS. Formal analysis: NS. Funding acquisition: DJR. Investigation: NS. Methodology: NS, DJR. Project administration: NS, DJR. Resources: DJR. Supervision: DJR. Validation: NS. Visualization: NS. Writing – original draft: NS. Writing – review and editing: NS, DJR.

Author ORCIDs

Nicole Scheunemann https://orcid.org/0000-0003-1845-6236
David J. Russell https://orcid.org/0000-0002-0129-0375

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

References

Adhikari K, Hartemink AE (2016) Linking soils to ecosystem services – A global review. Geoderma 262: 101–111. https://doi.org/10.1016/j.geoderma.2015.08.009

Bardgett RD (2005) The Biology of Soil: a community and ecosystem approach (1st edn.). Oxford University Press, Oxford.

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software 67(1): 1–48. https://doi.org/10.18637/jss.v067.i01

Birkhofer K, Diekötter T, Boch S, Fischer M, Müller J, Socher S, Wolters V (2011) Soil fauna feeding activity in temperate grassland soils increases with legume and grass species richness. Soil Biology & Biochemistry 43(10): 2200–2207. https://doi.org/10.1016/j.soilbio.2011.07.008

Comerford NB, Franzluebbers AJ, Stromberger ME, Morris L, Markewitz D, Moore R (2013) Assessment and evaluation of soil ecosystem services. Soil Horizons 54: 0. https://doi.org/10.2136/sh12-10-0028

Crowther TW, Boddy L, Jones TH (2011) Outcomes of fungal interactions are determined by soil invertebrate grazers. Ecology Letters 14(11): 1134–1142. https://doi.org/10.1111/j.1461-0248.2011.01682.x

Damm C (2013) Ecological restoration and dike relocation on the river Elbe, Germany. Scientific Annals of the Danube Delta Institute 19: 79–86. http://www.ddni.ro/index.php?page_id=194&siteSection=6&sectionTitle=ScientificAnnals

Filser J, Faber JH, Tiunov AV, Brussaard L, Frouz J, De Deyn G, Uvarov AV, Berg MP, Lavelle P, Loreau M, Wall DH, Querner P, Eijsackers H, Jiménez JJ (2016) Soil fauna: Key to new carbon models. Soil (Göttingen) 2(4): 565–582. https://doi.org/10.5194/soil-2-565-2016

Fox J, Weisberg S (2019) An R Companion to Applied Regression (3rd edn). https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Frouz J (2018) Effects of soil macro- and mesofauna on litter decomposition and soil organic matter stabilization. Geoderma 332: 161–172. https://doi.org/10.1016/j.geoderma.2017.08.039

Geissen V, Brümmer GW (1999) Decomposition rates and feeding activities of soil fauna in deciduous forest soils in relation to soil chemical parameters following liming and fertilization. Biology and Fertility of Soils 29(4): 335–342. https://doi.org/10.1007/s003740050562

Geissen V, Gehrmann J, Genssler L (2007) Relationships between soil properties and feeding activity of soil fauna in acid forest soils. Journal of Plant Nutrition and Soil Science 170(5): 632–639. https://doi.org/10.1002/jpln.200625050

Giese LAB, Aust WM, Kolka RK, Trettin CC (2003) Biomass and caron pools of disturbed riparian forests. Forest Ecology and Management 180(1–3): 493–508. https://doi.org/10.1016/S0378-1127(02)00644-8

Glaeser J, Wulf M (2009) Effects of water regimes and habitat continuity on the plant species composition of floodplain forests. Journal of Vegetation Science 20(1): 37–48. https://doi.org/10.1111/j.1654-1103.2009.05282.x

Gongalsky KB, Pokarzhevskii AD, Filimonova ZV, Savin FA (2004) Stratification and dynamics of bait-lamina perforation in three forest soils along a north-south gradient in Russia. Applied Soil Ecology 25(2): 111–122. https://doi.org/10.1016/j.apsoil.2003.09.001

Gongalsky KB, Persson T, Pokarzhevskii AD (2008) Effects of soil temperature and moisture on the feeding activity of soil animals as determined by the bait-lamina test. Applied Soil Ecology 39(1): 84–90. https://doi.org/10.1016/j.apsoil.2007.11.007

Hamel C, Schellenberg MP, Hanson K, Wang H (2007) Evaluation of the "bait-lamina test" to assess soil microfauna feeding activity in mixed grassland. Applied Soil Ecology 36(2–3): 199–204. https://doi.org/10.1016/j.apsoil.2007.02.004

Hassink J, Bouwman LA, Zwart KB, Bloem J, Brussaard L (1993) 57 Soil Structure/Soil Biota Interrelationships Relationships between soil texture, physical protection of organic matter, soil biota, and C and N mineralization in grassland soils. In: Brussaard L, Kooistra MJ (Eds) Soil Structure/Soil Biota Interrelationships, Elsevier B.V., 105–128. https://doi.org/10.1016/B978-0-444-81490-6.50059-5

Heger A, Becker JN, Vasconez Navas LK, Eschenbach A (2021) Factors controlling soil organic carbon stocks in hardwood floodplain forests of the lower middle Elbe River. Geoderma 404: e115389. https://doi.org/10.1016/j.geoderma.2021.115389

Helling B, Pfeiff G, Larink O (1998) A comparison of feeding activity of collembolan and enchytraeid in laboratory studies using the bait-lamina test. Applied Soil Ecology 7(3): 207–212. https://doi.org/10.1016/S0929-1393(97)00065-6

Irmler U (1998) Spatial heterogeneity of biotic activity in the soil of a beech wood and consequences for the application of the bait-lamina-test. Pedobiologia 24: 102–108.

ISO 18311 I (2018) Soil quality – Method for thesting effects of soil contaminants on the feeding activity of soil dwelling organisms – Bait-lamiina test (ISO 18311:2016). https://doi.org/10.31030/2825144

Kiss M, Cseh V, Tanacs E (2015) Carbon sequestration of different types of floodplain forssts in the Maros river valley (Hungary). In: Luc M, Somorowska U, Szmańda J (Eds) Landscape Analysis and Planning. Springer Geography. Springer, Cham, 159–171. https://doi.org/10.1007/978-3-319-13527-4_9

Klimek B, Niklińska M, Jaźwa M, Chodak M, Tarasek A (2015) Application of the bait-lamina method to measure the feeding activity of soil fauna in temperate forests. Polish Journal of Ecology 63(3): 414–423. https://doi.org/10.3161/15052249 PJE2015.63.3.011

Koenzen U (2009) Auenzustandsbericht – Flussauen in Deutschland. Bundesamt für Naturschutz, Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit, Berlin.

Koenzen U (2021) Auenzustandsbericht 2021, Flussauen in Deutschland. Bundesamt für Naturschutz, Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit, Berlin. https://doi.org/10.19217/brs211

Kratz W (1998) The bait-lamina test: General aspects, applications and perspectives. Environmental Science and Pollution Research International 5(2): 94–96. https://doi.org/10.1007/BF02986394

Krishna MP, Mohan M (2017) Litter decomposition in forest ecosystems: A review. Energy, Ecology & Environment 2(4): 236–249. https://doi.org/10.1007/s40974-017-0064-9

Lenth RV (2022) emmeans: Estimates Marginal Means; aka Least-Squares Means. https://cran.r-project.org/package=emmeans

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos PM, Stevens HH, Szoecs E, Wagner H (2020) vegan: Community Ecology Package. https://cran.r-project.org/package=vegan%0A

R Core Team (2021) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Austria.

Römbke J (2014) The feeding activity of invertebrates as a functional indicator in soil. Plant and Soil 383(1–2): 43–46. https://doi.org/10.1007/s11104-014-2195-5

Rozen A, Sobczyk Ł, Liszka K, Weiner J (2010) Soil faunal activity as measured by the bait-lamina test in monocultures of 14 tree species in the Siemianice common-garden experiment, Poland. Applied Soil Ecology 45(3): 160–167. https://doi.org/10.1016/j.apsoil.2010.03.008

Russell DJ (2008) Metacommunity responses of soil Collembola to inundation intensity in the Upper Rhine Valley. Peckiana 5: 127–143.

Russell DJ, Griegel A (2006) Influence of variable inundation regimes on soil Collembola. Pedobiologia 50(2): 165–175. https://doi.org/10.1016/j.pedobi.2006.02.002

Saccá ML, Caracciolo AB, Di Lenola M, Grenni P (2017) Ecosystem services provided by soil microorganisms. Soil Biological Communities and Ecosystem Resilience. https://doi.org/10.1007/978-3-319-63336-7

Shupe HA, Hartmann T, Scholz M, Jensen K, Ludewig K (2021) Carbon stocks of hardwood floodplain forests along the middle elbe: The influence of forest age, structure, species, and hydrological conditions. Water 13(5): e670. https://doi.org/10.3390/w13050670

Shupe HA, Jensen K, Oldeland J, Ludewig K (2022) Droughts decrease and floods increase carbon sequestration rates of *Quercus robur* in hardwood floodplain forests. Trees, Forests and People 9: e100294. https://doi.org/10.1016/j.tfp.2022.100294

Simpson JE, Slade E, Riutta T, Taylor ME (2012) Factors affecting soil fauna feeding activity in a fragmented lowland temperate deciduous woodland. PLoS ONE 7(1): e29616. https://doi.org/10.1371/journal.pone.0029616

Sulkava P, Huhta V, Laakso J (1996) Impact of soil faunal structure on decomposition and N-mineralization in relation to temperature and moisture in forest soils. Pedobiologia 40: 505–513.

Thakur MP, Reich PB, Hobbie SE, Stefanski A, Rich R, Rice KE, Eddy WC, Eisenhauer N (2018) Reduced feeding activity of soil detritivores under warmer and drier conditions. Nature Climate Change 8(1): 75–78. https://doi.org/10.1038/s41558-017-0032-6

Tockner K, Schiemer F, Baumgartner C, Kum G, Weigand E, Ward JV, Zweimu I (2002) The Danube restoration project: Species diversity patterns across connectivity gradients in the floodplain system. Regulated Rivers 15(1–3): 245–258. https://doi.org/10.1002/(SICI)1099-1646(199901/06)15:1/3<245::AID-RRR540>3.0.CO;2-G

Tordoff GM, Chamberlain PM, Crowther TW, Black HIJ, Jones TH, Stott A, Boddy L (2011) Invertebrate grazing affects nitrogen partitioning in the saprotrophic fungus *Phanerochaete velutina*. Soil Biology & Biochemistry 43(11): 2338–2346. https://doi.org/10.1016/j.soilbio.2011.07.005

van Gestel CAM, Koolhaas JE, Hamers T, van Hoppe M, van Roovert M, Korsman C, Reinecke SA (2009) Effects of metal pollution on earthworm communities in a contaminated floodplain area: Linking biomarker, community and functional responses. Environmental Pollution 157(3): 895–903. https://doi.org/10.1016/j.envpol.2008.11.002

Vásconez Navas LK, Becker JN, Heger A, Gröngröft A, Eschenbach A (2023) Are active and former floodplain soils of the lower middle Elbe similar? A study of soil characteristics and possible implications for forest restoration. Catena 222: e106814. https://doi.org/10.1016/j.catena.2022.106814

von Törne E (1990) Assessing feeding activity of soil-living animals. I. Bait-lamina-tests. Pedobiologia 34: 89–101.

Walling DE, Fang D, Nicholas AP, Sweet RJ (2006) River flood plains as carbon sinks. Sediment Dynamics and the Hydromorphology of Fluvial Systems, 460–470.

Ward JV, Tockner K, Schiemer F (1999) Biodiversity of floodplain river ecosystems: Ecotones and connectivity. Regulated Rivers 15(1–3): 125–139. https://doi.org/10.1002/(SICI)1099-1646(199901/06)15:1/3<125::AID-RRR523>3.0.CO;2-E

Weber A, Hatz M (2020) Hyd1d – algorithms to compute 1D water levels along german federal waterways Elbe and Rhine. Journal of Ecohydraulics 1.

Weber A, Rosenzweig S (2020) Hydflood – algorithms to compute flood extent and duration along German federal waterways Elbe and Rhine. Journal of Ecohydraulics 1: 1–1.

Wei T, Simko V (2021) R package "corrplot": Visualisation of a correlation matrix. https://github.com/taiyun/corrplot

Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York. https://doi.org/10.1007/978-3-319-24277-4

Zhang W, Yuan S, Hu N, Lou Y, Wang S (2015) Predicting soil fauna effect on plant litter decomposition by using boosted regression trees. Soil Biology & Biochemistry 82: 81–86. https://doi.org/10.1016/j.soilbio.2014.12.016

Supplementary material 1

Soil parameters of all study sites

Author: Nicole Scheunemann

Data type: pdf

Explanation note: Sampling sites in different hydrological regimes differed in the number of flooding days per year: sites in the active floodplain had been flooded at an average of 5.2 days per year between 1990 and 2016, while sites in the seepage water zone and floodplain of tributaries had not been flooded by the Elbe. The exception was one site in the seepage water zone that had been flooded at a single dyke breach during a historic flood event in 2002. All sites of the seepage water zone had been exposed to rising ground water tables depending on the Elbe water gauge, while groundwater tables in the floodplain of tributaries were in dependent of the Elbe flood regime (but were exposed to that of the respective small tributary). Sites differed mainly by leaf area index, pH and sand content, i.e. forests in the active floodplain forests were sparse and soils were of neutral pH and medium sand content, while forests in the seepage water zone were more dense and soils had more acidic pH and high sand content. Forest in the floodplain of tributaries were most dense and showed low pH and comparably low sand content. Soil moisture was lowest in the active floodplain, but the decrease of soil moisture from spring to autumn was most pronounced in tributary sites. Within the active floodplain, sites sampled in the forest development gradient mainly differed by forest age (156.8 years in old dense forests, 130.8 years in old sparse forest, 22.3 years in young forests and 0 in grasslands). In most aspects, the three developmental stages with growing trees were rather similar in their environmental factors, e.g. number of plant species and cover of vegetation layers, litter cover, pH, sand content of soil, mean soil temperature, etc. However, they differed in leaf area index with old dense and young forests being more shadowed (high leaf area index) than old sparse forests. Surprisingly, grasslands had an average higher number of flooding days per year (49 in grasslands as compared to a maximum of 28 in forests). As expected, vegetation parameters differed between grasslands and forests, but most other environmental variables were similar to those in forests.

Link: https://doi.org/10.3897/natureconservation.53.106260.suppl1

Supplementary material 2

PCA plot of sites and environmental parameters of the hydrology gradient

Author: Nicole Scheunemann

Data type: pdf

Explanation note: PCA of ecological site parameters for hydrological regimes sampling 2020; Gray triangles indicate factor coordinates of closest environmental variable. Site IDs in bold with frame with color indicating hydrological situation: turquoise = active floodplain, purple = seepage water zone, red = tributary floodplain.

Link: https://doi.org/10.3897/natureconservation.53.106260.suppl2

Supplementary material 3

PCA of ecological site parameters of all sites in the forest development gradient

Author: Nicole Scheunemann

Data type: pdf

Explanation note: PCA of ecological site parameters of forest development sampling 2019; gray triangles indicate factor coordinates of closest environmental variable. Site IDs in bold with frame, with color indicating forest development stage: dark blue = old dense forest, light blue = old sparse forest, gray = young forest plantation, orange = grassland.

Link: https://doi.org/10.3897/natureconservation.53.106260.suppl3

Supplementary material 4

Soil fauna feeding activity of single sites in hydrology gradient

Author: Nicole Scheunemann

Data type: pdf

Explanation note: Soil fauna feeding activity of single sites (means of three replicates) of hydrological situations in spring (above) and autumn (below) 2020; line types represent site ID, legend is valid for spring as well as autumn sampling.

Link: https://doi.org/10.3897/natureconservation.53.106260.suppl4

Supplementary material 5

Soil fauna feeding activity of single sites in forest development gradient

Author: Nicole Scheunemann

Data type: pdf

Explanation note: Soil fauna feeding activity of single sites (means of three replicates) of forest development stages in spring (above) and autumn (below) 2019; Line types represent site ID, legend is valid for spring as well as autumn sampling.

Link: https://doi.org/10.3897/natureconservation.53.106260.suppl5

Nature Conservation 53: 279–295 (2023)
DOI: 10.3897/natureconservation.53.104135

Research Article

Analysis of the effects of habitat characteristics, human disturbance and prey on felids presence using long-term community monitoring information

J. Roberto Sosa-López[1*], Nydia Nicté Díaz Bernal[1], Eugenio Padilla[1,2], Miguel Briones-Salas[1*]

1 *Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Oaxaca (CIIDIR, Oaxaca), Instituto Politécnico Nacional, México, Hornos 1003, Col. Nochebuena, Santa Cruz Xoxocotlán, Oaxaca, 71230, México*
2 *Comisión Nacional de Áreas Naturales Protegidas (CONANP), Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT), Oaxaca, México*
Corresponding author: Miguel Briones-Salas (mbriones@ipn.mx)

OPEN ACCESS

Academic editor: Cássio Cardoso Pereira
Received: 27 March 2023
Accepted: 28 September 2023
Published: 17 October 2023

ZooBank: https://zoobank.org/5EF6E1BB-8EDB-4DA1-87DA-49CB44D98927

Citation: Sosa-López JR, Díaz Bernal NN, Padilla E, Briones-Salas M (2023) Analysis of the effects of habitat characteristics, human disturbance and prey on felids presence using long-term community monitoring information. Nature Conservation 53: 279–295. https://doi.org/10.3897/natureconservation.53.104135

Abstract

Predator species are essential for ecosystems as they maintain the ecological integrity of the habitat. Particularly, felids populations have declined globally due to their sensitivity to habitat disturbances. Nevertheless, in Mexico, there are areas protected by indigenous communities to preserve a portion of their territory, benefiting multiple species, including felids. Although the National Commission of Natural Protected Areas of Mexico sponsors a long-term national-wide communal monitoring programme using camera traps, there is not a systematic analysis of the information generated by the programme. We assessed the occurrence of three felids species known to occur in a Zapotec indigenous community conservation area in Oaxaca, Mexico. Specifically, we evaluated how habitat characteristics, human disturbance and prey influence felids' occurrence across the protected area. None of the variables explained better than the null model the proportion of sites used by Pumas (*Puma concolor*). Bobcats and Margays favour areas with medium-sized prey. Our study shows the importance of community-based monitoring and information systems (CBMIS) for identifying communal reserve characteristics that contribute to the occupation of carnivores. Further, our results also suggest that management should consider the habitat requirements of felids´ prey. By understanding wildlife habitat use, communal authorities could improve sustainable forest management within the reserves.

Key words: biodiversity, CBMIS, conservation, co-occurrence, GLMM, Oaxaca

Introduction

The combination of natural resources and environmental conditions where organisms can reproduce and survive defines a species´ suitable habitat (Morrison et al. 1992). Habitat loss, through anthropogenic activities, affects the reproduction and survival of some species, impacting the diversity of ecosystems. The worldwide vertebrate populations declined by one-third between 1970 and 2006, the Tropics being the most affected ecosystem (CBD 2020). Estimations suggest that 24% of all mammals at extinction risk are mainly due

---

[*] Contributed equally as the first author.

to modifying habitats caused by human activities (PBD 2001). Within mammals, felids are indicators of the conservation status of an ecosystem (Boitani 2001). Most species of felids are susceptible to alterations in their habitat, particularly to changes triggered by anthropogenic activities (Cramer and Portier 2001; Ordiz et al. 2021), for example, roads (Basille et al. 2013) and habitat loss and fragmentation (Zanin et al. 2015). Thus, by protecting felids, other species habiting the same areas would benefit, maintaining the ecological integrity of these areas (Scognamillo et al. 2003; Ripple et al. 2014).

In Mexico, there are six species of felids (Puma, *Puma concolor*; Jaguarundi, *Herpailurus yagouaroundi*; Bobcat, *Lynx rufus*; Ocelot, *Leopardus pardalis*; Jaguar, *Panthera onca*; and Margays, *Leopardus wiedii*). Particularly, Pumas, Bobcats and Margays have been little studied. Studies of habitat use and abundance in pumas stand out (Lira and Naranjo 2003; Estrada 2008; Monroy et al. 2009; Rodríguez-Soto et al. 2013; Prude and Cain 2021), in Bobcats (Monroy-Vilchis and Velázquez 2002; Burton et al. 2003; Elizalde et al. 2012; Espinosa-Flores and López-González 2017; Flores-Morales et al. 2019; Lavariega et al. 2022) and in Margays (Carvajal et al. 2012; Pérez-Irineo et al. 2017). All six species of felids that live in Mexico have been recorded in areas protected by indigenous communities, mainly in the Mexican tropics (Briones-Salas et al. 2016).

Since 2008, the Mexican government officially recognised some of these areas through a protection scheme called Voluntary Destined Areas for Conservation (ADVC by its Spanish acronym). The ADVCs must be designated voluntarily by the communities or private owners to get institutional recognition (Elizondo and López-Merlín 2009). This scheme allows indigenous and peasant communities to get involved in ecosystem conservation programmes and the sustainable use of their natural resources. These spaces are managed by the inhabitants through social consensus, which establishes rules of use, including restrictions on hunting, looting plant, species and removing plant cover for agricultural and livestock activities (Anta-Fonseca & Mondragón-Galicia 2006). ADVCs cover variable extensions of forests and aim to protect the most fragile natural environments.

Within the ADVCs, one essential activity is wildlife monitoring, which is carried out by locals called “monitores comunitarios” (community monitors). Some authors have questioned this activity for not following a systematised monitoring scheme or lacking analysis of the information generated (e.g. Burton (2012); Méndez-López et al. (2015)). However, there are examples of success in biodiversity monitoring and conservation activities in indigenous conservation areas, either directly or with the support of other actors, such as technicians, academics and government institutions (e.g. DeCaro and Stokes (2008); Lavariega et al. (2020)). Most of these achievements are mainly due to the active participation of community monitors (Méndez-López et al. 2015).

The National Commission of Natural Protected Areas of Mexico (CONANP by its Spanish acronym) sponsors a national-wide community monitoring programme to increase the knowledge of biodiversity in various country regions by fomenting the use of camera traps to detect wildlife. In Oaxaca, southern Mexico, this programme has been used successfully to assess the status of Jaguar populations (Lavariega et al. 2020), the herpetofauna (Simón-Salvador et al. 2021) and the study of birds in the Chinantla Region (Noria-Sánchez et al. 2015). From the governmental level, there is no difference in the management regime between community monitoring in the ADVCs and monitoring in the ANPs. The monitoring that is carried out in the ANPs of Oaxaca of the Sierra Juárez Mixteca direction (including the ADVC that attends) is carried out with the training, economic support, equipment and accompaniment of CONANP technicians. The results are discussed with the community authorities and sometimes in assemblies. For instance, during the last 10 years, communal monitors at the Voluntary Conservation Area La Cruz-Corral de Piedra in San Pablo Etla have been using camera traps to understand habitat use in three felid species found in the reserve (i.e. Pumas, Bobcats and Margays). However, despite a great deal of information generated by the community monitoring programme, there is no systematic approach to analyse the data. Furthermore, due to the limited number of studies carried out in Mexico and Latin America, little is known about habitat use, prey and conflicts with humans in Pumas, Bobcats and Margay.

Here, we analysed the information generated by a long-term wildlife monitoring programme at an ADVC protected by a Zapotec indigenous community to understand habitat use in three felids species (Pumas, Bobcats and Margays). The objective of this study was to evaluate the effect of habitat characteristics, human disturbance and the presence of prey which influence the occurrence of felines in the protected area. We used GLMM models to assess the association between landscape variables related to habitat characteristics, human disturbances and the presence of prey on felids occurrence within the protected area. We hypothesised that felids' occurrence would be explained by habitat characteristics and prey presence (e.g. Nowell and Jackson (1996); Laundré and Hernández (2010)) and limited by pressures related to human settlements or roads (e.g. Angelieri et al. (2016); Horn et al. (2020); Mayer et al. (2022)). We anticipate that the acquired results will be instrumental in aiding decision-making processes by indigenous communities concerning the preservation of the biodiversity they safeguard.

Materials and methods

Study area and community monitors

We carried out the study at the Voluntary Conservation Area La Cruz-Corral de Piedra in San Pablo Etla, Oaxaca, Mexico, in a Zapotec ethnic group (17°07' and 17°12'N, 96°39' and 96°48'W; Fig. 1). The reserve is in a mountainous area of the Sierra Madre de Oaxaca, its area is 23.35 km^2, its height ranges from 1,500 to 3,300 m a.s.l., it has an average annual temperature of 17.8 °C and an average annual rainfall of 1022 mm. The dominant vegetation types are pine forest: 5.5 km^2, pine-oak forest: 15.56 km^2 and oak forest: 2.22 km^2 (Ojeda-Lavariega et al. 2019). CONANP technicians trained 12 community members in three workshops in 2013, 2016 and 2019. During the workshops, the technicians and communal monitors established the objectives and designed the monitoring programme. The community monitors have been installing the trap cameras periodically with the help of CONANP technicians since 2013.

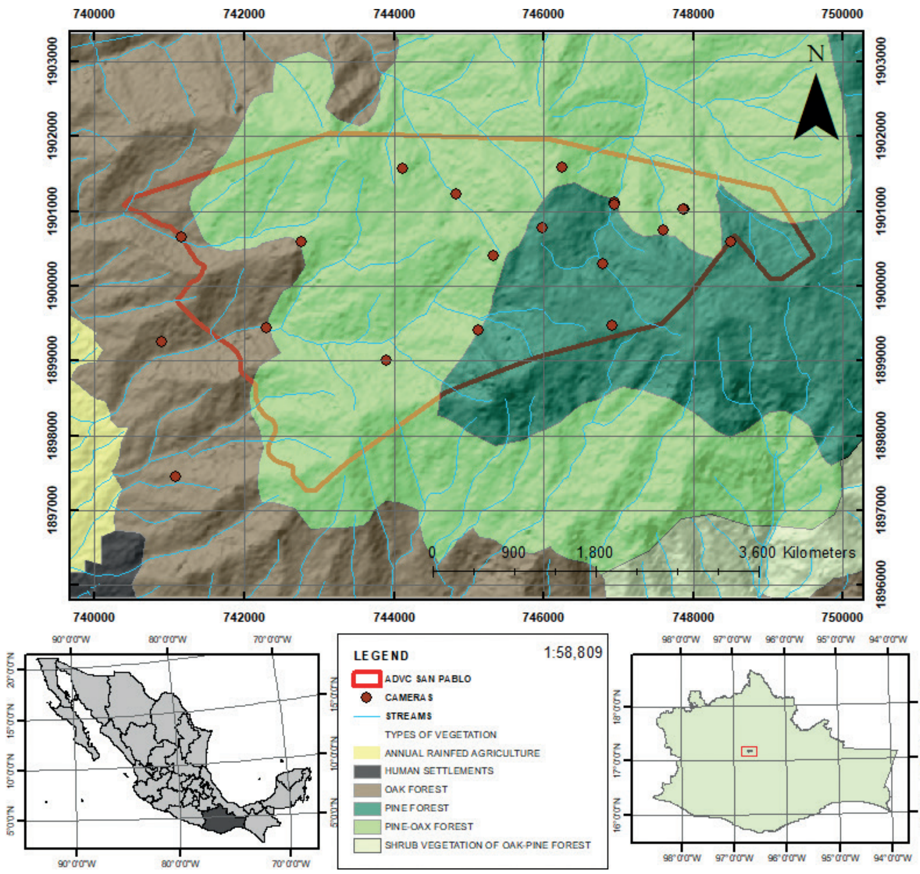


Figure 1. The geographical location of the Voluntary Conservation Areas "La Cruz-Corral de Piedra" in the Northern Sierra of Oaxaca, Mexico (red line). Circles and triangles show camera traps installed by community monitors and during our study, respectively. Dark green indicates vegetation dominated by pine, light green by pine-oak and light brown by oak.

Sampling sites selection

The sampling was carried out from January 2013 to June 2017 using 23 camera traps (12 Bushnell Trophy Cam and X8; nine Cuddeback Expert and Capture; two Simmons 119234C). We generated a grid spanning all the ADVC polygons using the ArcGIS programme (v.10.3). We followed previous studies with medium-sized felines to establish the sizes of the cells and generated 26 cells of 1.5 x 1.5 km (Burton et al. 2003; Lira and Briones 2012; Pérez-Irineo et al 2017). Community monitors installed 15 camera traps in the centre of 15 quadrants mainly east of the ADVC between January 2013 and June 2017 (Suppl. material 1: table S1). We installed eight additional camera traps in the centre of the unsampled cells between June 2016 and March 2017 to extend the sampling in most of the ADVC territory. The 23 camera traps were installed on natural trails, ravines, dry streams and riverbanks, at a height between 40 and 50 cm from the ground and spaced from each other at 1.5 km so as not to leave larger areas unsampled and assuming that this distance is large enough to achieve statistical independence between the trapping stations. The circuit of these cameras was programmed to remain active for 24 hours. The position of each one of them was georeferenced with a geopositioned Garmin etrex model.

Monitoring programme design

The monitoring programme established by the community allows us to have monthly information regarding the presence/absence of multiple species at each sampling site over time. However, the monitoring programme was not regular for all sampling sites, with maximum sampling periods that ranged from two to 54 months (Suppl. material 1: table S1). The cameras were programmed to remain active 24 hours a day with a 30-second separation between each shot and were checked monthly to change batteries and download the information. The following cases were considered as independent photographic records: a) consecutive photographs of different individuals and b) consecutive photographs of the same species separated by 24 hours (this criterion was applied when it was not clear if a series of photographs corresponded to the same individual, so photographs taken before 24 hours were considered as a single record) (Maffei et al. 2002; Lira et al. 2014). We reviewed all photographs identifying all mammal and bird species with the help of field guides. We elaborated tables with the records of the three species of felids studied. The community monitoring sampling effort was 16,200 days, which, according to Shannon et al. (2014), is higher than the minimum required in a monitoring programme.

Habitat variables

We gathered 11 habitat variables associated with each sampling site, following Mostacedo and Fredericksen (2000) and Mueller-Dombois and Ellenberg (2002). We used the point-centred quarter method for the vegetation traits, which involves establishing four 25-metre transects at each of the four cardinal points, taking the location of the camera trap as the central point. We marked five points in each transect, separated by 5 m, for a total of 20 points and each point was divided into four quadrants, encompassing 2500 square metres (following Cottam and Curtis 1956).

We assessed shrub layer density, defined as the number of plants per area with > 40 cm and < 2 m in height. Density was estimated using the correction factor proposed by Warde and Petranka (1981), as some quadrants had no shrubs layer; the basal area of the trees was obtained by assessing the diameter at breast height (DBH) at a height of 1.3 m above the ground (Mostacedo and Fredericksen 2000) of trees taller than 2 m using a BEN MEADOWS diametric tape. The basal area of the trees was estimated using the following formula: basal area = pi*DBH2/4, where: pi = 3.141592 and DBH = diameter at breast height.

We also calculated the importance value (IV) of the genus *Quercus*, the genus *Pinus* and other tree species (we pooled together plants of the genus: *Litesea*, *Arbutus*, *Abies*, *Alnus* and *Buddleja*), as these species were found mostly concentrated in sites, such as glens and creeks, following Cottam and Curtis (1956). We calculated IV as the sum of the relative tree density, relative tree frequency and relative tree dominance, given by the following formula:

$$\text{Importance Value (IV)} = \text{relative density} + \text{relative dominance} + \text{relative frequency}$$

We assessed seven variables associated with terrain characteristics and human presence: five continuous variables: altitude, slope (assessed with a

Clinometer SUUNTO PM-5/360PC); closest distances between the camera trap to any water body (e.g. rivers, dams or waterfalls); closest distances between the camera-trap to any main road within the Reserve (i.e. roads with a width of 7 to 10 m, where cars can circulate); and closest distances to human settlements (i.e. one or more people living in at least one building) and two binary variables: the presence of crags, defined as steep or rugged cliffs; the presence of trails, defined as narrow paths (≤ 2 m wide), formed by the passage of animals or people and located within the vegetation sampling quadrant. For the variables, closest distance to bodies of water, to the main road of the Reserve and human settlements, high-definition topographic maps of San Pablo Etla were reviewed and analysed in the ArcGis programme (version 10.3) generating the Euclidean distance between the sampling stations and the characteristics of the area evaluated.

Prey species

We identified 16 species as possible prey for the three felids. We classified all potential prey species into three categories, based on size and generated a detection history for each category. In the small-size prey category, we included: *Bassariscus astutus*, *Sylvilagus floridanus*, *Sylvilagus cunicularis*, *Conepatus leuconotus*, *Mephitis macroura*, *Sciurus aureogaster*, *Pipilo ocai*, *Cyanocitta stelleri* and *Dendrortyx macroura*. In the medium-size prey category, we included *Eira barbara*, *Nasua narica*, *Procyon lotor*, *Didelphis virginiana* and *Cuniculus paca*. In the large-size prey category, we included *Odocoileus virginianus* and *Dicotyles angulatus*.

Statistical analysis

We used generalised linear mixed models (GLMM) to assess the association between habitat and prey variables with the presence/absence for Pumas, Bobcats and Margays. We defined sampling units as station-month combinations, thus ‘stacking’ the month detection histories, achieving a larger effective sample size. The total sample size for this dataset is n = 531 sites/month combinations for each of the three felid species.

We generated GLMMs with Binomial error and complementary log-log function, as the probability of an event (presence) in our database was small and tended to fit better the data than logistic and probit. We included the presence/absence of Pumas, Bobcats or Margays as a dependent factor, while habitat variables and prey presence/absence were included as fixed factors. We included CameraID and Years as random factors. Including year as a random factor allowed us to meet the assumption of close populations at survey locations since presence/absence in one year is independent of occupancy in the other year. Models were constructed following a forward stepwise procedure, by adding one predictor variable at a time, starting with an empty model (null model) and selecting the variable that provides the best fit to the data according to the Akaike Information Criterion (AIC). We screened sets of predictor variables included in multivariable models to avoid having correlated (r > 0.6) variables together within models. We chose the most parsimonious models using the Akaike Information Criterion (ΔAIC < 2; Burnham and Anderson (2002)). As the study site was smaller than the average home ranges of the species being assessed, which violates the assumption of closure, we interpreted our results as “the proportion of sampled sites used by the species” rather than the “probability of occupancy”, following Madsen et al. (2020). Values are reported as means (± standard error). GLMM was performed in SPSS statistical analysis software (v.25 IBM Corp., Armonk, NY, USA).

Results

We recorded the three focal felids species *P. concolor*, *L. rufus* and *L. wiedii* (Fig. 2), in addition to other species of meso-predators, such as *Puma yagouaroundi*, *Canis latrans*, *Urocyon cinereoargenteus* and some species, such as potential prey: *Odocoileus virginianus*, *Pecari tajacu*, *Bassariscus astutus*, *Nasua narica*, *Didelphis virginiana*, *Sylvilagus floridanus*, *Sylvilagus cunicularis*, *Conepatus leuconotus*, *Mephitis macroura*, *Sciurus aureogaster*, *Cuniculus paca* and birds such as: *Pipilo ocai*, *Cyanocitta stelleri* and *Dendrortyx macroura* (Fig. 3).

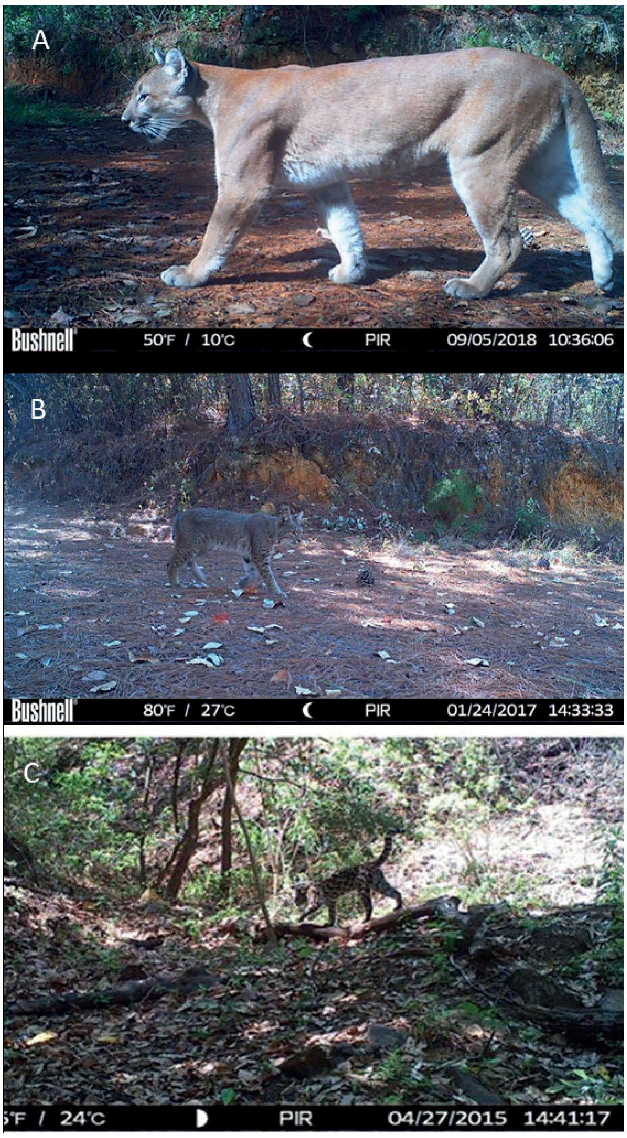


Figure 2. Images of Puma (*Puma concolor*: **A**), Bobcat (*Lynx rufus*: **B**) and Margay (*Leopardus wiedii*: **C**), obtained by camera trapping in the Voluntary Conservation Area La Cruz-Corral de Piedra in San Pablo Etla, Oaxaca, Mexico.

Figure 3. Images of feline prey: white-tailed deer (*Odocoileus virginianus*: **A**), squirrel (*Sciurus aureogaster*: **B**), collared peccary (Dicotyles angulatus: **C**) and white-nosed coati (*Nasua narica*), obtained by camera trapping in the Voluntary Conservation Area La Cruz -Corral de Piedra in San Pablo Etla, Oaxaca, Mexico.

Specifically, the Puma was detected 58 times in seven sampling stations; the Lynx was detected 29 times in five sampling stations and the Margay was detected 20 times in six sampling stations.

For the Puma, the presence was best explained by the null model and the model that included medium-size prey (F = 0.69, P = 0.40, Fig. 4A) (Table 1), indicating that Puma occurrence increased in sites with the presence of medium-size prey (Fig. 4A; Table 2). The most parsimonious model suggests that the sites used by Bobcats and Margay were better explained by the variable medium-size prey (Table 1), indicating that Bobcats and Margay occurrence increased in sites with the presence of medium-size prey F = 0.43, P = 0.51, Fig. 4B and F = 0.20, P = 0.65, Fig. 4C, respectively; Table 2). Full sets of tested GLMMs and averaged estimates of the function slopes of variables for the three felid species are presented in the Suppl. material (Suppl. material 1: table S2 for Puma, table S3 for Bobcats and table S4 for Margay).

Discussion

In the study, it was observed that the occurrence of the three felid species was high in areas where their prey species were present, indicating a potential influence of prey on the occupation patterns of these felines. None of the other variables explained the presence of felines; however, there are studies suggesting that human settlements or urbanisation have a negative effect on Pumas, Bobcats and Margays distribution (e.g. Angelieri et al. (2016); Horn et al. (2020); Mayer et al. (2022)). A possible explanation is that access to the ADVC is restricted by the community, with few visitors and motor cars all year round, resulting in a non-negative effect on the occurrence of the felid in the area.

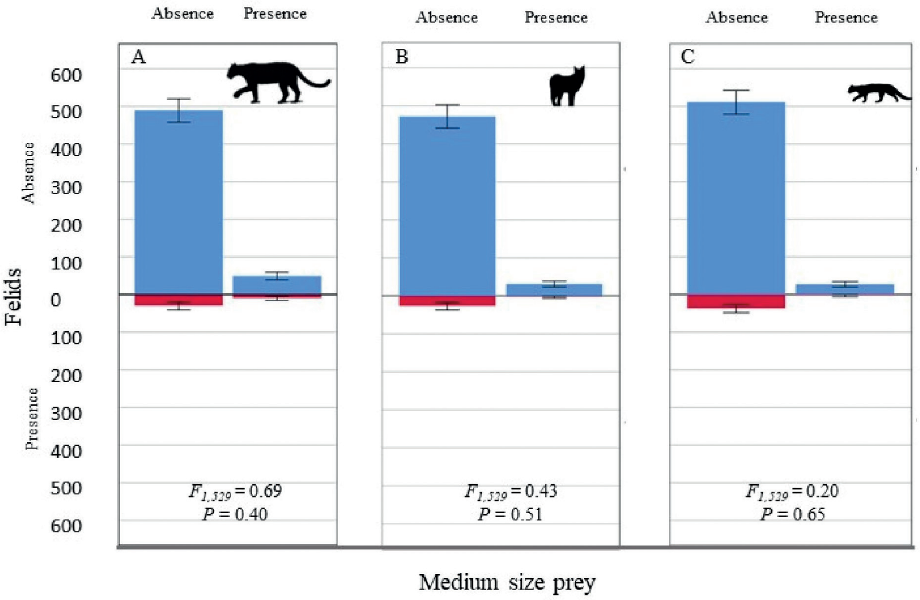


Figure 4. Graphs depicting the relationship between the presence of medium-size prey and Puma (*Puma concolor*: **A**), Bobcat (*Lynx rufus*: **B**) and Margay (*Leopardus wiedii*: **C**). The y axis indicates counts. Model with predictive accuracy by adding one predictor variable at a time, starting with an empty model (null model) and selecting the variable that provides the best fit to the data according to the Akaike Information Criterion (AIC). *P*-values evaluate the null hypothesis, not model performance. Error bars show standard error. Blue bars refer to absence and red bars refer to presence.

Table 1. Best generalised linear mixed models (GLMM) describing the proportion of sampled sites used by the three species felid species. The best five models are presented for each species.

Model	-2log likelihood	AIC	ΔAIC	AICw
Puma				
Null	1907.21	1911.23	0.00	0.51
Medium-size prey	1907.33	1911.35	0.12	0.48
Medium-size prey * Presence of trails	1921.16	1925.18	13.95	0.00
Presence of trails	1923.71	1927.73	16.50	0.00
Medium-size prey + Presence of trails	1923.89	1927.91	16.68	0.00
Bobcat				
Medium-size prey	2021.75	2025.77	0.00	0.70
Medium-size prey + Season	2024.69	2028.72	2.95	0.16
Null	2025.80	2029.82	4.05	0.09
Season	2027.92	2031.94	6.17	0.03
Medium-size prey + Presence of trails	2028.93	2032.95	7.18	0.02
Margay				
Medium-size prey	2024.41	2028.41	0.00	0.69
Medium-size prey + Season	2026.69	2030.71	2.30	0.22
Null	2094.48	2033.48	5.07	0.05
Medium-size prey + Presence of trails	2031.55	2035.57	7.16	0.02
Season	2031.79	2035.79	7.38	0.02

The Akaike Information Criterion score (AICc), the -2log, the difference between the given model and the most parsimonious model (Δ) and the Akaike weight (w) are listed.

Table 2. Averaged estimates of the function slopes of variables present in the most parsimonious GLMMs. Estimates of radon factors are shown. Standard errors (SE) and 95% confidence limits (CL) are shown. Tests of significance of variables (*F* and *P*) are also given. The asterisk (*) indicates the reference variable.

Variables	Estimate	SE	Lower 95% CL	Upper 95% CL	*F* (df1, df2)	*P*
Puma						
Null	0.24	0.19	0.82	1.66		< 0.001
Medium-size prey					0.69 (1,529)	0.40
Absence	0.21	0.255	-0.28	0.74		
Presence	0*					
CameraID	0.37	0.16	0.15	0.88		0.02
Year	0.05	0.05	0	0.42		0.34
Bobcat					0.43 (1,529)	0.51
Medium-size prey						
Absence	0.19	0.29	-0.39	0.78		
Presence	0*					
CameraID	0.25	0.11	0.10	0.62		0.02
Year	0	0.07	0	0.49		0.46
Margay						
Medium-size prey					0.20 (1,529)	0.65
Absence	0.13	0.3	-0.45	0.73		
Presence	0*					
CameraID	0.27	0.12	0.11	0.66		0.02
Year	0	0.11	0.21	0.60		0.69

Although the best model suggests that Pumas' presence was not associated with any of the variables assessed in this study, the second-best model in our study suggests that the occurrence of the Puma was related to the occurrence of medium-size prey, as has been seen in other investigations. (e.g. Aranda and Sánchez-Cordero (1996); Hass (2009); Hernández-SaintMartín et al. (2015); Prude and Cain (2021)). Unlike other studies, our results suggest no relationship with pine-oak forests even though they are ideal places to ambush their prey, with easy access to resting and refuge sites (Cox et al. 2006; Land et al. 2008; Laundré and Hernández 2010).

Regarding Margay, it is one of the least-studied cats (Brodie 2009), highlighting the importance of our study. Recognising the association between prey and the presence of Margays is valuable for residents, as it indicates that to preserve this endangered feline (SEMARNAT-NOM-059; IUCN; CITES) in their territories, they must also prioritise efforts to conserve prey species. In this study, we analysed data from camera traps capturing individuals at ground level, indicating successful observations of Margays. The Margay species have been observed to engage in both ground and tree-based-hunting behaviour, with the interesting behaviour of climbing trees to consume prey captured on the ground (Aranda 2005). Furthermore, some authors mention that Margays undertake exploratory tours on the ground when not actively foraging for food (De Oliveira 1998; Hodge 2014). High densities of shrubs have been associated with the presence of Margays, as it facilitates the presence of prey (small mammals of Order: Didelphomorphia and Rodentia, principally; Bianchi et al. (2011)) and provides shelter for Margays (Goulart et al. 2009; Hodge 2014).

Bobcat diet is mainly constituted by lagomorphs and rodents and, to a lesser extent, by opossums, coatis and birds (e.g. Aranda et al. (2002); Hass (2009)), while Margay's diet is mainly constituted by small mammals, lagomorphs, rodents, as well as birds and reptiles (e.g. Sunquist and Sunquist (2002)). Our results indicate that medium-size prey is positively associated with the presence of Bobcats and Margays, supporting the high encounter hypothesis for Bobcats, but not for Margays. Other studies have mentioned the utilisation of the habitat by bobcat is correlated to the abundance of their prey (Litvaitis et al. 1986).

We acknowledge that the study area is very small, considering all three felid species have large home ranges – Margay: 10–21 km^2 (Konecny 1989), Bobcat:11 km^2 (Monroy and Briones-Salas 2012), Puma: 83 km^2 (Nuñez-Pérez and Miller 2019), that the number of camera-traps was relatively small and that the sampling periods between cameras were not the same. Thus, the results should be taken with caution. However, this study highlights the importance of community monitoring of wildlife in protected areas by indigenous communities. In addition to incentivising local people, we believe that community wildlife monitoring is a viable alternative since it is relatively cheaper in the long run and is more effective in drawing on local experiences. Further, currently, the monitoring programme is operating on several communal reserves across Mexico; we encourage communal authorities and CONANP to conduct a global analysis of the information generated by the monitoring programme to implement a monitoring protocol that will lead to better management of forest practices national-wide.

Conclusions

This study highlights that community monitoring (in this case, indigenous monitoring) contributes to scientific knowledge. In this study, information was obtained on the local-scale habitat use of three felids; for one of them, the region represents its southernmost distribution area (*L. rufus*). Bobcats and Margays favour areas with medium-sized prey. Pumas' presence did not correlate with the assessed variables. Additionally, the high occurrence of all three felid species in areas with their prey suggests prey influence on their habitat selection. By understanding the relationship between these carnivores, habitat characteristics, human disturbances and the presence of prey, community authorities could improve sustainable forest management. Considering that many indigenous communities around the world protect their natural resources – even without official recognition – (Farhan Ferrari et al. 2015; Jurrius and López Rodríguez 2020), community conservation efforts are essential for preserving biodiversity and environmental services.

Acknowledgements

We thank the communal authorities and residents of San Pablo Etla for access to their lands. To CONANP for providing information on various species of wildlife obtained in San Pablo Etla through the programme "Biological Monitoring of Biodiversity in Protected Natural Areas, Sierra Juárez Mixteca Directorate". R.E. Galindo contributed with revisions and contributions to the document. MBS thanks the Operation and Promotion of Academic Activities Committee (COFAA) at the IPN. MBS and JRSL thanks the Research Performance Stimulus Program (EDI) at the IPN, as well as the National System of Researchers (SNI) for its recognition and support.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was supported by the National Council of Science and Technology (CONACYT) through a scholarship for Master's studies to NNDB at the Instituto Politécnico Nacional (717791/591006) and the Basic Scientific found a programme (250910 granted to JRSL); and IPN Research and Postgraduate Secretariat (SIP: 20195164 and SIP: 20200713 granted to MBS, and SIP: 20220208 and 20231274 granted to JRSL).

Author contributions

All authors have contributed equally.

Author ORCIDs

J. Roberto Sosa-López https://orcid.org/0000-0002-0120-0704
Eugenio Padilla https://orcid.org/0000-0001-7883-3166
Miguel Briones-Salas https://orcid.org/0000-0003-1413-9946

Data availability

NNDB, MBS and JRSL conceived and designed the study. NNDB and EP compiled and identified the species for generating the database. NNDB and JRSL performed the statistical analyses. All authors contributed to the interpretation of results and the writing of the manuscript.

References

Angelieri CCS, Adams-Hosking C, Ferraz KMPMB, de Souza MP, McAlpine CA (2016) Using species distribution models to predict potential landscape restoration effects on puma conservation. PLoS ONE 11(1): e014523. https://doi.org/10.1371/journal.pone.0145232

Anta-Fonseca S, Mondragón-Galicia F (2006) El Ordenamiento Territorial y los estatutos comunales: el caso de Santa Cruz Tepetotutla, Usila, Oaxaca. In: Anta S, Arreola AV, González MA, Acosta J (Eds) Ordenamiento Territorial Comunitario: un debate de la sociedad civil hacia la construcción de políticas públicas. México. Secretaría de Medio Ambiente y Recursos Naturales; Instituto Nacional de Ecología; Instituto para el Desarrollo Sustentable en Mesoamérica, AC. Grupo Autónomo para la Investigación Ambiental, A.C. Grupo de Estudios Ambientales, A.C. Methodus Consultora, S, 191–208.

Aranda M (2005) *Leopardus wiedii* (Schinz, 1821). In: Ceballos G, Oliva G (Eds) Los mamíferos silvestres de México. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, Fondo de Cultura Económica, México, 361–362.

Aranda M, Sánchez-Cordero V (1996) Prey spectra of jaguar (*Panthera onca*) and puma (*Puma concolor*) in tropical forests of Mexico. Studies on Neotropical Fauna and Environment 31: 65–67. https://doi.org/10.1076/snfe.31.2.65.13334

Aranda M, Rosas O, Ríos JJ, García N (2002) Análisis comparativo de la alimentación del gato montés (Lynx rufus) en dos diferentes ambientes de México. Acta Zoológica Mexicana 87(87): 99–109. https://doi.org/10.21829/azm.2002.87871803

Basille M, Van Moorter B, Herfindal I, Martin J, Linnell JD, Odden J, Andersen R, Gaillard JM (2013) Selecting habitat to survive: The impact of road density on survival in a large carnivore. PLoS ONE 8(7): e65493. https://doi.org/10.1371/journal.pone.0065493

Bianchi RDC, Rosa AF, Gatti A, Mendes SL (2011) Diet of margay, *Leopardus wiedii*, and jaguarundi, *Puma yagouaroundi*, (Carnivora: Felidae) in Atlantic rainforest, Brazil. Zoologia 28(1): 127–132. https://doi.org/10.1590/S1984-46702011000100018

Boitani L (2001) Carnivore introductions and invasions. In: Gittleman J, Funk SM, Macdonald DW, Wayne RK (Eds) Carnivore Conservation. Cambridge University Press, Cambridge.

Briones-Salas M, Lavariega MC, Cortés-Marcial M, Monroy-Gamboa AG, Masés-García AC (2016) Iniciativas de conservación para los mamíferos de Oaxaca. In: Briones-Salas M, Hortelano-Moncada Y, Magaña-Cota G, Sánchez-Rojas G, Sosa-Escalante JE (Eds) Riqueza y Conservación de los Mamíferos en México a nivel Estatal. Instituto de Biología, Universidad Nacional Autónoma de México, Asociación Mexicana de Mastozoología, A.C. y Universidad de Guanajuato, Ciudad de México, México.

Brodie J (2009) Is research effort allocated efficiently for conservaction? Felidae as a global case study. Biodiversity and Conservation 18(11): 2927–2939. https://doi.org/10.1007/s10531-009-9617-3

Burnham KP, Anderson DR (2002) Model Selection and Multimodel Inference: A Practical Information-theoretic Approach (2nd edn.). Springer-Verlag, New York, 488 pp.

Burton AC (2012) Critical evaluation of a long-term, locally-based monitoring program in West Africa. Biodiversity and Conservation 21(12): 3079–3094. https://doi.org/10.1007/s10531-012-0355-6

Burton AM, Navarro S, Chávez C (2003) Bobcat ranging behavior in relation to small mammal abundance on Colima Volcano, México. Anales del Instituto de Biología, Universidad Nacional Autónoma de México. Serie Zoología 74: 67–82. https://www.redalyc.org/articulo.oa?id=45874107

Carvajal S, Caso A, Downey P, Moreno A, Tewes EM, Grassman L (2012) Spatial patterns of the margay (*Leopardus wiedii*; Felidae, Carnivora) at “El Cielo” Biosphera Reserve, Tamaulipas, Mexico. Mammalia 76(3): 237–244. https://doi.org/10.1515/mammalia-2011-0100

CBD (2020) Global Biodiversity Outlook 5—Summary for Policy Makers. Secretariat of the Convention on Biological Diversity, Montreal.

Cottam G, Curtis JT (1956) The Use of Distance Measures in Phytosociological Sampling. Ecology 37(3): 451–460. https://doi.org/10.2307/1930167

Cox JJ, Maehr SD, Larkin LJ (2006) Florida Panther Habitat Use: New Approach to an Old Problem. The Journal of Wildlife Management 70(6): 1778–1785. https://doi.org/10.2193/0022-541X(2006)70[1778:FPHUNA]2.0.CO;2

Cramer P, Portier K (2001) Modeling Florida panther movements in response to human attributes of the landscape and ecological settings. Ecological Modelling 140(1–2): 51–80. https://doi.org/10.1016/S0304-3800(01)00268-X

De Oliveira TG (1998) *Leopardus wiedii*. Mammalian Species 579: 1–6. https://doi.org/10.2307/3504400

DeCaro D, Stokes M (2008) Social-psychological principles of community-based conservation and conservancy motivation: Attaining goals within an autonomy-supportive environment. Conservation Biology 22(6): 1443–1451. https://doi.org/10.1111/j.1523-1739.2008.00996.x

Elizalde C, López JC, Hernández L, Laundré WJ, Cervantes AF, Alonso M (2012) Home range size and activity patterns of bobcats (*Lynx rufus*) in the southern part of their range in the Chihuahuan desert, Mexico. American Midland Naturalist 168(2): 247–264. https://doi.org/10.1674/0003-0031-168.2.247

Elizondo C, López-Merlín D (2009) Las áreas voluntarias de conservación en Quintana Roo. México DF, Mexico: CONABIO, Corredor Biológico Mesoamericano México, Serie Acciones No. 6.

Espinosa-Flores ME, López-González CA (2017) Landscape attributes determine bobcat (*Lynx rufus escuinapae*) presence in Central Mexico. Mammalia 81(1): 101–105. https://doi.org/10.1515/mammalia-2015-0049

Estrada GC (2008) Dieta, uso de hábitat y patrones de actividad del puma (*Puma concolor*) y el jaguar (*Panthera onca*) en la Selva Maya, Centro América. Revista Mexicana de Mastozoología 12(1): 113–130. https://doi.org/10.22201/ie.20074484e.2008.12.1.48

Farhan Ferrari M, de Jong C, Belohrad VS (2015) Community-based monitoring and information systems (CBMIS) in the context of the Convention on Biological Diversity (CBD). Biodiversity 16(2–3): 57–67. https://doi.org/10.1080/14888386.2015.1074111

Flores-Morales M, Vázquez J, Bautista A, Rodríguez-Martínez L, Monroy-Vilchis O (2019) Response of two sympatric carnivores to human disturbances of their habitat: The bobcat and coyote. Mammal Research 64(1): 53–62. https://doi.org/10.1007/s13364-018-0385-x

Goulart FVB, Cáceres NC, Graipel ME, Tortato MA, Ghizoni Jr IR, Oliveira-Santos LGR (2009) Habitat selection by large mammals in a southern Brazilian Atlantic Forest. Mammalian Biology 74(3): 182–190. https://doi.org/10.1016/j.mambio.2009.02.006

Hass CC (2009) Competition and coexistence in sympatric bobcats and pumas. Journal of Zoology 278(3): 174–180. https://doi.org/10.1111/j.1469-7998.2009.00565.x

Hernández-SaintMartín AD, Rosas-Rosas OC, Palacio-Núñez J, Tarango-Arambula LA, Clemente-Sánchez F, Hoogesteijn AL (2015) Food habits jaguar and puma in protected are and adjacent fragmented landscape of northeastern Mexico. Natural Areas Journal 35(2): 308–317. https://doi.org/10.3375/043.035.0213

Hodge AM (2014) Habitat selection of the margay (*Leopardus wiedii*) in the eastern Adean foot hills of Ecuador. Mammalia 78(3): 351–358. https://doi.org/10.1515/mammalia-2013-0070

Horn PE, Pereira MJR, Trigo TC, Eizirik E, Tirelli FP (2020) Margay (Leopardus wiedii) in the southernmost Atlantic Forest: Density and activity patterns under different levels of anthropogenic disturbance. PLoS ONE 15(5): e0232013. https://doi.org/10.1371/journal.pone.0232013

Jurrius I, López Rodríguez FV (2020) Monitoreo Comunitario y Participativo de los Manglares bajo Acuerdos de Uso y Custodia de Manglar en Ecuador. Investigation 14(14): 27–37. https://doi.org/10.31095/investigatio.2020.14.3

Konecny MJ (1989) Movement patterns and food habits of four sympatric carnivore species in Belize, Central America. In: Redford KH, EisenbergJF (Eds) Advances in Neotropical Mammalogy. The Sandhill Crane Press, Gainesville, 243–264.

Land DE, Shindle DB, Kawula RJ, Benson JF, Lots MA, Onorato DP (2008) Florida panther habitat selection analysis of concurrent GPS and VHF telemetry data. The Journal of Wildlife Management 72(3): 633–639. https://doi.org/10.2193/2007-136

Laundré JW, Hernández L (2010) What we know about pumas in Latin America. In: Hornocker MG, Negri S (Eds) Cougar: Ecology and Conservation. The University of Chicago Press, Chicago.

Lavariega MC, Ríos-Solís JA, Flores-Martínez JJ, Galindo-Aguilar RE, Sánchez-Cordero V, Juan-Albino S, Soriano-Martínez I (2020) Community-based monitoring of jaguar (*Panthera onca*) in the Chinantla region, Mexico. Tropical Conservation Science 13: 1–16. https://doi.org/10.1177/1940082920917825

Lavariega MC, Briones-Salas M, Monroy-Gamboa AG, Ramos D (2022) Population density and daily activity patterns of bobcat in its southernmost continental distribution. Animal Biodiversity and Conservation 45(2): 145–160. https://doi.org/10.32800/abc.2022.45.0145

Lira I, Briones MA (2012) Abundancia relativa y patrones de actividad de los mamíferos de los Chimalapas, Oaxaca, México. Acta Zoológica Mexicana 28(3): 566–585. https://doi.org/10.21829/azm.2012.283859

Lira I, Naranjo EJ (2003) Abundancia, preferencia de hábitat e impacto del ecoturismo sobre el puma y dos de sus presas en la Reserva de la Biosfera el Triunfo, Chiapas, México. Rev Mex Mastoz 7(1): 20–39. https://doi.org/10.22201/ie.20074484e.2003.7.1.178

Lira I, Briones-Salas M, Sánchez-Rojas G (2014) Abundancia relativa, estructura poblacional, preferencia de hábitat y patrones de actividad del tapir centroamericano *Tapirus bairdii* (Perissodactyla: Tapiridae), en la selva de los Chimalapas, Oaxaca, México. Revista de Biología Tropical 62(4): 1407–1419. https://doi.org/10.15517/rbt.v62i4.12584

Litvaitis JA, Sherburne JA, Bissonette JA (1986) Bobcat habitat use and home range size in relation to prey density. The Journal of Wildlife Management 50(1): 110–117. https://doi.org/10.2307/3801498

Madsen EK, Elliot NB, Mjingo EE, Masenga EH, Jackson CR, May RF, Røskaft E, Broekhuis F (2020) Evaluating the use of local ecological knowledge (LEK) in determining habitat preference and occurrence of multiple large carnivores. Ecological Indicators 118: e106737. https://doi.org/10.1016/j.ecolind.2020.106737

Maffei L, Cuellar E, Noss J (2002) Uso de trampas cámara para la evaluación de mamíferos en el ecotono Chaco-Chiquitanía. Revista Boliviana de Ecología y Conservación Ambiental 11: 55–65.

Mayer AE, McGreevy Jr TJ, Brown C, Ganoe LS, Gerber BD (2022) Transient persistence of bobcat (*Lynx rufus*) occurrence throughout a human-dominated landscape. Population Ecology 64(4): 1–13. https://doi.org/10.1002/1438-390X.12123

Méndez-López ME, García-Frapolli E, Ruiz-Mallén I, Porter-Bolland L, Reyes-García V (2015) From Paper to Forest: Local Motives for Participation in Different Conservation Initiatives. Case Studies in Southeastern Mexico. Environmental Management 56(3): 695–708. https://doi.org/10.1007/s00267-015-0522-0

Monroy G, Briones-Salas M (2012) Primeros datos sobre área de actividad de gato montés (*Lynx rufus*) en Oaxaca, México. Acta Zoológica Mexicana 28(2): 471–474. https://doi.org/10.21829/azm.2012.282848

Monroy O, Rodríguez C, Zarco M, Urios V (2009) Cougar and jaguar habitat use and activity patterns in central Mexico. Animal Biology 59(2): 145–157. https://doi.org/10.1163/157075609X437673

Monroy-Vilchis O, Velázquez A (2002) Distribución regional y abundancia del lince (*Lynx rufus escuinape*) y el coyote (Canis latrans cagottis), por medio de estaciones olfativas: Un enfoque espacial. Ciencia Ergo Sum 9(3): 293–300.

Morrison ML, Marcot BG, Mannan RW (1992) Wildlife-Habitat Relationships: Concepts and Applications. University of Wisconsin Press, Madison.

Mostacedo B, Fredericksen T (2000) Manual de Métodos Básicos de Muestreo y Análisis en Ecología Vegetal. Proyecto BOLFOR. Santa Cruz, Bolivia.

Mueller-Dombois D, Ellenberg H (2002) Aims and Methods of Vegetation Ecology. Blackburn Press. Caldwell, USA.

Noria-Sánchez JL, Prisciliano J, Patiño J (2015) Monitoreo comunitario de aves en la Chinantla, Oaxaca: un esfuerzo para la conservación de la biodiversidad. In: Ortega-Álvarez R, Sánchez-González LA, Berlanga-García H (Eds) Plumas de Multitudes, Integración Comunitaria en el Estudio y Monitoreo de Aves en México. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, México.

Nowell K, Jackson P (1996) Wild cats. Status Survey and Conservation Action Plan. IUCN/SSC Cat Specialist Group, Gland.

Nuñez-Perez R, Miller B (2019) Movements and home range of jaguars (*Panthera onca*) and mountain lions (Puma concolor) in a tropical dry forest of western Mexico. Movement ecology of neotropical forest mammals: focus on social animals: 243–262. https://doi.org/10.1007/978-3-030-03463-4_14

Ojeda-Lavariega E, Vásquez-Dávila MA, Padilla-Gómez E, Manzanero-Medina GI (2019) Usos de mamíferos silvestres medianos y grandes en San Pablo Etla, Oaxaca, México. AICA 14: 42–46.

Ordiz A, Aronsson M, Persson J, Støen OG, Swenson JE, Kindberg J (2021) Effects of human disturbance on terrestrial apex predators. Diversity 13(2): 1–68. https://doi.org/10.3390/d13020068

PBD (2001) Enfoque Estratégico Para Integrar la Biodiversidad en la Cooperación Para el Desarrollo. Comisión Europea, Bruselas, Bélgica/UICN, Gland, Suiza y Cambridge, Reino Unido.

Pérez-Irineo G, Santos-Moreno A, Hernández-Sánchez A (2017) Density and activity pattern of *Leopardus wiedii* and *Leopardus pardalis* at Sierra Norte of Oaxaca, Mexico. Therya 8(3): 217–222. https://doi.org/10.12933/therya-17-487

Prude CH, Cain JW III (2021) Habitat diversity influences puma *Puma concolor* diet in the Chihuahuan Desert. Wildlife Biology 4(4): wlb-00875. https://doi.org/10.2981/wlb.00875

Ripple WJ, Estes JA, Beschta RL, Wilmers CC, Ritchie EG, Hebblewhite M, Wirsing AJ (2014) Status and ecological effects of the world's largest carnivores. Science 343(6167): e1241484. https://doi.org/10.1126/science.1241484

Rodríguez-Soto C, Hernández-Tellez M, Monroy-Vilchis O (2013) Distribución y uso de hábitat de Puma concolor en la Reserva Natural Sierra de Nanchititla. In: Monroy-Vilchis O, Soria-Díaz L (Eds) Ecología de *Puma concolor* en la Sierra Nanchititla, México. Universidad Autónoma del Estado de México, 45–64.

Scognamillo D, Maxit I, Sunquist M, Polisar J (2003) Coexistence of jaguar (*Panthera onca*) and puma (*Puma concolor*) in mosaic landscape in the Venezuela llanos. Journal of Zoology 259(3): 269–279. https://doi.org/10.1017/S0952836902003230

Shannon G, Lewis JS, Gerber BD (2014) Recommended survey designs for occupancy modelling using motion-activated cameras: Insights from empirical wildlife data. PeerJ 2: e532. https://doi.org/10.7717/peerj.532

Simón-Salvador PR, Arreortúa M, Flores CA, Santiago-Dionicio H, González-Bernal E (2021) The role of Indigenous and Community Conservation Areas in herpetofauna conservation: A preliminary list for Santa Cruz Tepetotutla, Oaxaca Mexico. ZooKeys 1029: 185–208. https://doi.org/10.3897/zookeys.1029.62205

Sunquist M, Sunquist F (2002) Wild Cats of the World. The University of Chicago Press, London, 452 pp. https://doi.org/10.7208/chicago/9780226518237.001.0001

Warde W, Petranka JW (1981) A correction factor table for missing point-center quarter data. Ecology 62(2): 491–494. https://doi.org/10.2307/1936723

Zanin M, Palomares F, Brito D (2015) What we (don't) know about the effects of habitat loss and fragmentation on felids. Oryx 49(1): 96–106. https://doi.org/10.1017/S0030605313001609

Supplementary material 1

Sampling sites and dates on which the camera-traps were installed and Generalized linear mixed models (GLMM) for Puma, Bobcat and Margay

Authors: J. Roberto Sosa-López, Nydia Nicté Díaz Bernal, Eugenio Padilla, Miguel Briones-Salas

Data type: docx

Explanation note: **table S1.** Sampling sites and dates on which the camera-traps were installed. **table S2.** Generalized linear mixed models (GLMM) for Puma. **table S3.** Generalized linear mixed models (GLMM) for Bobcat. **table S4.** Generalized linear mixed models (GLMM) for Margay.

Link: https://doi.org/10.3897/natureconservation.53.104135.suppl1

Nature Conservation 53: 297–317 (2023)
DOI: 10.3897/natureconservation.53.100631

Research Article

Distribution, conservation assessment and management perspectives of Chilean micro-snails of the family Charopidae

Gonzalo A. Collado[1,2], Nataly Flores[1], Marcela A. Vidal[1,2], Cristian Torres-Díaz[1,2], Moisés A. Valladares[2,3]

1 *Departamento de Ciencias Básicas, Facultad de Ciencias, Universidad del Bío-Bío, Avenida Andrés Bello 720, Chillán, Chile*
2 *Grupo de Investigación en Biodiversidad y Cambio Global, Universidad del Bío-Bío, Chillán, Chile*
3 *Laboratorio de Biología Evolutiva, Departamento de Ecología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile*
Corresponding author: Gonzalo A. Collado (gcollado@ubiobio.cl)

Abstract

The biodiversity of molluscs is highly threatened in marine, terrestrial and freshwater ecosystems worldwide. This research aimed at studying the distribution and conservation status of eight poorly-known micro-snails of the genera *Stephacharopa* and *Stephadiscus* in Chile. We performed a comprehensive review of literature and databases to determine the occurrences of the species, which were mapped on vector layers containing protected areas and human development infrastructure to find potential threats. Conservation status assessment was performed following the criteria and tools implemented by the International Union for the Conservation of Nature (IUCN) Red List and NatureServe. We also conducted species distribution models, based on maximum entropy, to identify areas that should be prioritised for conservation. Two species meet the criteria for IUCN listing as Critically Endangered (CR), four Endangered (EN), one Vulnerable (Vu) and one Least Concern (LC). This classification is rather coincident with equivalent categories obtained under the NatureServe standard, in which two species were ranked as Critically Imperiled (N1), five Imperiled (N2) and one Vulnerable (N3). We found that *Stephacharopa paposensis* is the most at-risk species, with only one occurrence not included in a protected area, followed by *Stephadiscus stuardoi*, with two occurrences, one of them within a protected area. *Stephadiscus lyratus* was the species with the greatest geographic range, accounting for 17 occurrences, seven matching a protected area. We found wider potential ranges in modelled species that may be useful for prioritising conservation measures. Considering distributional data, protected areas and more than 20 plausible threats identified, we propose potential *in situ* and *ex situ* conservation actions to protect these neglected micro-snails.

Key words: conservation biology, endemic species, imperiled micromolluscs, IUCN, land snails, NatureServe, niche modelling, protected areas

Academic editor: William Magnusson
Received: 16 January 2023
Accepted: 5 May 2023
Published: 25 October 2023

ZooBank: https://zoobank.org/FDECA679-FF86-4225-9199-9B3EFC7FA1FE

Citation: Collado GA, Flores N, Vidal MA, Torres-Díaz C, Valladares MA (2023) Distribution, conservation assessment and management perspectives of Chilean micro-snails of the family Charopidae. Nature Conservation 53: 297–317. https://doi.org/10.3897/natureconservation.53.100631

Introduction

In recent decades, the loss and degradation of ecosystems due to human activities has increased to alarming levels worldwide (Chivian 2001; Cardinale et al. 2012; FAO and UNEP 2020). Habitat loss, fragmentation and alteration have been considered as the main cause of species extinction (Rawat and Agarwal 2015). However, environmental pollution, invasive species and overexploitation, amongst other factors, have also contributed significantly to the disappearance or decline of populations (Thomas et al. 2004; Clavero and García-Berthou 2005; Mason 2015; Maxwell et al. 2016). As entities that cause ecosystem disturbances, humankind is also responsible for preserving biodiversity considering that it is not only important for ecological, aesthetic or scientific reasons, but also because human existence depends on the use of biological resources and ecosystem services (Luck et al. 2003; Barker et al. 2010).

Knowledge of geographic distribution of species is crucial for conservation status assessment (Anderson and Martinez-Meyer 2004), acquiring relevance in endemic species (Behroozian et al. 2020). The isolation of the Chilean continental territory generated by the Atacama Desert in the north, the Andes Mountain range to the east, the Pacific Ocean to the west and the Antarctic Ocean to the south, has produced a relatively high biodiversity in the country, reaching 33,000 native species, of which about 25% are endemic (MMA 2022).

Molluscs are highly threatened in marine, terrestrial and freshwater ecosystems worldwide (Régnier et al. 2009; Burlakova et al. 2011). In fact, they have been considered one of the most endangered groups of animals on the planet, even surpassing the number of extinctions of all other taxa combined (Régnier et al. 2009). However, this could be even more serious because the phylum contains a range of poorly-known groups, with many endemic species restricted to one or a few localities. Endemism is one of the most important factors used by the public in conservation biology, surpassing other useful aspects that these could have in the field of conservation (Meuser et al. 2009). On the other hand, from the geographic point of view, molluscs also contain a lot of rare species, rarity being another factor that influences the probability of extinction of organisms (Gaston 1998; Mace et al. 2008).

The International Union for Conservation of Nature (IUCN) Red List of Threatened Species and Natural Heritage Network Element Ranking Systems (NatureServe 2006) are two institutions that aim to list species in conservation categories at different geographical scales (IUCN 2001, 2012, 2019, 2022; NatureServe 2006, 2021). However, the need to apply a legal instrument soon led many countries to create their own classification systems to categorise threatened species (Gärdenfors 2001; IUCN 2003). The first classification of wild species in conservation categories in Chile was published in 2005 as part of Law No. 19,300 (Decreto Supremo No. 75 of 2004, Ministerio Secretaría General de la Presidencia), where 35 species were evaluated. Currently, the conservation categories in Chile are based on those proposed by the IUCN (Squeo et al. 2010), following the Decreto Supremo N° 29 of 2011 of the Chilean Ministry of the Environment (MMA 2022). On the other hand, ecosystems and biota are protected in the Sistema Nacional de Áreas Silvestres Protegidas del Estado (SNASPE) (in English: National System of State Protected Wilderness Areas) (Root-Bernstein et al. 2013; Subsecretaría de Turismo 2015) and its administration oversees the Corporación Nacional Forestal (CONAF) (National Forestry Corporation).

In the present study, we applied the tools, criteria and categories developed by the IUCN and NatureServe to assess the conservation status of terrestrial micro-snails of the family Charopidae Hutton, 1884. This taxon is represented by species from Central and South America, South Africa, Australia, New Zealand and some islands of the Pacific, Atlantic and Indian Oceans (Stuardo and Vega 1985; Valdovinos Zarges 1999; Muratov et al. 2005; Salvador et al. 2020). Most of the Charopidae species are from humicolous habitats so they can be found in leaf litter in humid wooded areas (Miquel and Ramírez 2011; Miquel and Araya 2013; Araya and Catalán 2014). Multiple threats have been recognised potentially affecting the species of the group, including habitat loss, fragmentation and degradation, land uses changes, agriculture, logging and subsistence harvesting of wood, tourism, global warming, rainfall, floods, snowfall storms, volcanism, fires, invasive species and domestic and feral animals (Herbert 2004; Barker 2012; Vermeulen et al. 2014; Cuezzo et al. 2021; Collado et al. 2023).

In Chile, charopids reach a high diversity (Miquel and Cádiz Lorca 2008; Miquel and Barker 2009), accounting for 17 genera (Stuardo and Vega 1985; Valdovinos Zarges 1999; Miquel et al. 2007; Miquel and Cádiz Lorca 2008; Miquel and Araya 2013; Araya et al. 2017; Araya and Miquel 2018). *Stephacharopa* Miquel & Araya, 2013 and *Stephadiscus* Hylton Scott, 1981, the focus of this research, are two taxa represented by species of small size, usually less than 4 mm (Miquel and Cádiz Lorca 2008; Miquel and Ramírez 2011; Araya and Miquel 2018). Ectothermic traits of species, low vagility, probable low fecundity and particular habitat requirements (often wooded areas with leaf litter), all of them combined, makes species in the group potentially vulnerable (Cuezzo et al. 2021). Recently, several species in the genus *Radiodiscus* Pilsbry in Pilsbry & Ferriss, 1906 (Charopidae) were listed as critically endangered or endangered in Chile (Collado et al. 2023). At present, however, the conservation status or risk of extinction has not been assessed for none of the species of *Stephacharopa* or *Stephadiscus* in the country.

The aims of this study were to determine the distribution of micro-snail species in the genera *Stephacharopa* and *Stephadiscus* in Chile and assess their conservation status using the criteria and tools implemented by IUCN and NatureServe and propose conservation actions. In addition, we identified potential distributions of species or habitats with similar environmental conditions that can be incorporated into future management plans.

Methods

Species records and occurrence data

The genera *Stephacharopa* and *Stephadiscus* comprise eight species in Chile, four in each genus (Gould 1846; Hylton Scott 1969, 1970, 1973, 1979; Stuardo and Vega 1985; Valdovinos Zarges 1999; Tablado and Mantinian 2004; Miquel and Cádiz Lorca 2008; Miquel and Barker 2009; Rodríguez et al. 2012; Araya and Miquel 2018; Aldea et al. 2019; Cuezzo et al. 2021; MolluscaBase 2021a, 2021b). The genus *Stephacharopa* is represented by *S. calderaensis* Miquel & Araya, 2013, *S. distincta* (Hylton Scott, 1970), *S. paposensis* Miquel & Araya, 2018 and *S. testalba* (Hylton Scott, 1970). The genus *Stephadiscus* comprises *S. celinae* (Hylton Scott, 1969), *S. lyratus* (Couthouy in Gould, 1846), *S. rumbolli* (Hylton Scott, 1973) and *S. stuardoi* Miquel & Barker, 2009. The dataset on species including the geographic coordinates of occurrences was obtained from literature and the Global Biodiversity Information Facility (GBIF) database (https://www.gbif.org/occurrence/search?q=Stephadiscus) (Suppl. material 1). Presence occurrences without geographic coordinates were georeferenced using the radius-point method (Wieczorek et al. 2004; Escobar et al. 2016) in the GOOGLE EARTH (v.7.3.3.7786, Google Inc.) software (Suppl. material 1).

IUCN conservation assessment

Under the IUCN guidelines, the species can be classified in one conservation category according to five criteria, which can work independently or combined (IUCN 2001, 2019). Criterion A covers population size reduction, Criterion B geographic range, Criterion C small population size and decline, Criterion D very small or restricted population and Criterion E quantitative analysis of the probability of extinction. The IUCN has established nine conservation categories: Extinct (EX), Extinct in the Wild (EW), Critically Endangered (CR), Endangered (EN), Vulnerable (VU), Near Threatened (NT), Least Concern (LC), Data Deficient (DD) and Not Evaluated (NE).

We applied Criteria B and D (D2) to assess the conservation status under the IUCN guidelines due to the difficulty of applying criteria A, C and E since they lack information regarding Chilean micro-snail species. The application of Criterion B requires the estimation of the extent of occurrence (EOO) (sub-criterion B1), which represents the area in km^2 formed by the polygon that forms the line that encloses all the occurrences of the species and/or area of occupancy (AOO) (sub-criterion B2), the area within its extent of occurrence obtained from the sum of each occurrence with respect to a 2 × 2 km^2 grid cell and then meeting at least two of these conditions: a) severely fragmented or known to exist in no more than 1 ≤ 5 or ≤ 10 locations, b) continuing decline or c) extreme fluctuation. For the taxa studied, we used conditions a) and b) considering available data. In the last case, we used sub-condition (iii): continuous decline projected in area, extent and/or quality of habitat. The EOO and AOO parameters were obtained in the Geospatial Conservation Assessment Tool (GeoCAT) portal (Bachman et al. 2011), which uses a file of geographic coordinates of the species to provide a preliminary semi-automatic evaluation of the conservation status of a taxon. In the case of the EOO, we use the minimum convex polygon (MCP) and, for the AOO, a 2 × 2 km^2 grid cell around the occurrence (IUCN 2012, 2019). For species with few occurrences, we also use criterion D, specifically D2. According to the IUCN (2019, 2022), a species qualifies for Vulnerable D2 if the area of occupancy is very restricted (typically less than 20 km^2) or exists at typically five or fewer locations and if there is a plausible natural or anthropogenic threat.

NatureServe conservation assessment

NatureServe weighs three different factors at once: rarity, threats and population trends. Rarity refers to range extent and area of occupancy, population size and number of occurrences and/or percentage of area with good ecological viability and/or ecological integrity. Threats consider the average impact of different threats facing or potentially facing species, while population trends include long- and short-term temporal variation (NatureServe 2006; Master et al. 2009; Faber-Langendoen et al. 2012). The system allows researchers to categorise species and ecosystems according to the geographical rank to be applied: Global (G), National (N) and Sub-national (S). There are five NatureServe conservation categories: Critically Imperiled (1), Imperiled (2), Vulnerable (3), Apparently Secure (4) and Secure (5). For example, if a species is classified as Critically Imperiled at the Global Rank, it will be ranked as (G1), but if it is classified at the National Rank as Critically Imperiled, it will be ranked as (N1). To assess the conservation status of species, we use the parameters EOO, AOO, number of occurrences, threats and ecological viability (habitat availability), entering the values or estimates in the Conservation Rank Calculator developed by the organisation (NatureServe 2021). Population trends were set as "unknown" due to lack of information. The threats and habitat available for species were obtained examining satellite images on the software Google Earth and merging species occurrences with road works, hydrographic and urban development vector layers available in the Biblioteca del Congreso Nacional de Chile (Library of the National Congress of Chile) (https://www.bcn.cl/siit/mapas_vectoriales), which were qualitatively examined in QGIS v.3.22.7 software (QGIS Development Team 2021). Literature, government sources and the media were also used to identify threats that subsequently allowed us to propose conservation measures.

Mapping of protected areas

To find out whether populations are located inside protected areas, we merged species occurrences with the SNASPE vector layer available at the Library of National Congress of Chile in QGIS, together with a topographic layer representing the Chilean territory obtained from the same source. Distribution maps, including MCPs, occurrences, country regions and SNASPE areas were made using GeoCAT and QGIS.

Species distribution models

We use 19 bioclimatic variables (Fick and Hijmans 2017) (Suppl. material 3), four environmental variables (elevation, evaporation, solar radiation and wind) obtained from WorldClim v.2.0 (www.worldclim.org) and the topographic layer of the Chilean territory. The 23 climatic and environmental variables were downloaded as raster format (30 s, ~ 1 km^2). Each of them was superimposed on the topographic variable and then cut with respect to its limits and transformed into a variable in ASCII format in the ArcGIS v.10.8 software (ESRI 2011). Species distribution analysis was carried out in the MaxEnt v.3.4.4 software (Phillips et al. 2006), which predicts the potential distribution of species according to the environmental conditions of the studied region. For this analysis, the occurrence records of each species and the ASCII variables were loaded into MaxEnt. Models were built using the default parameters and others with 25% testing, setting the logistic output format. The validation of the models was based on the Area Under the Curve (AUC), which represents the total area under the receiver operating characteristic (ROC) curve, whose maximum value is 1. AUC values greater than 0.7–0.8 have useful application in the model (Manel et al. 2001; Loo et al. 2007). The contribution of each variable individually was evaluated using the Jackknife test. Species with a single or two occurrence records were not analysed.

Results

Conservation categories

The genus *Stephacharopa* is distributed in Chile from the Antofagasta Region to the Los Lagos Region, while *Stephadiscus* from the Bío-Bío Region to the Magallanes y de la Antártica Chilena Region (Figs 1A–D, 2A–D). Of these species, *Stephacharopa paposensis* and *Stephadiscus stuardoi* are endemic to Chile, while *Stephacharopa calderaensis*, *Stephacharopa testalba*, *Stephacharopa distincta*, *Stephadiscus celinae*, *Stephadiscus lyratus* and *Stephadiscus rumbolli* are native to Chile and Argentina. Geographical distribution updates show that *Stephacharopa calderaensis* and *Stephacharopa paposensis* inhabit northern Chile, with occurrences in the Atacama Desert, while *Stephacharopa distincta* and *Stephacharopa testalba* inhabit the south of the country. Of these species, *Stephacharopa paposensis* is restricted to one locality (Table 1). *Stephadiscus celinae* and *Stephadiscus stuardoi* occur in southern Chile, while *Stephadiscus rumbolli* and *Stephadiscus lyratus* are confined to austral Patagonia. *Stephadiscus lyratus* poses the largest distribution and number of occurrences in Chile (17), scattered from Los Ríos Region to the Magallanes y de la Antártica Chilena Region (Table 1). *Stephadiscus stuardoi* is restricted to two locations, one in Bío-Bío Region, another in austral Patagonia (Aysén).

GeoCAT analyses recovered EOO values ranging between 0.000 and 285,317 km^2, whereas AOO values between 4 and 64 km^2, considering both genera. When EOO values are lower than AOO, the former must be increased to equal the AOO values according to IUCN (2019). Thus, two EOO values less than AOO must be equal to the AOO value in Table 1.

Based on GeoCat and the IUCN sub-criterion B1 (EOO), two species were listed as Critically Endangered (CR), one Endangered (EN), three Vulnerable (Vu) and two as Least Concern (LC) (Table 1). Applying IUCN sub-criterion B2 (AOO), two species were listed as Critically Endangered (CR), four Endangered (EN), one Vulnerable (Vu) and one Least Concern (LC) (Table 1). *Stephacharopa paposensis* was assessed as Critically Endangered (CR) B1ab(iii) given that EOO < 100 km^2 (B1), it occurs in one locality (a) and projected decline in habitat quality due to disturbance and transformation of its area of occupancy [b(iii)]. Similarly, it was evaluated as Critically Endangered (CR) B2ab(iii) given that AOO < 10 km^2, it occurs in one locality (a) plus the condition b(iii) (Table 1). *Stephadiscus stuardoi* was evaluated as Critically Endangered (CR) B1ab(iii) given that EOO < 100 km^2 (B1), it occurs in two localities very distant from each other (a) plus the condition b(iii) and as CR B2ab(iii) given that AOO < 10 km^2 plus the conditions (a) and b(iii). *Stephacharopa testalba* was evaluated as Endangered (EN) B1ab(iii) given that EOO < 5000 km^2 (B1), it occurs in five locations (a) plus the condition b(iii) and as Endangered (EN) B2ab(iii) given that AOO < 500 km^2 (B2), five locations (a) plus the condition b(iii). *Stephacharopa calderaensis* was evaluated as Vulnerable (Vu) B1ab(iii) given that EOO < 20,000 km^2 (B1), it occurs in six locations (a) plus the condition b(iii) and as Vulnerable (Vu) B2ab(iii) given that AOO < 2000 km^2 (B2) plus the same conditions (a) and b(iii). *Stephacharopa distincta* and *Stephadiscus rumbolli* were evaluated as Vulnerable (Vu) B1ab(iii) given that EOO < 20,000 km^2 (B1), < 10 localities (a) plus the condition b(iii) and as Endangered (EN) B2ab(iii) given that AOO < 500 km^2 (B2), < 5 localities (a) plus the condition b(iii). This latter classification [EN B2ab(iii)] also fits *Stephadiscus celinae*, which was also listed as Least Concern (LC). According to NatureServe, *Stephacharopa paposensis* and *Stephadiscus stuardoi* were ranked as Critically Imperiled (N1), *Stephacharopa calderaensis*, *Stephacharopa distincta*, *Stephacharopa testalba*, *Stephadiscus celinae* and *Stephadiscus rumbolli* as Imperiled (N2), while *Stephadiscus lyratus* Vulnerable (N3) (Table 1, Suppl. material 2).

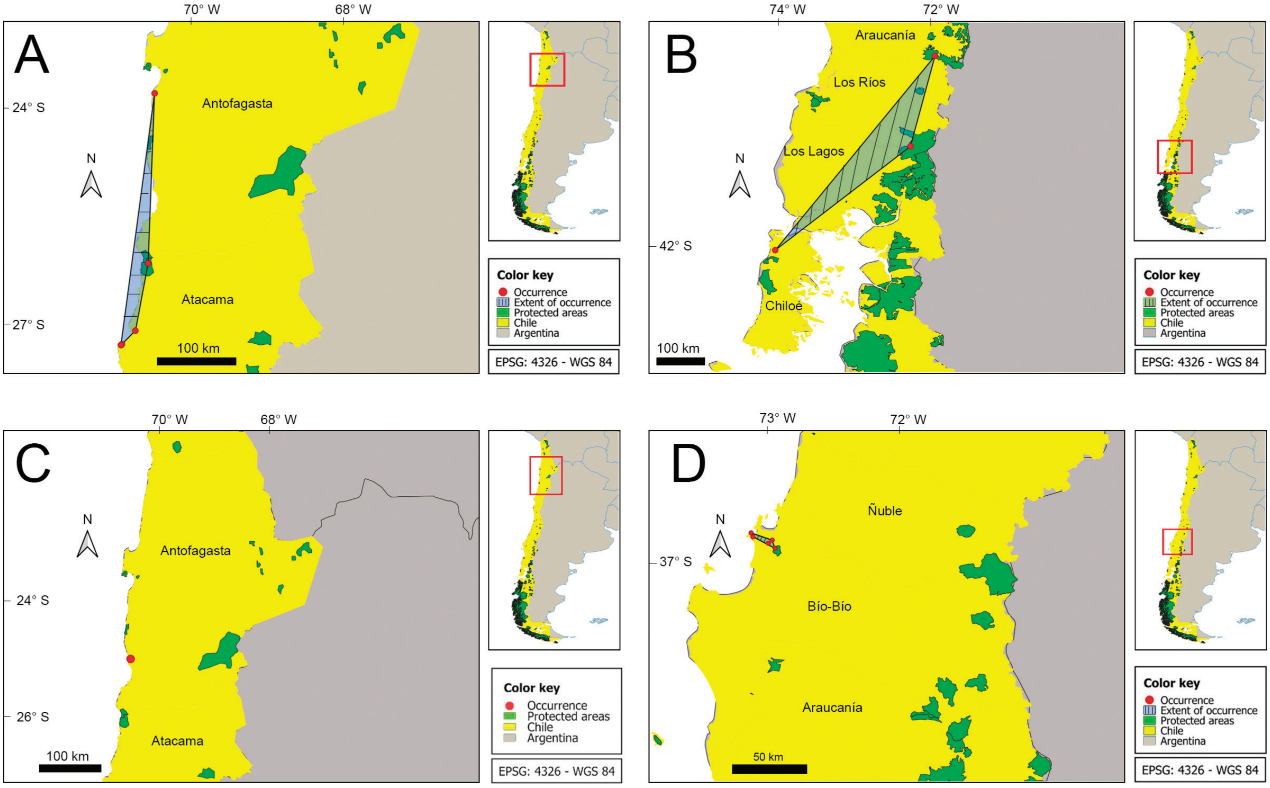


Figure 1. Distribution and occurrences of the Chilean micro-snails of the genus *Stephacharopa*, including protected areas. **A** *Stephacharopa calderaensis* **B** *Stephacharopa distincta* **C** *Stephacharopa paposensis* **D** *Stephacharopa testalba*. The maps were created using GeoCAT and QGIS software (Maps: G.A. Collado).

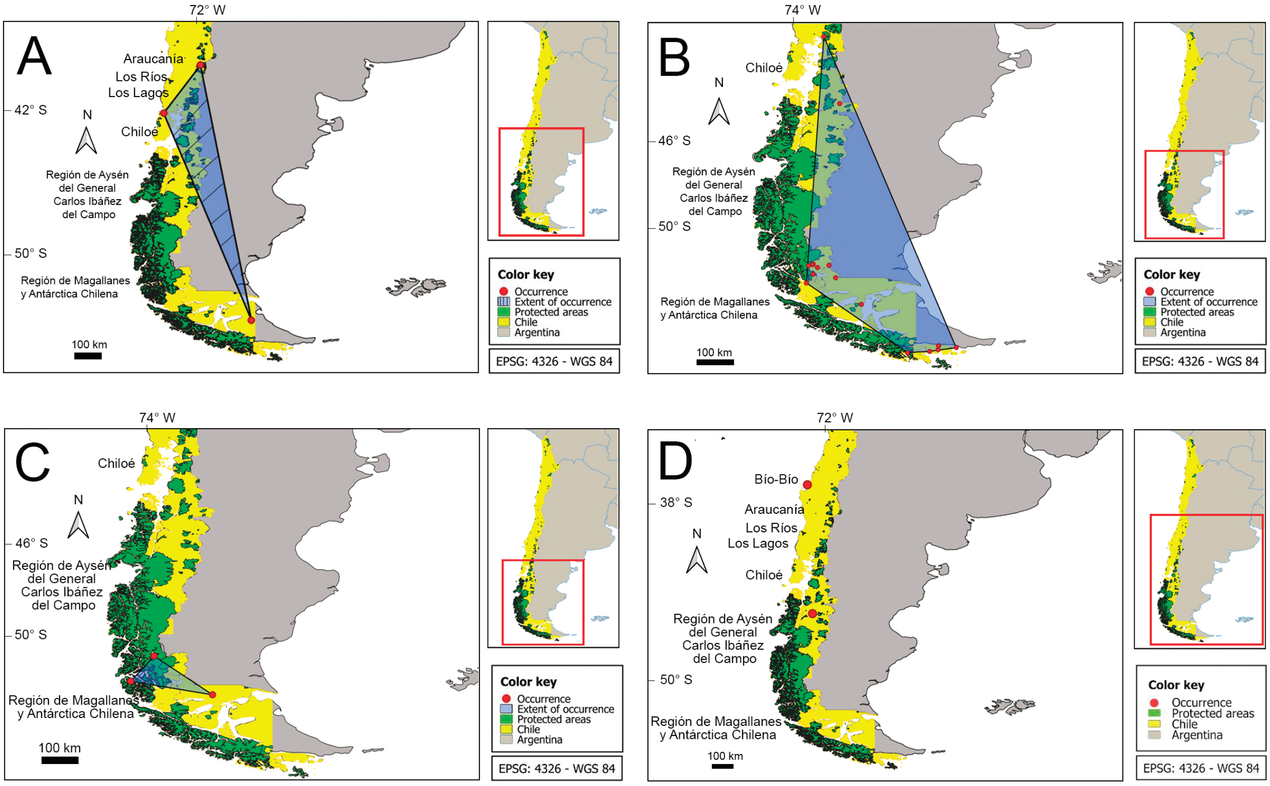


Figure 2. Distribution and occurrences of the Chilean micro-snails of the genus *Stephadiscus*, including protected areas. **A** *Stephadiscus celinae* **B** *Stephadiscus lyratus* **C** *Stephadiscus rumbolli* **D** *Stephadiscus stuardoi*. The maps were created using GeoCAT and QGIS software (Maps: G.A. Collado).

Table 1. Distributional parameters and conservation status for Chilean species of the genera *Stephacharopa* and *Stephadiscus*, based on IUCN and NatureServe guidelines. Abbreviations: AOO, area of occupancy; CR, Critically Endangered; D2, Vulnerable; EN, Endangered; EOO, extent of occurrence; LC, Least Concern; N1, Critically Imperiled; N2, Imperiled; N3, Vulnerable; NS, NatureServe; O, occurrences; OPA, occurrences in protected areas; Vu, Vulnerable. *Values of EOO should be changed to make them equal to AOO (IUNC 2019).

Species	O	OPA	EOO (km²)	AOO (km²)	IUCN			NS
					B1 (EOO)	B2 (AOO)	D	
Stephacharopa								
S. calderaensis	6	1	4,674	16	Vu B1ab(iii)	Vu B2ab(iii)	–	N2
S. distincta	3	0	7,795	12	Vu B1ab(iii)	EN B2ab(iii)	D2	N2
S. paposensis	1	0	4*	4	CR B1ab(iii)	CR B2ab(iii)	D2	N1
S. testalba	5	1	65	20	EN B1ab(iii)	EN B2ab(iii)	D2	N2
Stephadiscus								
S. celinae	3	1	170,807	12	LC	EN B2ab(iii)	D2	N2
S. lyratus	17	7	285,317	64	LC	LC	–	N3
S. rumbolli	3	2	16,800	12	Vu B1ab(iii)	EN B2ab(iii)	D2	N2
S. stuardoi	2	1	8*	8	CR B1ab(iii)	CR B2ab(iii)	D2	N1

Threats

In Chile, the species studied are threatened by urbanisation, habitat loss, habitat alteration, pollution, extreme temperatures, droughts, storms, floods, tsunamis, volcanism, climate change, soil erosion, landslides, earthquakes, tourism and recreational activities, forest fires, highways and roads, livestock, mining, hydroelectric plants and invasive species (Suppl. material 1). Considering the number of occurrences and threats, *Stephacharopa distincta*, *Stephacharopa paposensis*, *Stephacharopa testalba*, *Stephadiscus celinae*, *Stephadiscus rumbolli* and *Stephadiscus stuardoi* also meet the conditions for Vulnerable D2 status since they have five locations or fewer (Table 1) and several plausible threats (Suppl. material 1).

Potential ranges

The mapping of the occurrences of *Stephacharopa* on the protected areas indicates that only one population of *Stephacharopa calderaensis* and *Stephacharopa testalba* fell within a protected area, the Pan de Azúcar National Park and Parque Botánico Hualpén, respectively. In *Stephadiscus*, of the 17 occurrences of *Stephadiscus lyratus*, seven are found within protected areas. Each of the three remaining species included at least one population within a protected area (Table 1).

Species distribution models were performed on all species, except for *Stephacharopa paposensis* and *Stephadiscus stuardoi* because they have few occurrences (one and two, respectively). All models performed in MaxEnt gave AUC values greater than 0.9 in five cases and 0.7 in one (Suppl. material 3). Results from the analysis showed a gain in coverage with respect to the current range in all modelled species, covering a greater number of protected areas, which was remarkable in *Stephacharopa distincta*, *Stephacharopa testalba* and *Stephadiscus celinae* (Fig. 3). The variables that contributed the most information to the mod-

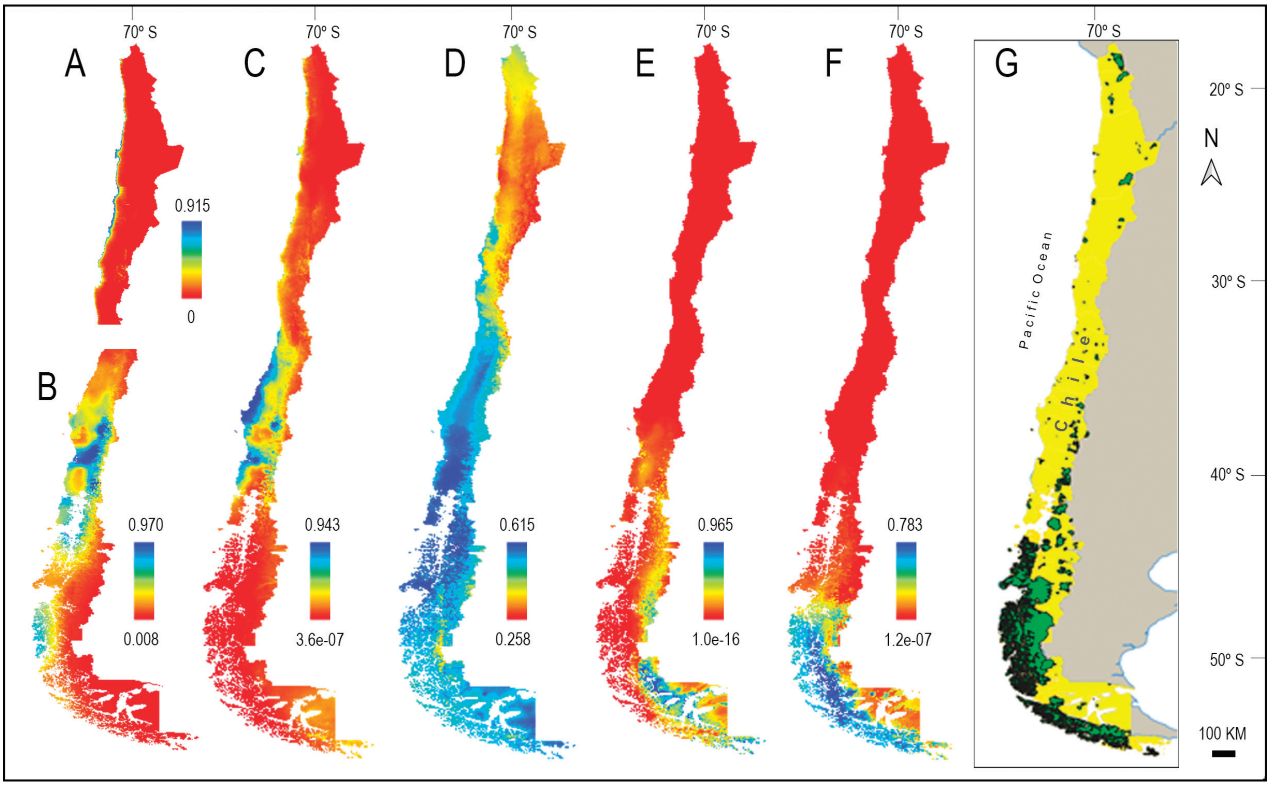


Figure 3. Potential distribution and species habitats of the genera *Stephacharopa* and *Stephadiscus* in Chile according to the modelling performed in Maxent. **A** *Stephacharopa calderaensis* **B** *Stephacharopa distincta* **C** *Stephacharopa testalba* **D** *Stephadiscus celinae* **E** *Stephadiscus lyratus* **F** *Stephadiscus rumbolli* **G** map showing Chilean territory (yellow) and protected areas (dark green). The maps were created using ArcGIS software (Maps: G.A. Collado).

els in *Stephacharopa calderaensis*, *Stephacharopa distincta* and *Stephacharopa testalba* were temperature annual range (bio7), precipitation of the coldest quarter (bio19) and precipitation of driest month (bio14), respectively, while in *Stephadiscus celinae*, *Stephadiscus lyratus* and *Stephadiscus rumbolli*, it was solar radiation. The contribution of all variables per species is shown in Suppl. material 3.

Discussion

Considering the IUCN sub-criterion B2 (AOO), which frequently classifies species in more serious conservation categories than sub-criterion B1 (EOO) and that it should be the final conservation category assigned according to the IUCN, two species were classified as Critically Endangered (CR), four Endangered (EN), one Vulnerable (Vu) and one Least Concern (LC). Under NatureServe, the assessment was rather equivalent to this one, ranking the same first two species as Critically Imperiled (N1), five Imperiled (N2) and one Vulnerable (N3). On the other hand, based on the IUCN D2 sub-criterion, six species were listed as Vulnerable (D2) given the low number of populations and the plausible threats that were identified.

Within the genus *Stephacharopa*, *S. paposensis* is the most at-risk species of the four evaluated since it was listed as Critically Endangered (CR) by the IUCN and Critically Imperiled (N1) by NatureServe. This snail is restricted to its type locality, Quebrada Paposo, an area that presents several and serious threats (Suppl. material 1). High contamination by garbage and debris has been observed in this ravine, as well as earth movements resulting from road works. The disappearance of a population of freshwater snails from a small spring in the area was previously reported (Collado 2012). A similar situation occurs for *Stephadiscus stuardoi*, but in this case, its presence has been reported from two localities separated by hundreds of kilometres. Both localities present several threats, which makes the future of the species uncertain. We avoid labelling species with few occurrences as Data Deficient (DD) by treating them as highly endemic species circumscribed to one or a few localities, as recommended by the IUCN (2019). Besides, classifying a species as Data Deficient (DD) practically nullifies any possibility of management.

As Chile comprises a long territory from north to south, with different climatic and hydrographic conditions, to which are added variations in the density of human population and urban development, not all species are exposed to the same threats. *Stephacharopa calderaensis* and *Stephacharopa paposensis* are the only northern taxa. In the xeric environments of Atacama Desert, with little urban development, these species are threatened by floods and droughts, mining, climate change, habitat loss, pollution and soil landslides, amongst other factors (Collado 2012; Burgos 2015; present study). *Stephacharopa distincta*, *Stephacharopa testalba*, *Stephadiscus celinae* and *Stephadiscus stuardoi* occupy the centre-south area of the country, with a Mediterranean climate and mesomorphic characteristics and where the largest number of people and cities of the country are concentrated. These species are threatened by urbanisation, habitat loss, forest fires, pollution, forestry activities, hydroelectric plants, roads, tourism and recreational activities, droughts and climate change (Araya 2016; Arnaboldi 2016; Subsecretaría de Turismo 2017; Díaz et al. 2018; Morales Flores 2019; Enríquez 2021; Robles 2021; present study). On the other hand, *Stephadiscus celinae* and *Stephadiscus rumbolli* are distributed in the austral region of Chile, where there is a humid, cold temperate climate with oceanic influence and hygromorphic characteristics. Species are affected by storms and floods, habitat alteration, tourism and recreational activities, pollution and invasive species (Martinic 2004; DAPMA 2009; Magallanes 2012; Graells et al. 2015; Valderrama et al. 2018; Arredondo et al. 2019; El Heraldo Austral 2019; GEF 2019; CONAF 2020; SONAMI 2021; present study).

The AUC results obtained in the species distribution models of the species analysed (greater than 0.9 and 0.7) indicate high reliability. The modelling results showed that the species have broader potential ranges, being useful for prioritising conservation actions. The analysis also revealed the climatic and environmental variables that influence the habitat of the species (Carvajal-Hernández et al. 2020), which contributes to the limited knowledge of these micro-snails. However, Pearson et al. (2007) have pointed out that the results of modelling in species with few occurrences should be interpreted as revealing geographic areas that present environmental conditions like those existing in current habitats, not necessarily indicating potential distributions.

Temperature is one of the most influential environmental predictors affecting the distribution of species (Austin and Meyers 1996) and it was the most important variable regarding the distribution of *Stephacharopa calderaensis*, *Stephacharopa distincta* and *Stephacharopa testalba*. Solar radiation is also an influential environmental variable affecting the habitat conditions in some species of plants and animals (Austin and Meyers 1996; Bennie et al. 2008), but not always. For example, the occurrence of the green toad, *Bufotes viridis* (Laurenti, 1768) was associated with a high level of solar radiation in the Middle East area, but it did not influence the distribution of the salamander *Salamandra infraimmaculata* Martens, 1885 in the same geographic area (Blank and Blaustein 2012).

Different measures have been proposed regarding conservation biology for some species of flora and fauna threatened with extinction. *Ex situ* conservation measures include translocation and breeding or culture of species outside the natural habitat, such as zoos, government and conservation institutions or natural parks (Bloxam et al. 1984; Tonge and Bloxam 1991; CBD 1992; Pearce-Kelly et al. 1997; Mace et al. 1998; Hadfield et al. 2004; IUCN/SSC 2014). Likewise, different *in situ* conservation measures have been proposed, including the installation of environmental interpretation trails, the creation of micro-reserves, the creation/extension of protected areas and the eradication of invasive species (Berkmüller and Savasdiasara 1981; CBD 1992; Balmford et al. 1995; IUCN 2002; Laguna et al. 2004; Anonymous 2005; Kadis et al. 2013; Trias-Blasi et al. 2017). For the species with few occurrence records (*Stephacharopa paposensis*, *Stephadiscus stuardoi* and *Stephacharopa distincta*, *Stephadiscus celinae* and *Stephadiscus rumbolli*), we propose to establish short-term *ex situ* conservation measures, such as breeding of specimens in captivity due to the high risk of extinction. In land snails, there are several examples that were successful after implementing these types of actions (Bloxam et al. 1984; Tonge and Bloxam 1991; Pearce-Kelly et al. 1997; Mace et al.1998; Hadfield et al. 2004). At the same time, the creation of micro-reserves around the type localities would also be a priority measure concerning their proven effectiveness (Laguna et al. 2004; Kadis et al. 2013; Trias-Blasi et al. 2017). However, this measure is more difficult to implement, considering landowners, personnel costs, infrastructure, logistics etc. With due precautions, specimen translocations can also be carried out, which have given positive results in different taxa (Balmford et al. 1995; Berger-Tal et al. 2020).

For species with five to six populations (*Stephacharopa testalba*, *Stephacharopa calderaensis*), we recommend the creation of micro-reserves (Laguna et al. 2004; Kadis et al. 2013; Trias-Blasi et al. 2017) in at least one locality, as well as the installation of environmental interpretation trails. Although this last initiative has been somewhat questioned (Navrátil et al. 2016), the heart of the idea seems good enough, having been implemented successfully (Berkmüller and Savasdiasara 1981; Anonymous 2005). Translocations are also appropriate. For *Stephadiscus celinae*, an additional measure would be to extend the Chiloé National Park by a few kilometres to the north, incorporating the locality registered on the island. Immediate measures to conserve the species *Stephadiscus lyratus* seem not to be critical considering the 17 populations in which the species is subdivided in Chile. This species also has several occurrences within protected areas (Cuezzo et al. 2021; present study). However, much of its southernmost distribution range is also occupied by the invasive beaver *Castor canadensis* Kuhl, 1820 (Graells et al. 2015; Molina et al. 2018), which is very damaging in invaded ecosystems, so it is recommended to eradicate this species.

Biodiversity hotspots, endemism areas and species with restricted distribution are key concepts in biodiversity studies (Reid 1998). In addition, knowing the threat status of species is crucial in conservation biology (Kahraman et al. 2012). In the present study, we evaluated the conservation status of eight species of land micromolluscs from different genera, most of them resulting in a high risk of extinction for which we proposed several conservation measures. However, the lack of knowledge of the life cycle, ecological and reproductive aspects of many Chilean mollusc species, including those studied here, is an issue that should be considered in the short term.

Conclusions

In this study, one species of the genus *Stephacharopa* (*S. paposensis*) was listed as Critically Endangered (CR), two Endangered (EN) (*S. distincta*, *S. testalba*) and one Vulnerable (Vu) (*S. calderaensis*) using the IUCN standards. Under NatureServe, *S. paposensis* was ranked as Critically Imperiled (N1), while *S. calderaensis*, *S. distincta* and *S. testalba* as Imperiled (N2). Likewise, one species of the genus *Stephadiscus* (*S. stuardoi*) was listed as Critically Endangered (CR), two Endangered (EN) (*S. celinae*, *S. rumbolli*) and one Least Concern (LC) (*S. lyratus*) using the IUCN standards. Under NatureServe, *S. stuardoi* was ranked as Critically Imperiled (N1), *S. celinae*, and *S. rumbolli* as Imperiled (N2), while *S. lyratus* Vulnerable (N3). Species distribution analysis showed that all species modelled have wider habitat ranges. Prompt conservation actions regarding micro-snail species are necessary to apply in Chile considering the high extinction risk of endemic and native species analysed in this study.

Acknowledgments

The authors thank Gustavo Darrigran for comments and anonymous referees for improving the manuscript.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

No funding was reported.

Author contributions

Conceptualization: MAV, GAC, CTD. Formal analysis: GAC, NF. Investigation: GAC. Methodology: NF, GAC. Project administration: GAC. Software: GAC. Supervision: GAC. Validation: MAV, GAC, MAV, CTD. Visualization: MAV, GAC. Writing – original draft: GAC. Writing – review and editing: NF, CTD, GAC, MAV, MAV.

Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

References

Aldea C, Novoa L, Rosenfeld S (2019) Lista sistemática actualizada de los Moluscos de Chile. v1.4, Universidad de Magallanes. http://gbif-chile.mma.gob.cl/ipt/resource?r=moluscos-chile [Accessed on 11.12.2021]

Anderson RP, Martinez-Meyer E (2004) Modelling species geographic distributions for preliminary conservation assessments: An implementation with the spiny pocket mice (*Heteromys*) of Ecuador. Biological Conservation 116: 167–179. https://doi.org/10.1016/S0006-3207(03)00187-3

Anonymous (2005) Environmental interpretation manual for protected areas in the Mesoamerican barrier reef system region. Technical Document No. 25. Belize City, Belize, 49 pp. https://docslib.org/doc/2760317/environmental-interpretation-manual-for-protected-areas-in-the-mbrs [Accessed on 11.12.2021]

Araya JF (2016) On some land snails (Mollusca: Gastropoda) of Los Molles, central Chile. Revista Mexicana de Biodiversidad 87(4): 1365–1368. https://doi.org/10.1016/j.rmb.2016.10.002

Araya JF, Catalán R (2014) A review of the non-bulimulid terrestrial Mollusca from the Region of Atacama, northern Chile. ZooKeys 398(398): 33–51. https://doi.org/10.3897/zookeys.398.4282

Araya JF, Miquel SE (2018) A new *Stephacharopa* (Gastropoda: Punctoidea: Charopidae) from Paposo, Northern Chile. Journal of Conchology 43: 129–133. https://doi.org/10.1127/arch.moll/1869-0963/142/227-235

Araya JF, Miquel SE, Martínez E (2017) New records of terrestrial mollusks (Gastropoda: Stylommatophora) from Antofagasta, northern Chile. Revista Mexicana de Biodiversidad 88(3): 769–772. https://doi.org/10.1016/j.rmb.2017.07.015

Arnaboldi T (2016) ¿Qué le hemos hecho al Laja? Outdoors. https://outdoors.cl/que-le-hemos-hecho-al-laja/ [Accessed on 11.12.2021]

Arredondo C, Eisenman E, Püschel N, Teneb E, Kusch A, Dougnac C, Vila A (2019) Diagnóstico de la relación entre la ganadería y los carnívoros en la Isla Grande de Tierra del Fuego, Chile: situación de los perros asilvestrados y los zorros chilla y culpeo. Wildlife Conservation Society, Punta Arenas, Chile. https://www.magyp.gob.ar/sitio/areas/depredadores/documentos/wcs-cl/WCS-Chile%20Diagnostico%20-Isla%20Grande-de-Tierra%20del-Fuego-2019.pdf [Accessed on 11.12.2021]

Austin M, Meyers J (1996) Current approaches to modelling the environmental niche of eucalypts: Implication for management of forest biodiversity. Forest Ecology and Management 85(1–3): 95–106. https://doi.org/10.1016/S0378-1127(96)03753-X

Bachman S, Moat J, Hill AW, Scott B (2011) Supporting Red List threat assessments with GeoCat: Geospatial conservation assessment tool. ZooKeys 150: 117–126. https://doi.org/10.3897/zookeys.150.2109

Balmford A, Leader-Williams N, Green MJB (1995) Parks or arks: Where to conserve threatened mammals? Biodiversity and Conservation 4(6): 595–607. https://doi.org/10.1007/BF00222516

Barker G (2012) *Sinployea monstrosa*. The IUCN Red List of Threatened Species 2012: e.T195594A2391518. https://doi.org/10.2305/IUCN.UK.2012.RLTS.T195594A2391518.en

Barker T, Mortimer M, Perrings C (2010) Biodiversity, ecosystems and ecosystem services. In: Kumar P (Ed.) The Economics of Ecosystems and Biodiversity: Ecological and Economic. Earthscan, Foundations London, UK, 41–104.

Behroozian M, Ejtehadi H, Peterson AT, Memariani F, Mesdaghi M (2020) Climate change influences on the potential distribution of *Dianthus polylepis* Bien. ex Boiss. (Caryophyllaceae), an endemic species in the Irano-Turanian region. PLoS ONE 15(8): e0237527. https://doi.org/10.1371/journal.pone.0237527

Bennie J, Huntley B, Wiltshire A, Hill MO, Baxter R (2008) Slope, aspect and climate: Spatially explicit and implicit models of topographic microclimate in chalk grassland. Ecological Modelling 216(1): 47–59. https://doi.org/10.1016/j.ecolmodel.2008.04.010

Berger-Tal O, Blumstein D, Swaisgood R (2020) Conservation translocations: A review of common difficulties and promising directions. Animal Conservation 23(2): 121–131. https://doi.org/10.1111/acv.12534

Berkmüller K, Savasdiasara T (1981) Guidelines and techniques for environmental interpretation. Ann Arbor, Michigan, University of Michigan.

Blank L, Blaustein L (2012) Using ecological niche modeling to predict the distributions of two endangered amphibian species in aquatic breeding sites. Hydrobiologia 693(1): 157–167. https://doi.org/10.1007/s10750-012-1101-5

Bloxam QMC, Tonge SJ, Horton R (1984) A preliminary account of the history and management of populations of endangered Moorean tree snail *Partula* spp. at the Jersey Wildlife Preservation Trust. The Dodo : journal of the Jersey Wildlife Preservation Trust 20: 73–79.

Burgos P (2015) Diagnóstico, estado y tendencias de la biodiversidad: Región de Antofagasta. Informe final. Diagnóstico del estado y tendencia de la biodiversidad en las regiones de Chile. Proyecto N° 82692: Planificación Nacional de la Biodiversidad para apoyar la implementación del Plan Estratégico de la Convención de Diversidad Biológica (CDB) 2011–2020. https://es.scribd.com/document/358657602/DIAGNOSTICO-ESTADO-Y-TENDENCIAS-DE-LA-BIODIVERSIDAD-REGION-DE-LA-ARAUCANIA [Accessed on 11.12.2021]

Burlakova L, Karatayev A, Karatayev V, May M, Bennett D, Cook M (2011) Endemic species: Contribution to community uniqueness, effect of habitat alteration, and conservation priorities. Biological Conservation 144(1): 155–165. https://doi.org/10.1016/j.biocon.2010.08.010

Cardinale B, Duffy J, Gonzalez A, Hooper D, Perrings C, Venail P, Narwani A, Tilman D, Wardle D, Kinzig A, Daily G, Loreau M, Grace J, Larigauderie A, Srivastava D, Naeem S (2012) Biodiversity loss and its impact on humanity. Nature 486(7401): 59–67. https://doi.org/10.1038/nature11148

Carvajal-Hernández CI, Juárez-Fragoso MA, Armenta-Montero S, Demeneghi-Calatayud AP, Vázquez-Torres M (2020) Different criteria for evaluation of the conservation status of *Ceratozamia miqueliana* (Zamiaceae). Tropical Conservation Science 13: 1–12. https://doi.org/10.1177/1940082920952682

CBD [Convention on Biological Diversity] (1992) Secretariat of the Convention on Biological Diversity. United Nations, Montreal, Canada. http://wbbb.wb.gov.in/pdf/ConventiononBiologicalDiversity1992.pdf [Accessed on 11.12.2021]

Chivian E (2001) Environment and health: 7. Species loss and ecosystem disruption — the implications for human health. Canadian Medical Association Journal 164: 66–69.

Clavero M, García-Berthou E (2005) Invasive species are a leading cause of animal extinction. Trends in Ecology & Evolution 20(3): 110. https://doi.org/10.1016/j.tree.2005.01.003

Collado GA (2012) Nuevo registro de distribución geográfica y antecedentes de historia natural de *Heleobia chimbaensis* (Biese, 1944) (Caenogastropoda: Cochliopidae) en la costa del desierto de Atacama: implicancias para su conservación. Amici Molluscarum 20(2): 13–18.

Collado GA, Salvador RB, Vidal M, Parra Aravena F, Delgado V, Torres-Díaz C (2023) Distribution, conservation status and proposed measures for preservation of *Radiodiscus* microgastropods in Chile. PeerJ 11: e14027. https://doi.org/10.7717/peerj.14027

CONAF [Corporación Nacional Forestal] (2020) Conmemoran el 10º aniversario del Parque Nacional Alerce Costero. https://www.conaf.cl/conmemoran-10-o-aniversario-del-parque-nacional-alerce-costero/ [Accessed on 11.12.2021]

Cuezzo MG, Medina R, Nieto C (2021) Geographic distribution modeling and taxonomy of *Stephadiscus lyratus* (Cothouny in Gould, 1846) (Charopidae) reveal potential distributional areas of the species along the Patagonian Forests. PeerJ 9: e11614. https://doi.org/10.7717/peerj.11614

DAPMA [Departamento Áreas Protegidas y Medio Ambiente] (2009) Plan de manejo Reserva Nacional Alacalufe (No. 536). Gobierno de Chile, Ministerio de Agricultura, CONAF. http://areasprotegidas.mma.gob.cl/areas-protegidas/ [Accessed on 11.12.2021]

Díaz P, Jaque E, Ojeda C (2018) Presión hídrica en ambientes lacustres de alta montaña: Entre el cambio climático y el desarrollo energético. Laguna del Laja, Chile. Diálogo Andino 55(55): 143–158. https://doi.org/10.4067/S0719-26812018000100143

El Heraldo Austral (2019) Guardaparques del Parque Nacional Vicente Pérez Rosales realizan limpieza en Petrohué. https://www.eha.cl/noticia/local/guardaparques-del-parque-nacional-vicente-perez-rosales-realizan-limpieza-en-petrohue-3876 [Accessed on 11.12.2021]

Enríquez J (2021) Extinguen incendios forestales en el Parque Nacional Villarrica. biobiochile.cl. https://www.biobiochile.cl/noticias/nacional/region-de-la-araucania/2021/02/14/extinguen-incendios-forestales-en-el-parque-nacional-villarrica.shtml [Accessed on 11.12.2021]

Escobar D, Jojoa LM, Díaz SR, Rudas E, Albarracín RD, Ramírez C, Gómez JY, López CR, Saavedra J, Ortiz R (2016) Georreferenciación de localidades: Una guía de referencia para colecciones biológicas, versión 4.0. Bogotá, Colombia: Instituto de Investigación de Recursos Biológicos Alexander von Humboldt y Instituto de Ciencias Naturales, Universidad Nacional de Colombia, 144 pp. https://hdl.handle.net/20.500.11761/35180 [Accessed on 11.12.2021]

ESRI (2011) ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute.

Faber-Langendoen D, Nichols J, Master L, Snow K, Tomaino A, Bittman R, Hammerson G, Heidel B, Ramsay L, Teucher A, Young B (2012) NatureServe Conservation Status Assessments: Methodology for Assigning Ranks. NatureServe, Arlington, VA, 44 pp. https://www.natureserve.org/publications/natureserve-conservation-status-assessments-methodology-assigning-ranks

FAO and UNEP (2020) The State of the World's Forests 2020. Forests, biodiversity and people. Rome. https://doi.org/10.4060/ca8642en [Accessed on 11.12.2021]

Fick SE, Hijmans RJ (2017) WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. International Journal of Climatology 37(12): 4302–4315. https://doi.org/10.1002/joc.5086

Gärdenfors U (2001) Classifying threatened species at national versus global levels. Trends in Ecology & Evolution 16(9): 511–516. https://doi.org/10.1016/S0169-5347(01)02214-5

Gaston KJ (1998) Rarity as double jeopardy. Nature 394(6690): 229–230. https://doi.org/10.1038/28288

GEF [Global Environment Facility] (2019) Proyecto GEF Castor confirma presencia de la especie invasora en todos los ríos de la Reserva Laguna Parrillar. Conservando los ecosistemas de la Patagonia chilena manejo, prevención y control del castor. https://gefcastor.mma.gob.cl/proyecto-gef-castor-confirma-presencia-de-la-especie-invasora-en-todos-los-rios-de-la-reserva-laguna-parrillar/ [Accessed on 11.12.2021]

Gould AA (1846) Descriptions of new shells, collected by the United States Exploring Expedition. Proceedings of the Boston Society of Natural History 2: 87–196. https://doi.org/10.5962/bhl.part.3318

Graells G, Corcoran D, Aravena JC (2015) Prospección y datación de la fecha de colonización del área del Río Hollemberg, provincia de Última Esperanza, el punto más septentrional de presencia de *Castor canadensis* (Castoridae) en Sudamérica. Anales del Instituto de la Patagonia 43(2): 61–67. https://doi.org/10.4067/S0718-686X2015000200004

Hadfield MG, Holland BS, Olival KJ (2004) Contributions of ex-situ propagation and molecular genetics to conservation of Hawaiian trees snails. In: Gordon MG, Bartol SM (Eds) Experimental approaches to conservation biology. University of California Press, Berkeley, 16–34. https://doi.org/10.1525/california/9780520240247.003.0003

Herbert DG (2004) *Trachycystis clifdeni*. The IUCN Red List of Threatened Species 2004: e.T44710A10938455. https://doi.org/10.2305/IUCN.UK.2004.RLTS.T44710A10938455.en

Hylton Scott MI (1969) Endodóntidos neotropicales IV (Moll. Pulm.). Neotrópica 15: 59–63.

Hylton Scott MI (1970) Endodóntidos de la región austral americana (Gastropoda-Pulm.). Revista del Museo Argentino de Ciencias Naturales "Bernardino Rivadavia". Zoologia 10(18): 267–296.

Hylton Scott MI (1973) Endodóntidos neotropicales IV (Gastropoda Pulmonata). Neotrópica 19: 104–109.

Hylton Scott MI (1979) Información malacológica de la Campaña de la Transecta de Botánica de Patagonia Austral. Neotrópica 25: 3–8.

IUCN (2001) IUCN Red List Categories and Criteria: version 3.1. IUCN Species Survival Commision. Switzerland and Cambridge, IUCN, UK.

IUCN (2002) Technical Guidelines on the Management of Ex-situ populations for Conservation. International Union for the Conservation of Nature, Gland, Switzerland. https://portals.iucn.org/library/efiles/documents/Rep-2002-017.pdf [Accessed on 11.12.2021]

IUCN (2003) Guidelines for Application of IUCN Red List Criteria at Regional Levels, Version 3.0. Gland, Switzerland and Cambridge, UK. https://portals.iucn.org/library/node/8255 [Accessed on 26 March 2022]

IUCN (2012) IUCN Red List categories and criteria, version 3.1. 2nd edn. IUCN Gland, Switzerland. https://portals.iucn.org/library/node/10315 [Accessed on 11.12.2021]

IUCN/SSC (2013) Guidelines for reintroductions and other conservation translocations. Version 1.0. IUCN, Gland, Switzerland. https://portals.iucn.org/library/efiles/documents/2013-009.pdf [Accessed on 11.12.2021]

IUCN (2019) Guidelines for using the IUCN Red List categories and criteria. Version 14. Prepared by the Standards and Petitions Committee. https://www.iucnredlist.org/resources/redlistguidelines [Accessed on 11.12.2021]

IUCN (2022) Guidelines for using the IUCN Red List categories and criteria. Version 15. Prepared by the Standards and Petitions Committee. [Accessed on 11.12.2021]

IUCN/SSC (2014) Guidelines on the use of *ex situ* management for species conservation. Version 2.0. IUCN Species Survival Commission, Gland. https://portals.iucn.org/library/sites/library/files/documents/2014-064-Es.pdf

Kadis C, Thanos CA, Laguna Lumbreras E (2013) Plant micro-reserves: from theory to practice. Utopia Publishing.

Kahraman A, Bagherpour S, Karabacak E, Doğan M, Dogan H, Uysal I, Celep F (2012) Reassessment of conservation status of the genus *Salvia* (Lamiaceae) in Turkey II. Turkish Journal of Botany 36: 103–124. https://doi.org/10.3906/bot-1007-2

Laguna E, Deltoro VI, Pèrez-Botella J, Pèrez-Rovira P, Serra L, Olivares A, Fabregat C (2004) The role of small reserves in plant conservation in a region of high diversity in eastern Spain. Biological Conservation 119(3): 421–426. https://doi.org/10.1016/j.biocon.2004.01.001

Loo SE, Keller R, Leung B (2007) Freshwater invasions: Using historical data to analyse spread. Diversity & Distributions 13(1): 23–32. https://doi.org/10.1111/j.1366-9516.2006.00313.x

Luck GW, Daily GC, Ehrlich PR (2003) Population diversity and ecosystem services. Trends in Ecology & Evolution 18(7): 331–336. https://doi.org/10.1016/S0169-5347(03)00100-9

Mace GM, Pearce-Kelly P, Clarke D (1998) An integrated conservation programme for the tree snails (Partulidae) of Polynesia: A review of captive and wild elements. Memoirs of the Museum of Victoria 56: 431–433. https://doi.org/10.24199/j.mmv.1997.56.34

Mace GM, Collar NJ, Gaston KJ, Hilton-Taylor C, Akcakaya HR, Leader-Williams N, Milner-Gulland EJ, Stuart SN (2008) Quantification of extinction risk: IUCN's system for classifying threatened species. Conservation Biology 22(6): 1424–1442. https://doi.org/10.1111/j.1523-1739.2008.01044.x

Magallanes G (2012) Estrategia Regional de Desarrollo Magallanes y Antártica chilena 2012–2020. Gobierno Regional de Magallanes y Antártica Chilena, Chile. https://www.goremagallanes.cl/sitioweb/documentos/ERD%20Magallanes2020.pdf

Manel S, Williams HC, Ormerod SJ (2001) Evaluating presence-absence models in ecology: The need to account for prevalence. Journal of Applied Ecology 38(5): 921–931. https://doi.org/10.1046/j.1365-2664.2001.00647.x

Martinic M (2004) La minería del carbón en Magallanes entre 1868–2003. Historia (Santiago) 37: 129–167.

Mason RA (2015) The sixth mass extinction and chemicals in the environment: Our environmental deficit is now beyond nature's ability to regenerate. Journal of Biological Physics and Chemistry 15(3): 160–176. https://doi.org/10.4024/10MA15F.jbpc.15.03

Master L, Faber-Langendoen D, Bittman R, Hammerson G, Heidel B, Nichols J, Ramsay L, Tomaino A (2009) Natureserve conservation status assessments: factors for assessing extinction risk. NatureServe, Arlington, VA.

Maxwell SL, Fuller RA, Brooks TM, Watson JE (2016) The ravages of guns, nets and bulldozers. Nature 536(7615): 144–145. https://doi.org/10.1038/536143a

Meuser E, Harshaw HW, Mooers AØ (2009) Public preference for endemism over other conservation-related species attributes. Conservation Biology 23(4): 1041–1046. https://doi.org/10.1111/j.1523-1739.2009.01257.x

Miquel SE, Araya JF (2013) A new Charopidae from Chile and Argentina, *Stephacharopa calderaensis* n. gen. and n. sp., with remarks on the taxonomy of the genus *Stephadiscus* Hylton Scott 1981 (Mollusca: GastropodaPulmonata). Archiv für Molluskenkunde 142: 227–235. https://doi.org/10.1127/arch.moll/1869-0963/142/227-235

Miquel SE, Barker GM (2009) New Charopidae from Chilean-Argentine Patagonia: (Mollusca: Gastropoda: Stylommatophora). Archiv für Molluskenkunde 138(1): 53–61. https://doi.org/10.1127/arch.moll/0003-9284/138/053-061

Miquel SE, Cádiz Lorca JF (2008) *Araucocharopa gallardoi* n. gen. et n. sp. de Charopidae (Mollusca Gastropoda Stylommatophora) del sur de Chile. Revista del Museo

Argentino de Ciencias Naturales, Nueva Serie 10: 329–340. https://doi.org/10.22179/REVMACN.10.287

Miquel SE, Ramírez R (2011) First records of actual and fossil *Stephadiscus* outside of Patagonia, and the description of a new Amazonian species (Mollusca: Pulmonata: Charopidae). Archiv für Molluskenkunde 140(1): 49–56. https://doi.org/10.1127/arch.moll/1869-0963/140/049-056

Miquel SE, Ramírez R, Thomé JW (2007) Biodiversidad y taxonomía de micromoluscos Punctoidea del sur de Brasil, con la descripción de una nueva especie de *Radiodiscus* de la Mata Atlántica (Mollusca, Gastropoda, Pulmonata). Revista del Museo Argentino de Ciencias Naturales, Nueva Serie 9: 205–230. https://doi.org/10.22179/REVMACN.9.308

MMA [Ministerio del Medio Ambiente] (2022) Inventario nacional de especies de Chile. Ministerio del Medio Ambiente de Chile, Chile. https://clasificacionespecies.mma.gob.cl/procesos-de-clasificacion/ [Accessed on 17.12.2022]

Molina R, Soto N, Tapia A (2018) Estado actual de la distribución del castor *Castor canadensis* Kuhl 1820 (Rodentia) en el área continental de la región de Magallanes, Chile. Anales del Instituto de la Patagonia 46(3): 7–15. https://doi.org/10.4067/S0718-686X2018000300007

MolluscaBase (2021a) *Stephacharopa* Miquel and Araya, 2013. https://www.molluscabase.org/aphia.php?p=taxdetailsandid=996035 [Accessed on 17.12.2021]

MolluscaBase (2021b) *Stephadiscus* Hylton Scott, 1981. https://www.molluscabase.org/aphia.php?p=taxdetailsandid=996036 [Accessed on 17.12.2021]

Morales Flores A (2019) Concepción Cota 50: Interfaz urbano-forestal como proyecto de paisaje. MSc thesis, Pontificia Universidad Católica de Chile, Chile, 175 pp.

Muratov IV, Abdou A, Bouchet P (2005) Charopid land snails (Gastropoda Pulmonata Charopidae) from Mayotte, Comores: Alive and well. Tropical Zoology 18(2): 171–208. https://doi.org/10.1080/03946975.2005.10531220

NatureServe (2006) NatureServe Explorer: An online encyclopedia of life (web application). Version 6.1. NatureServe, Arlington, VA. http://www.natureserve.org/explorer/ranking.htm [Accessed on 17.12.2021]

NatureServe (2021) Products. Conservation Rank Calculator. https://www.natureserve.org/products/conservation-rank-calculator [Accessed on 17.12.2021]

Navrátil J, Knotek J, Pícha K (2016) The significance of self-guided interpretive trails in protected areas for the environmental education of visitors. Socijalna Ekologija 24: 5–21. https://doi.org/10.17234/SocEkol.24.1.3

Pearce-Kelly P, Clarke D, Walker C, Atkin P (1997) A conservation programme for the partulid tree snails of the Pacific region. Memoirs of the Museum of Victoria 56(2): 431–433. https://doi.org/10.24199/j.mmv.1997.56.34

Pearson R, Raxworthy C, Nakamura M, Peterson A (2007) Predicting species distributions from small numbers of occurrence records: A test case using cryptic geckos in Madagascar. Journal of Biogeography 34(1): 102–117. https://doi.org/10.1111/j.1365-2699.2006.01594.x

Phillips SJ, Anderson RP, Schapire RE (2006) Maximum entropy modeling of species geographic distributions. Ecological Modelling 190(3–4): 231–259. https://doi.org/10.1016/j.ecolmodel.2005.03.026

QGIS Development Team (2021) QGIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org [Accessed on 11.12.2021]

Rawat US, Agarwal NK (2015) Biodiversity: Concept, threats and conservation. Environmental Conservation 16(3): 19–28. https://doi.org/10.36953/ECJ.2015.16303

Régnier C, Fontaine B, Bouchet P (2009) Not knowing, not recording, not listing: Numerous unnoticed mollusk extinctions. Conservation Biology 23(5): 1214–1221. https://doi.org/10.1111/j.1523-1739.2009.01245.x

Reid WV (1998) Biodiversity hotspots. Trends in Ecology & Evolution 13(7): 275–280. https://doi.org/10.1016/S0169-5347(98)01363-9

Robles C (2021) Retiran más de 3 mil kilos de basura desde el Parque Nacional Laguna del Laja. La Tribuna. https://www.latribuna.cl/noticias/2021/08/14/retiran-mas-de-3-mil-kilos-de-basura-desde-el-parque-nacional-laguna-del-laja.html [Accessed on 11.12.2021]

Rodríguez PE, Miquel SE, Tauber AA, Krapovickas JM (2012) First record of land gastropods (Mollusca, Pulmonata, Charopidae) in the early-middle Miocene from Santa Cruz province, Austral Patagonia, Argentina. Archiv für Molluskenkunde 141: 57–66. https://doi.org/10.1127/arch.moll/1869-0963/141/057-066

Root-Bernstein M, Montecinos Carvajal Y, Ladle R, Jepson P, Jaksic F (2013) Conservation easements and mining: The case of Chile. Earth's Future 1(1): 33–38. https://doi.org/10.1002/2013EF000136

Salvador RB, Brook FJ, Shepherd LD, Kennedy M (2020) Molecular phylogenetic analysis of Punctoidea (Gastropoda, Stylommatophora). Zoosystematics and Evolution 96(2): 397–410. https://doi.org/10.3897/zse.96.53660

SONAMI [Sociedad Nacional de Minería] (2021) Mapa minero. https://consejominero.cl/nosotros/mapa-minero/ [Accessed on 11.12.2021]

Squeo FA, Estades C, Bahamonde N, Cavieres L, Rojas G, Benoit I, Parada E, Fuentes A, Avilés R, Palma A, Solís R, Guerrero S, Montenegro G, Torres-Mura JC (2010) Revisión de la clasificación de especies en categorías de amenaza en Chile. Revista Chilena de Historia Natural 83(4): 511–529. https://doi.org/10.4067/S0716-078X2010000400006

Stuardo JR, Vega R (1985) Synopsis of the land Mollusca of Chile. With remarks on distribution. Studies on Neotropical Fauna and Environment 20(3): 125–146. https://doi.org/10.1080/01650528509360682

Subsecretaría de Turismo (2015) Plan de acción de turismo sustentable en Áreas Protegidas del Estado 2014-2018. Subsecretaría de Turismo, Santiago, Chile. https://ccps.mma.gob.cl/documentos/plan-de-accion-de-turismo-sustentable-en-areas-protegidas-del-estado-2014-2018/ [Accessed on 11.12.2021]

Subsecretaría de Turismo (2017) Proyecto puesta en valor Parque Nacional Villarrica, Región de la Araucanía. Gobierno de Chile, SERNATUR, CONAF, Santiago, Chile. http://www.subturismo.gob.cl/wp-content/uploads/2015/11/FICHA-VILLARRICA_13032017.pdf [Accessed on 11.12.2021]

Tablado A, Mantinian J (2004) Catálogo de ejemplares tipo de la División Invertebrados del Museo Argentino de Ciencias Naturales. II. Mollusca. Revista del Museo Argentino de Ciencias Naturales, Nueva Serie 6: 363–384. https://doi.org/10.22179/REVMACN.6.95

Thomas CD, Cameron A, Green RE, Bakkenes B, Beaumont LJ, Collingham YC, Erasmus BFN, Ferriera de Siqueira M, Grainger A, Hannah L, Hughes L, Huntley B, Van Jaarsveld AS, Midgley GF, Miles L, Ortega-Huerta MA, Peterson AT, Phillips OL, Williams SE (2004) Extinction risk from climate change. Nature 427(6970): 145–148. https://doi.org/10.1038/nature02121

Tonge S, Bloxam Q (1991) A review of the captive-breeding programme for Polynesian tree snails *Partula* spp. International Zoo Yearbook 30(1): 51–59. https://doi.org/10.1111/j.1748-1090.1991.tb03465.x

Trias-Blasi A, Gücel S, Özden Ö (2017) Current distribution and conservation status re-assessment of the Cyprus Tulip (*Tulipa cypria*: Liliaceae), new data from Northern Cyprus. Plant Biosystems 151(3): 394–402. https://doi.org/10.1080/11263504.2016.1174177

Valderrama L, Contreras-Reyes JE, Carrasco R (2018) Ecological impact of forest fires and subsequent restoration in Chile. Resources 7(2): 26. https://doi.org/10.3390/resources7020026

Valdovinos Zarges C (1999) Biodiversidad de moluscos chilenos: Base de datos taxonómica y distribucional. Gayana (Concepción) 63: 111–164.

Vermeulen J, Marzuki M, Seddon MB (2014) *Charopa lafargei*. The IUCN Red List of Threatened Species 2014: e.T64835554A64864208. https://doi.org/10.2305/IUCN.UK.2014-3.RLTS.T64835554A64864208.en

Wieczorek J, Guo Q, Hijmans R (2004) The point-radius method for georeferencing locality descriptions and calculating associated uncertainty. International Journal of Geographical Information Science 18(8): 745–767. https://doi.org/10.1080/13658810412331280211

Supplementary material 1

List of geographical coordinates and threats affecting species

Authors: Gonzalo A. Collado, Nataly Flores, Marcela A. Vidal, Cristian Torres-Díaz, Moisés A. Valladares

Data type: Geographical coordinates and threats (.xlsx file)

Link: https://doi.org/10.3897/natureconservation.53.100631.suppl1

Supplementary material 2

NatureServe assessment

Authors: Gonzalo A. Collado, Nataly Flores, Marcela A. Vidal, Cristian Torres-Díaz, Moisés A. Valladares

Data type: table (.docx file)

Explanation note: Input data of Chilean species for NatureServe assessment.

Link: https://doi.org/10.3897/natureconservation.53.100631.suppl2

Supplementary material 3

Species distribution models

Authors: Gonzalo A. Collado, Nataly Flores, Marcela A. Vidal, Cristian Torres-Díaz, Moisés A. Valladares

Data type: table (.docx file)

Explanation note: Relative contributions (in percentage) of the climate and environmental variables in species of *Stephacharopa* and *Stephadiscus* and model validation results.

Link: https://doi.org/10.3897/natureconservation.53.100631.suppl3

Nature Conservation

Nature Conservation 53: 319–320 (2023)
DOI: 10.3897/natureconservation.53.113548

Corrigenda

Corrigendum: Using drone imagery to obtain population data of colony-nesting seabirds to support Canada's transition to the global Key Biodiversity Areas program. Nature Conservation 51: 155–166. doi:10.3897/natureconservation.51.96366

Lindsay A. R. Lalach[1], David W. Bradley[1], Douglas F. Bertram[2], Louise K. Blight[3,4]

1 *Birds Canada, Delta, BC, V4K 2T9, Canada*
2 *Institute of Ocean Sciences, Environment Canada Wildlife Research Division, 9860 West Saanich Rd, P.O. Box 6000, Sidney, BC, V8L 4B2, Canada*
3 *School of Environmental Studies, University of Victoria, Victoria, British Columbia V8P 5C2, Canada*
4 *Procellaria Research & Consulting, Salt Spring Island, British Columbia V8K 1G2, Canada*
Corresponding author: Lindsay A. R. Lalach (lindsay.lalach@gmail.com)

In the recent paper "Using drone imagery to obtain population data of colony-nesting seabirds to support Canada's transition to the global Key Biodiversity Areas program" by Lalach et al. (2023), the authors used quantitative global and national thresholds to determine whether an Important Bird Area (IBA) for the Glaucous-winged Gull (*Larus glaucescens*) met the criteria for conversion to a Key Biodiversity Area (KBA). The threshold values used were taken from the Birds Canada IBA-KBA crosswalk database (https://kba-maps.deanrobertevans.ca/). Because national estimates of bird populations (mostly derived from Breeding Bird Survey data and resulting Partner in Flight estimates) are thought to be negatively biased in Canada, this database instead uses continental population estimates for national thresholds (Sólymos et al. 2020; D.R. Evans pers. comm). In the paper, these national thresholds were erroneously referred to as being based on national population estimates, when they are actually based on the continental estimates.

This error means that the national KBA threshold of 4,400 individuals reported in Lalach et al. (2023) is based on the continental population estimate for this species (as per the National KBA Standard Protocol for this ongoing IBA to KBA conversion) (KBA Canada Coalition 2021). This resulted in a higher threshold value than would be expected based on the actual national Glaucous-winged Gull population estimates of 47,800 individuals (Rodway et al. 2023). If the IBA-KBA crosswalk were to use this national estimate, a site with only 478 breeding individuals would trigger the National KBA criterion.

OPEN ACCESS

This article is part of:
Remote Sensing Applications to Monitor Ecosystem Services
Edited by Javier Martinez-Lopez, Domingo Alcaraz, Simon Willcock, Javier Cabello, Francisco J. Bonet & Joris de Vente

Academic editor: Francisco J. B. García
Received: 1 October 2023
Accepted: 7 November 2023
Published: 20 November 2023

ZooBank: https://zoobank.org/757A8B39-4ACD-4621-8756-941808EFF099

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Citation: Lalach LAR, Bradley DW, Bertram DF, Blight LK (2023) Corrigendum: Using drone imagery to obtain population data of colony-nesting seabirds to support Canada's transition to the global Key Biodiversity Areas program. Nature Conservation 51: 155–166. doi:10.3897/natureconservation.51.96366. Nature Conservation 53: 319–320. https://doi.org/10.3897/natureconservation.53.113548

Funding

No funding was reported.

Author contributions

All authors have contributed equally.

Author ORCIDs

Lindsay A. R. Lalach https://orcid.org/0000-0002-3154-5412
Louise K. Blight https://orcid.org/0000-0001-6610-7773

Data availability

All of the data that support the findings of this study are available in the main text.

References

KBA Canada Coalition (2021) A National Standard for the Identification of Key Biodiversity Areas in Canada v. 1.0. Wildlife Conservation Society Canada and Key Biodiversity Area Canada Coalition, Toronto. https://doi.org/10.19121/2021.Report.39502

Lalach LAR, Bradley DW, Bertram DF, Blight LK (2023) Using drone imagery to obtain population data of colony-nesting seabirds to support Canada's transition to the global Key Biodiversity Areas program. Nature Conservation 51: 155–166. https://doi.org/10.3897/natureconservation.51.96366

Rodway MS, Campbell WR, Lemon MJF [Preliminary Edition] (2023) Seabird Colonies of British Columbia. Vol. 4: Salish Sea: A history to 2022.

Sólymos P, Toms JD, Matsuoka SM, Cumming SG, Barker NKS, Thogmartin WE, Stralberg D, Crosby AD, Dénes FV, Haché S, Mahon CL, Schmiegelow FKA, Bayne EM (2020) Lessons learned from comparing spatially explicit models and the partners in flight approach to estimate population sizes of boreal birds in Alberta, Canada. The Condor 122(2): duaa007. https://doi.org/10.1093/condor/duaa007

Nature Conservation 53: 321–340 (2023)
DOI: 10.3897/natureconservation.53.113052

Research Article

Small reserve but high diversity: butterfly community across an altitudinal gradient in the Brazilian Atlantic Forest

Andrêsa G. Andrade[1], Cássio Cardoso Pereira[2], Vinícius F. Sperandei[3], Tatiana Cornelissen[4]

1 *Programa de Pós-Graduação em Ecologia, Universidade Federal de São João del-Rei, 36301-160, São João del-Rei, MG, Brazil*
2 *Programa de Pós-Graduação em Ecologia, Conservação e Manejo da Vida Silvestre, Departamento de Genética, Ecologia e Evolução, Universidade Federal de Minas Gerais, 31270-901, Belo Horizonte, MG, Brazil*
3 *Universidade de Rio Verde, Núcleo de Disciplina Comum, 75901-970, Rio Verde, GO, Brazil*
4 *Centro de Síntese Ecológica e Conservação, Departamento de Genética, Ecologia e Evolução, Universidade Federal de Minas Gerais, 31270-901, Belo Horizonte, MG, Brazil*
Corresponding author: Cássio Cardoso Pereira (cassiocardosopereira@gmail.com)

Abstract

The present study aimed to describe the composition of the butterfly community in relation to the altitudinal gradient in the Reserva Particular do Patrimônio Natural (RPPN) Alto-Montana, Serra da Mantiqueira, Minas Gerais, Brazil, and to provide a list of species for the area. We collected samples in the RPPN Alto-Montana along an altitudinal gradient from 1400 to 2100 m, between the dry and rainy seasons of 2018 and 2019. During this period, the sampling method utilizing Van-Someren Rydon traps totaled 3,936 hours and the effort using sweep nets totaled 246 hours. A total of 1,253 butterflies distributed across 124 species and six families of diurnal butterflies were observed (Hesperiidae, Lycaenidae, Nymphalidae, Papilionidae, Pieridae, and Riodinidae). Nymphalidae was the most representative family, followed by Hesperiidae, Pieridade, Lycaenidae, Papilionidae and Riodinidae. Moreover, we recorded 37 species with only one individual (*singletons*) and 20 species with only two individuals (*doubletons*), totalling 57 species, which corresponds to 46% of all sampled richness. The rarefaction curve did not reveal a tendency toward stabilization. However, the indices showed slightly higher values for the 124 species sampled. The analysis performed using the Bootstrap estimator predicted a total of 143.22 species (± 10.87 SE), with a further 19 additional species than observed. Chao 1 predicted 153.42 (± 11.82 SE), and Jackknife 1 predicted 164.00 (± 16.29 SE) species, with 29 and 40 additional species than the observed, respectively. Our study contributes to the knowledge of butterfly biodiversity in Serra da Mantiqueira and reveals a high species richness for the RPPN Alto Montana, especially considering the relatively small area. In addition, our study provides the first inventory of butterflies for the RPPN Alto Montana, thus supporting further studies investigating the butterfly richness in the Serra da Mantiqueira region. Finally, our findings of endemic, rare, and endangered butterfly species highlight the relevance of further conservation strategies to be considered for the Protected Area's Management Plan.

Key words: Conservation, Hesperiidae, inventory, Lepidoptera, Nymphalidae, species richness, threatened species

Academic editor: Daniel Silva
Received: 20 September 2023
Accepted: 15 November 2023
Published: 29 November 2023

ZooBank: https://zoobank.org/F3ED8426-677E-4BA3-B156-5BBE804B5FEC

Citation: Andrade AG, Pereira CC, Sperandei VF, Cornelissen T (2023) Small reserve but high diversity: butterfly community across an altitudinal gradient in the Brazilian Atlantic Forest. Nature Conservation 53: 321–340. https://doi.org/10.3897/natureconservation.53.113052

Introduction

Covering part of the Atlantic coast of South America, the Atlantic Forest Domain is composed of a mosaic of tropical vegetation with typical forest formations, altitude grasslands, restingas, and mangroves (Joly et al. 2014; Soldati et al. 2019; Marques and Grelle 2021). With the urbanization of recent centuries, resource exploitation, and land-use change, much of the Atlantic Forest's cover has been deforested and fragmented, with today only around 11% of its original area remaining (Ribeiro et al. 2009; de Lima 2020). Approximately 90% of its extension is located in Brazilian territory (Ribeiro et al. 2009; Colli-Silva et al. 2020), and its great richness of fauna and flora associated with high rates of endemism make this Domain a biodiversity hotspot (Myers et al. 2000). This richness is reflected in the fact that the Atlantic Forest is home to an enormous diversity of Lepidoptera. One of the reasons for this diversity is the vegetation associated with altitudinal and climatic variations, which provides heterogeneous environments that favor the occurrence of several species of butterflies (Soldati et al. 2019).

The butterfly richness for the Atlantic Domain is approximately 2,100 species (Francini et al. 2011). These values correspond to about 59% of the total butterfly richness for Brazil - 3,517 species (Casagrande and Duarte 2023) and 23% of the total butterfly richness for Latin America - 9,000 species (Raven et al. 2020). This high diversity can be explained by environmental characteristics such as habitat heterogeneity, and also by inter and intra specific interactions such as competition, factors that reflect on the different colors, body shape, and diet of these organisms in their different life stages - juvenile or adult (Stevens et al. 2012). Different life stages allow butterflies to occupy a wide range of habitats, mainly because they do not compete for food. When in the juvenile stage, they have mandibles and feed on leaves, flowers, and other plant parts such as stems (Stevens et al. 2012). When adults, they have proboscis and are classified into two major guilds: Fruit-feeding when food is based on fermented fruits, sap, and/or exudates, and nectarivores when flower nectar is their food resource (DeVries et al. 1997). Butterflies are also involved in various ecological interactions such as pollination, herbivory, plant population dynamics, and serving as food for higher trophic level organisms (DeVries et al. 1997).

All these characteristics classify them as bioindicators of environmental quality and illustrate the capacity with which this group responds quickly to landscape and climate changes (Ribeiro et al. 2012). Thus, butterflies are an excellent study group because they are sensitive to environmental disturbances, allowing to better evaluate the impacts caused to the environment (DeVries et al. 1997). In addition, they are organisms that are relatively easy to capture, having short life cycles and well-known taxonomy (Ribeiro et al. 2012). That way, even though they are an abundant group, butterflies have been suffering major population declines (Sánchez-Bayo and Wyckhuys 2019). This decline comes from the increasing loss of habitat caused by the exploitation of natural resources, deforestation, intensive agriculture, pollution, climate change, and species introduction (Sánchez-Bayo and Wyckhuys 2019). Today, many species are listed as endangered species (Chowdhury et al. 2023). Given this scenario, studies aimed at understanding patterns of distribution and diversity of butterflies are important tools to better support conservation actions, such as the creation of conservation units and improved wildlife management (Chowdhury et al. 2023).

Although studies with butterflies have increased in recent decades, inventories for this group in mountainous regions are still scarce (Quintero and Jetz 2018; Girardello et al. 2019; Shirai et al. 2019). Some explanations for this scarcity are these places' logistical and natural difficulties, such as rugged terrain and access difficulties (Quintero and Jetz 2018; Girardello et al. 2019). In the Serra da Mantiqueira region, some efforts aimed at understanding the composition of the butterfly community were carried out by Zikán and Zikán (1968), Freitas et al. (2011) and Vieira et al. (2022). This study aims to complement the knowledge about the butterfly community of Serra da Mantiqueira, particularly for the southern region of Minas Gerais. Therefore, our objective is to describe the composition of the butterfly community related to the altitudinal gradient in the Reserva Particular do Patrimônio Natural (RPPN) Alto-Montana, Serra da Mantiqueira, Minas Gerais, Brazil, and to provide a list of species for the area.

Materials and methods

Study area

The study was carried out in the Reserva Particular do Patrimônio Natural (RPPN) Alto-Montana, in the municipality of Itamonte, in the southern region of the state of Minas Gerais, Brazil (22°21'55"S, 44°48'32"W, Fig. 1). The reserve has about 672 ha and is part of the Serra da Mantiqueira Environmental Protection Area (Mazza et al. 2018; Souza et al. 2021). The landscape is quite rugged, with a granite outcrop that stands out in the landscape, ranging from 1,400 to 2,500 m in altitude. Its climate is subtropical highland climate (Cwb), with mild, dry winters and rainy summers (Alvares et al. 2013). The average annual temperature is 18.6 °C, and the average rainfall is 1,749 mm, with periods of heavy rain from December to January (Cruz et al. 2014; Vilanova 2015; ICMBio 2018). The vegetation is diverse, being composed of typical phytophysiognomies of the Atlantic Domain, with the presence of Floresta Estacional Semidecidual, Floresta Ombrófila Densa Montana, Floresta Ombrófila Densa Altomontana (Cloud Forest), Floresta Ombrófila Mista and Campos de Altitude in the higher altitudes (Fig. 2A, B). The RPPN Alto Montana is one of the only places in Minas Gerais with the native presence of *Araucaria angustifolia* (Bertol.) Kuntze, the dominant species of Floresta Ombrófila Mista (Cruz et al. 2014; Pompeu et al. 2014; ICMBio 2018).

Data sampling

The butterfly samplings were carried out along the altitudinal gradient in an access trail in the northern area of the RPPN Alto-Montana. This region of the protected area has a slope of 700 meters starting from 1,400 m to a maximum altitude of 2,100 m above sea level. In order to carry out the sampling of butterflies along the gradient, eight altitudinal elevations were delimited with the aid of a Garmin® GPS, distributed across every 100 m of altitude, over a period of one year, in four collection periods, contemplating the rainy season, the dry season, and the transition between the two. Sampling in different seasons ensures that species that have life cycles in different periods can be captured and sampled,

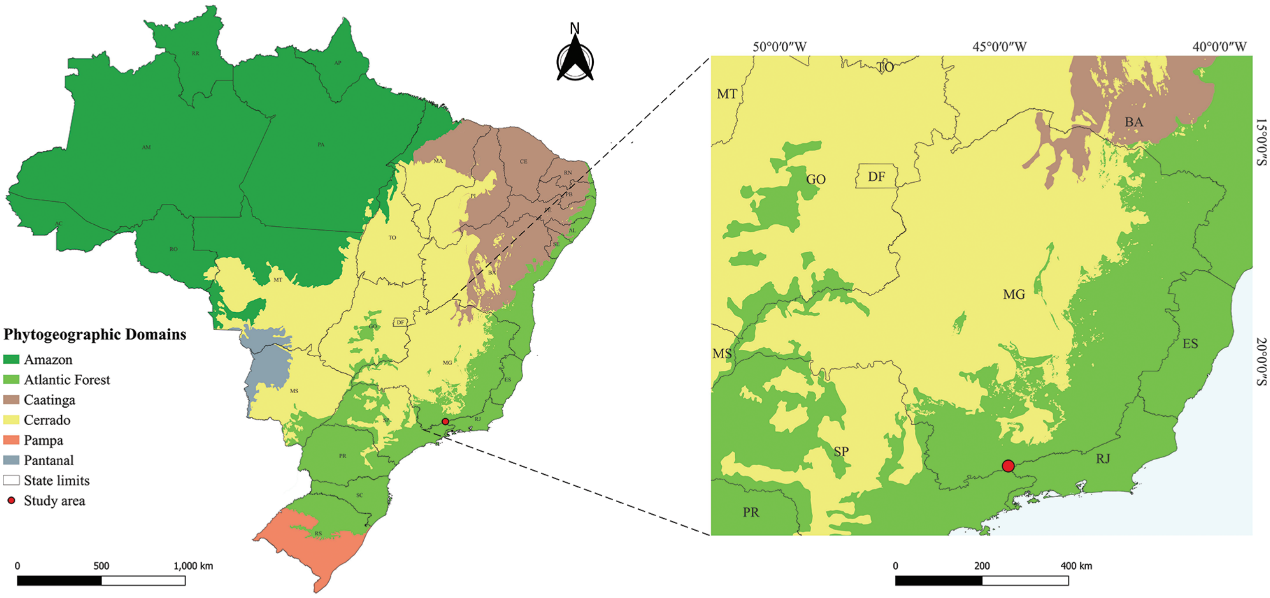


Figure 1. Map of the geographic location of the study area in Reserva Particular do Patrimônio Natural (RPPN) Alto-Montana, Itamonte, Minas Gerais, Brazil. The boundaries of Brazilian phytogeographic domains were adapted from shapefiles available from the IBGE - Instituto Brasileiro de Geografia e Estatística (2023), from the global ecoregions of Dinerstein et al. (2017), and from the map of vegetations on rocky outcrops of the Cerrado domain by Newton Barbosa. Map design: Cássio Cardoso Pereira.

thus generating more robust results (Ribeiro et al. 2016). Therefore, to contemplate the transition between the rainy season of 2017 and the dry season of 2018, the samplings were carried out in February 2018 (rainy season, three days) and April 2018 (dry season, three days). Subsequently, we carried out samplings in July 2018 (dry season, four days) and January 2019 (rainy season, three days). In this way, we totaled thirteen days of collection throughout the evaluated period.

For the capture of frugivorous butterflies, 40 traps of the Van-Someren Rydon type were distributed in groups of five traps for each altitudinal level (Fig. 2C, D), separated by at least 20 m and placed at a height of approximately 1 m from the ground, in all altitude ranges. The traps contained a bait made out of a mixture of banana and sugarcane molasses that underwent 48 hours of fermentation and were left on site for 48 hours (e.g., Uehara-Prado et al. 2009; Melo et al. 2019). During this period, the traps were inspected after 24 hours to remove captured individuals (Uehara-Prado et al. 2009; Santos et al. 2011; Henriques et al. 2019).

The collection and active search for butterflies along the gradient were carried out via sweep nets in each altitudinal range, during the same period of 1 year, to capture nectarivorous or even frugivorous butterflies that were eventually flying or foraging. The time for these samplings comprised an interval between 9 am and 4 pm when the butterflies are more active and thus more susceptible to active capture. The collections lasted around 50 minutes for each of the eight gradients, alternating the starting points, that is, on one day we started at 1400 m at 9 am and finished at 2100 m at 4 pm. The next day, we started at 2100 m at 9 am and ended at 1400 m at 4 pm (Santos et al. 2011; Henriques et al. 2019). All individuals sampled were placed in entomological envelopes with records of altitude, date, and place of collection. Subsequently, they were sacrificed, mounted, and identified via guides and/or consultations with entomological collections and specialists. All species were checked for

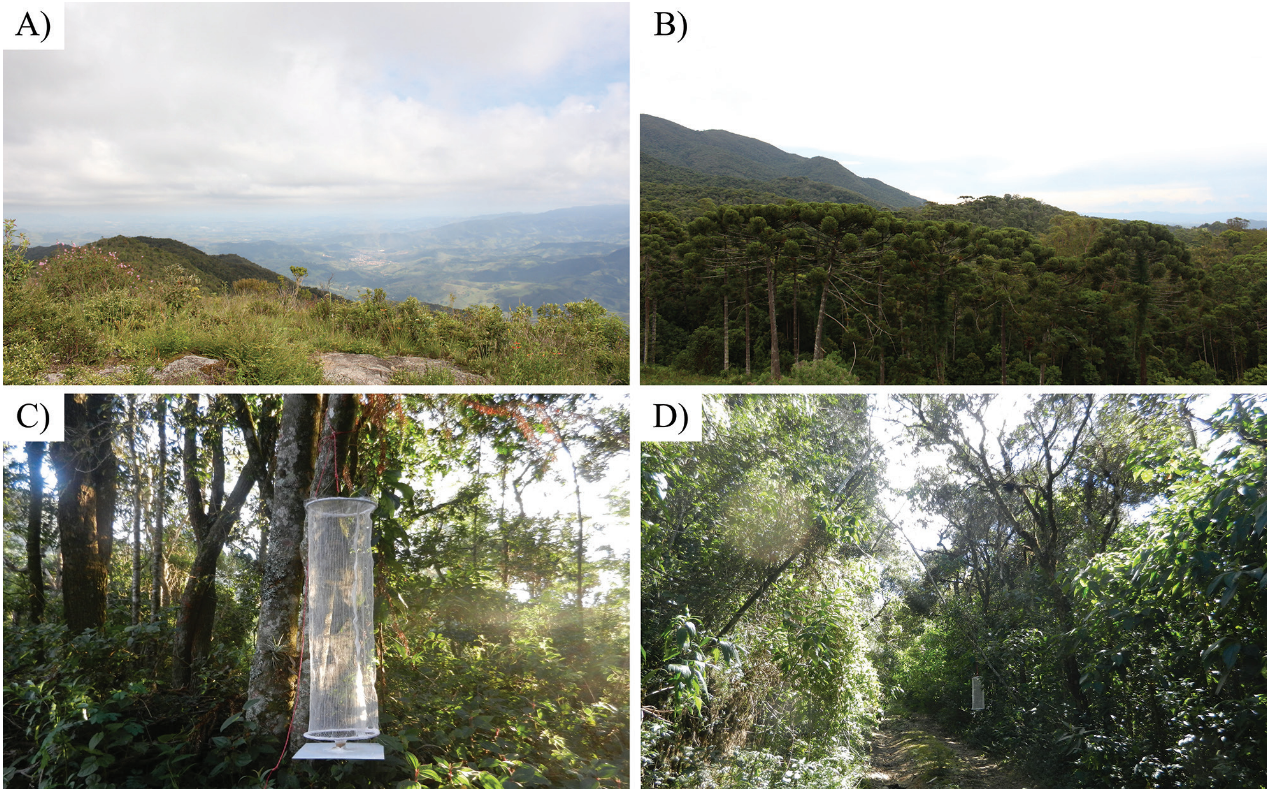


Figure 2. Sampling areas of the Reserva Particular do Patrimônio Natural (RPPN) Alto-Montana, Itamonte, Minas Gerais, Brazil **A** Campo de Altitude **B** Floresta Ombrófila Mista **C, D** Van-Someren Rydon type trap installed.

their conservation status by consulting the International Union for Conservation of Nature – IUCN website (2023). The taxonomy adopted for this study is according to the database of the Global Biodiversity Information – GBIF (2022). The reference collection was organized and deposited in the Entomological Collection of the Departamento de Ciências Naturais of the Universidade Federal de Minas Gerais (UFMG).

The sampling effort was calculated based on the hours of active collections and baited traps. The sampling effort per baited trap was obtained by multiplying the total number of traps placed in the field for all collection days and the total number of hours the traps were available to butterflies on selected sites. The sampling effort per active collection was calculated by multiplying the number of hours (for each collection day) by the number of sweep nets used. Finally, the total sampling effort was obtained by adding the efforts by active collection and baited trap of each day sampled. Thirteen field trips were carried out in the (RPPN) Alto-Montana. During this period, the sampling effort by traps totaled 3,936 hours and the effort by sweep nets totaled 246 hours.

Data analysis

The total richness was estimated for the entire length of the altitudinal gradient. All species were analyzed and classified according to their frequency of occurrence in order to detect rare species. Species in which only one individual was observed were classified as *singletons*. Species with two individuals observed were classified as *doubletons*, and species with more than two individuals were considered common species (Chao 1984; Ferraz et al. 2009; Colwell 2013).

To assess sampling sufficiency, a rarefaction curve was constructed using the *specaccum* function in Vegan (Oksanen 2013) in R software (R Core Team 2021). The *specaccum* function acts by randomizing the sample and describing the average species accumulation curve (plus standard deviation) from the application of a thousand permutations of the data, eliminating the temporal bias (Oksanen 2013). The rarefaction method was based on the 13 sampling periods. Using the specpool function in Vegan (Oksanen 2013), three richness estimator indices (Bootstrap, Chao 1, Jackknife 1) were calculated. Jackknife 1 is an index based on the number of species that occur only once in the original sample (*singletons*), being an analysis sensitive to rare species (Quenouille 1956; Ferraz et al. 2009; Colwell 2013). Chao 1, like Jackknife 1, is also a sensitive estimator for rare species (Chao 1984; Chao and Shen 2004; Colwell et al. 2012). However, in Chao 1 the estimated richness takes into account not only the number of species represented by a single individual (*singletons*) but also the number of species with an abundance of two individuals (*doubletons*) (Chao 1984; Ferraz et al. 2009). Finally, Bootstrap uses data from all collected species to estimate total richness without giving greater weight to species that appear less often in the sample (Efron 1979; Smith and Van Belle 1984; Colwell 2013).

Results

A total of 1,253 butterflies distributed in 124 species and six families of diurnal butterflies (Hesperiidae, Lycaenidae, Nymphalidae, Papilionidae, Pieridae, and Riodinidae) were observed (Table 1, see some of these species in Figs 3–5). Nymphalidae (65 species, about 52.42% of the total) was the most representative family, followed by Hesperiidae (27 species, 21.77%), Pieridade (14 species, 11.29%), Lycaenidae (8 species, 6.45%), Papilionidae (6 species, 4.84%) and Riodinidae (4 species, 3.23%). Nymphalidae was also the most abundant family with 841 individuals sampled, corresponding to 67.12% of the total abundance. Pieridae was the second most abundant family (230 individuals, 18.36%), followed by Hesperiidae (126 individuals, 10.05%), Papilionidae (37 individuals, 2.95%) Lycaenidae (11 individuals, 0.88%), and Riodinidae (8 individuals, 0.64%).

The most abundant species were *Morpho portis* (Hübner, 1821) (Nymphalidae: Satyrinae, N = 249, Fig. 4F) followed by *Dismorphia thermesia* (Godart, 1819) (Pieridae: Dismorphiinae, N = 128, Fig. 3F), *Foetterleia schreineri* (Foetterle, 1902) (Nymphalidae: Satyrinae, N = 85, Fig. 3I), *Forsterinaria necys* (Godart, 1824) (Nymphalidae: Satyrinae, N = 78), *Heliconius besckei* (Ménétriés, 1857) (Nymphalidae: Heliconiinae, N = 62, Fig. 4A), *Eurema albula* (Cramer, 1775) (Pieridae: Coliadinae, N = 58), and *Praepedaliodes phanias* (Hewitson, 1861) (Nymphalidae: Satyrinae, N = 40). Together, these species account for about 56% of the total abundance. Of these, only *M. portis* was recorded in a single season of the year (rainy season - January 2019), the other species occurred in all seasons. Considering the altitudinal gradient, only *M. portis* and *F. necys* occurred at all altitudinal points. Thirty-seven species with only one individual (*singletons*) and 20 species with only two individuals (*doubletons*) were recorded, totaling 57 species, which corresponds to 46% of all sampled richness.

Table 1. List of butterfly species from Reserva Particular do Patrimônio Natural (RPPN) Alto-Montana, Itamonte, Minas Gerais states, Brazil. S = richness; m = meters; n = number of individuals. IUCN Status: NE = Not Evaluated; LC = Least Concern.

Family/Species	Altitudinal gradient (m)/Abundance (n)								Total	IUCN Status
	1400	1500	1600	1700	1800	1900	2000	2100		
Hesperiidae (S = 27)										
Hesperiinae (S = 15)										
Callimormus saturnus (Herrich-Schäffer, 1869)	–	–	–	1	–	–	–	–	1	NE
Calpodes ethlius (Stoll, 1782)	–	2	–	2	3	5	3	–	15	NE
Cobalopsis nero (Herrich-Schäffer, 1869)	–	1	–	–	–	–	–	–	1	NE
Cumbre cumbre (Schaus, 1902)	–	–	–	–	–	3	–	–	3	NE
Cymaenes gisca (Evans, 1955)	2	1	–	2	7	5	8	4	29	NE
Cymaenes lepta (Hayward, 1938)	–	–	–	–	–	–	–	1	1	NE
Cymaenes tripunctata (Latreille, 1824)	–	–	–	–	1	–	–	–	1	NE
Cymaenes tripunctus (Herrich-Schäffer, 1865)	1	1	–	1	6	1	1	–	11	NE
Lucida lucia (Capronnier, 1874)	–	1	–	–	–	–	–	–	1	NE
Vehilius clavicula (Plötz, 1884)	–	–	–	–	2	–	–	–	2	NE
Vettius aurelius (Plötz, 1882)	–	–	1	2	1	–	–	–	4	NE
Vettius diversa (Herrich-Schäffer, 1869)	–	–	1	–	–	–	–	–	1	NE
Vettius phyllus (Cramer, 1777)	–	–	–	1	1	–	–	–	2	NE
Vettius ploetzii (Capronnier, 1874)	–	–	1	–	6	5	1	–	13	NE
Zariaspes mys (Hübner, 1808)	–	–	–	1	–	–	–	–	1	NE
Eudaminae (S = 1)										
Autochton neis (Geyer, 1832)	–	–	–	–	–	1	–	–	1	NE
Pyrginae (S = 11)										
Achlyodes busirus (Stoll, 1782)	–	–	–	–	–	1	2	1	4	NE
Astraptes fulgerator (Walchs, 1775)	1	1	1	–	–	–	–	–	3	NE
Epargyreus socus (Hübner, 1925)	–	1	1	–	–	–	–	–	2	NE
Heliopetes ochroleuca (Zikán, 1938)	2	3	1	–	1	1	–	–	8	NE
Oechydrus chersis (Herrich-Schäffer, 1869)	5	1	–	–	–	–	–	–	6	NE
Pyrgus orcus (Stoll, 1780)	–	–	–	–	–	1	–	–	1	NE
Pythonides lancea (Hewitson, 1868)	1	1	–	–	–	–	–	–	2	NE
Sostrata cronion (C. Felder & R. Felder, 1867)	2	–	–	–	–	–	–	–	2	NE
Theagenes dichrous (Mabille, 1878)	–	–	–	–	–	–	–	1	1	NE
Urbanus dorantes (Stoll, 1790)	–	–	–	–	1	–	–	–	1	NE
Urbanus teleus (Hübner, 1821)	1	2	4	–	1	–	–	1	9	NE
Lycaenidae (S = 8)										
Theclinae (S = 8)										
Arawacus meliboeus (Fabricius, 1793)	–	–	–	–	–	1	–	–	1	NE
Arawacus tadita (Hewitson, 1877)	–	–	–	–	–	1	–	–	1	NE
Laothus phydela (Hewitson, 1867)	–	–	–	1	–	–	–	–	1	NE
Strymon bubastus (Stoll, 1780)	–	–	–	1	–	1	–	–	2	NE
Symbiopsis lenitas (Druce, 1907)	–	–	1	–	1	–	1	–	3	NE
Theritas deniva (Hewitson, 1874)	–	–	–	–	–	1	–	–	1	NE
Theritas triquetra (Hewitson, 1865)	–	–	–	–	–	–	–	1	1	NE
Thestius azaria (Hewitson, 1867)	–	–	–	–	–	–	–	1	1	NE

Family/Species	Altitudinal gradient (m)/Abundance (n)								Total	IUCN Status
	1400	1500	1600	1700	1800	1900	2000	2100		
Nymphalidae (S = 65)										
Biblidinae (S = 4)										
Catonephele sabrina (Hewitson, 1852)	1	–	2	–	6	2	–	1	12	NE
Diaethria candrena (Godart, 1823)	2	–	–	–	–	1	–	–	3	NE
Epiphile hubneri Hewitson, 1861	1	4	13	2	3	–	–	–	23	NE
Epiphile orea (Hübner, 1823)	–	4	8	7	1	6	–	–	26	NE
Charaxinae (S = 4)										
Archaeoprepona chalciope (Hübner, 1823)	1	1	–	–	–	–	–	–	2	NE
Memphis moruus (Fabricius, 1775)	–	1	2	–	–	–	–	–	3	NE
Polygrapha suprema (Schaus, 1920)	–	–	–	–	4	5	3	5	17	NE
Zaretis itys (Cramer, 1777)	–	–	2	–	–	–	–	–	2	NE
Danainae (S = 7)										
Episcada carcinia (Schaus, 1902)	–	–	–	1	–	–	–	–	1	NE
Episcada philoclea (Hewitson, 1854)	–	1	–	–	–	–	–	1	2	NE
Epityches eupompe (Geyer, 1832)	–	–	2	–	1	–	1	–	4	NE
Hypothyris ninonia (Hübner, 1806)	1	1	1	–	–	–	–	–	3	NE
Mechanitis lysimnia (Fabricius, 1793)	1	–	3	–	1	–	–	–	5	NE
Pseudoscada erruca (Hewitson, 1855)	2	–	1	–	–	–	–	–	3	NE
Pteronymia sylvo (Geyer, 1832)	1	–	1	–	4	–	–	–	6	NE
Heliconiinae (S = 12)										
Actinote alalia (Felder, 1860)	–	–	–	–	1	1	–	2	4	NE
Actinote bonita (Penz, 1996)	–	–	–	–	–	3	–	–	3	NE
Actinote conspicua (Jordan, 1913)	–	–	–	–	1	–	–	2	3	NE
Actinote dalmeidai (Francini, 1996)	–	–	–	–	–	–	–	1	1	NE
Actinote mantiqueira (Freitas, Francini, Paluch & Barbosa, 2018)	–	–	1	–	2	–	2	6	11	NE
Actinote surima (Schaus, 1902)	–	–	–	–	–	–	–	3	3	NE
Dione juno (Cramer, 1779)	–	–	–	–	–	1	–	–	1	NE
Eueides pavana (Ménétriés, 1857)	2	–	–	–	–	–	–	–	2	NE
Heliconius besckei (Ménétriés, 1857)	–	–	2	1	1	–	–	–	4	NE
Heliconius erato (Linnaeus, 1758)	11	9	11	12	14	4	1	–	62	NE
Heliconius ethilla (Latreille & Godart, 1819)	2	4	5	1	–	1	–	–	13	NE
Philaethria wernickei (Röber, 1906)	–	–	1	–	–	–	–	–	1	NE
Limenitidinae (S = 6)										
Adelpha calliphane (Fruhstorfer, 1915)	3	2	1	–	–	–	–	–	6	NE
Adelpha hyas (Boisduval, 1836)	–	–	–	–	–	–	–	1	1	NE
Adelpha mythra (Godart, 1823)	1	2	4	3	–	–	–	–	10	NE
Adelpha poltius (Hall, 1938)	–	–	–	1	2	–	1	–	4	NE
Adelpha syma (Godart, 1823)	–	–	–	–	–	–	1	1	2	NE
Adelpha thessalia (Felder, 1867)	–	–	–	1	–	–	–	–	1	NE
Nymphalinae (S = 4)										
Eresia lansdorfi (Godart, 1819)	–	–	1	–	–	–	–	–	1	NE
Tegosa anieta (Hewitson, 1864)	1	3	–	2	2	–	1	–	9	NE
Tegosa claudina (Eschscholtz, 1821)	6	3	4	–	–	1	–	–	14	NE
Vanessa braziliensis (Moore, 1883)	–	–	–	–	–	1	–	2	3	NE
Satyrinae (S = 28)										
Caligo arisbe (Hübner, 1822)	–	–	1	–	–	–	–	–	1	NE
Caeruleuptychia helena (Anken, 1994)	1	–	1	–	–	–	–	–	2	NE

Family/Species	Altitudinal gradient (m)/Abundance (n)								Total	IUCN Status
	1400	1500	1600	1700	1800	1900	2000	2100		
Carminda griseldis (Weymer, 1911)	–	–	–	1	–	–	1	–	2	NE
Carminda surpresa (Barbosa, Aguiar, Rosa, Zacca & Freitas, 2020)	1	–	–	–	–	–	–	–	1	NE
Carminda umuarama (Ebert & Dias, 1997)	1	–	–	1	–	1	8	7	18	NE
Cissia phronius (Godart, 1824)	–	–	1	1	2	3	–	–	7	NE
Eryphanis reevesii (Doubleday, 1849)	–	–	1	–	–	–	–	–	1	NE
Eteona tisiphone (Boisduval, 1836)	1	–	–	–	–	–	1	–	2	NE
Foetterleia schreineri (Foetterle, 1902)	1	2	–	–	16	23	18	25	85	NE
Forsterinaria necys (Godart, 1824)	5	2	9	6	22	21	10	3	78	NE
Forsterinaria pronophila (Butler, 1867)	2	–	1	1	–	–	1	1	6	NE
Forsterinaria quantius (Godart, 1824)	15	–	–	–	–	–	–	–	15	NE
Godartiana muscosa (Butler, 1870)	1	–	3	3	1	–	–	–	8	NE
Moneuptychia itapeva (Freitas, 2007)	–	–	–	–	–	–	–	13	13	NE
Moneuptychia montana (Freitas, 2015)	–	–	–	–	–	–	3	2	5	NE
Moneuptychia pervagata (Freitas, Siewert & Mielke, 2015)	1	–	–	–	1	1	–	2	5	NE
Morpho epistrophus (Fabricius, 1796)	7	–	–	–	–	–	–	–	7	NE
Morpho portis (Hübner, 1821)	5	20	38	40	71	39	27	9	249	NE
Narope cyllastros (Doubleday, 1849)	–	–	–	–	2	–	1	–	3	NE
Opoptera syme (Hübner, 1821)	–	–	1	1	–	–	–	–	2	NE
Paryphthimoides eous (Butler, 1866)	–	–	1	–	–	–	1	1	3	NE
Paryphthimoides poltys (Prittwitz, 1865)	1	–	–	1	–	–	–	–	2	NE
Praepedaliodes phanias (Hewitson, 1861)	–	–	–	2	7	11	18	2	40	NE
Pseudodebis ypthima (Hübner, 1821)	–	–	–	–	–	1	–	–	1	NE
Splendeuptychia libitina (Butler, 1870)	–	1	1	2	4	–	–	1	9	NE
Taygetis ypthima (Hübner, 1816)	–	–	1	–	–	–	–	–	1	NE
Yphthimoides angularis (Butler, 1867)	–	2	–	–	1	–	–	–	3	NE
Yphthimoides ochracea (Butler, 1867)	–	–	–	–	–	–	–	1	1	NE
Papilionidae (S = 6)										
Papilioninae (S = 6)										NE
Heraclides hectorides (Esper, 1794)	2	1	1	–	–	–	–	–	4	LC
Mimoides lysithous (Hübner, 1821)	4	–	–	–	–	–	–	–	4	LC
Parides agavus (Drury, 1782)	1	1	–	–	–	–	–	–	2	LC
Parides anchises (Linnaeus, 1758)	1	–	–	–	–	–	–	–	1	LC
Parides bunichus (Hübner, 1821)	6	9	–	–	–	–	–	–	15	LC
Parides proneus (Hübner, 1831)	4	6	–	1	–	–	–	–	11	LC
Pieridae (S = 14)										
Coliadinae (S = 6)										
Eurema albula (Cramer, 1775)	8	16	30	2	1	1	–	–	58	NE
Eurema deva (Doubleday, 1847)	1	–	2	–	–	1	–	1	5	NE
Eurema elathea (Cramer, 1777)	3	–	–	–	–	–	–	–	3	NE
Eurema lirina (Bates, 1861)	3	3	–	1	–	–	–	–	7	NE
Eurema phiale (Cramer, 1775)	–	–	–	–	–	–	–	1	1	NE
Pyrisitia nise (Cramer, 1775)	–	–	–	–	1	–	–	–	1	NE
Dismorphiinae (S = 3)										
Dismorphia amphione (Cramer, 1779)	–	1	–	1	–	–	–	–	2	NE
Dismorphia astyocha (Hübner, 1831)	–	–	1	–	–	1	–	–	2	NE

Family/Species	Altitudinal gradient (m)/Abundance (n)								Total	IUCN Status
	1400	1500	1600	1700	1800	1900	2000	2100		
Dismorphia thermesia (Godart, 1819)	6	23	24	40	32	2	1	–	128	NE
Pierinae (S = 5)										
Archonias brassolis (Fabricius, 1776)	–	1	–	–	–	–	–	–	1	NE
Catasticta bithys (Hübner, 1831)	–	–	–	–	5	1	2	1	9	NE
Hesperocharis anguitia (Godart, 1819)	–	–	–	–	–	–	–	1	1	NE
Hesperocharis erota (Lucas, 1852)	–	–	–	–	1	1	5	3	10	NE
Pereute swainsoni (Gray, 1832)	–	1	–	–	–	–	1	–	2	NE
Riodinidae (S = 4)										
Riodininae (S = 4)	–	–	–	–	–	–	–	–		
Charis cadytis (Hewitson, 1866)	–	1	1	1	–	–	–	–	3	NE
Mesosemia rhodia (Godart, 1824)	–	–	–	1	2	–	–	–	3	NE
Synargis paulistina (Stichel, 1910)	–	–	–	–	–	–	1	–	1	NE
Synargis regulus (Fabricius, 1793)	1	–	–	–	–	–	–	–	1	NE

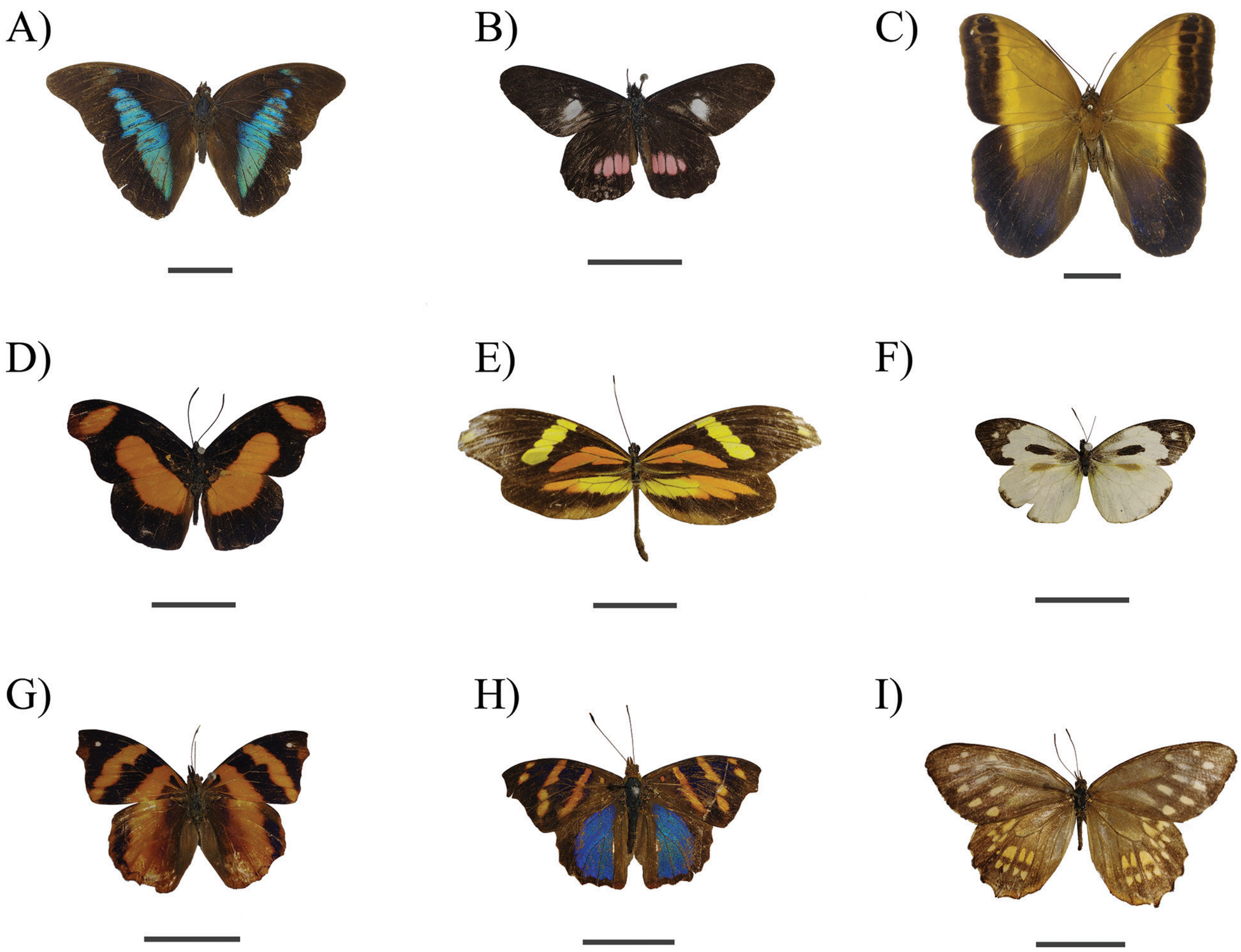


Figure 3. Butterfly species recorded in Reserva Particular do Patrimônio Natural (RPPN) Alto-Montana, Itamonte, Minas Gerais, Brazil **A** *Archaeoprepona chalciope* (Hübner, 1823) **B** *Archonias brassolis* (Fabricius, 1776) **C** *Caligo arisbe* (Hübner, 1922) **D** *Catonephele sabrina* (Hewitson, 1852) **E** *Dismorphia astyocha* (Hübner, 1831) **F** *Dismorphia thermesia* (Godart, 1819) **G** *Epiphile hubneri* (Hewitson, 1861) **H** *Epiphile orea* (Hübner, 1823) **I** *Foetterleia schreineri* (Foetterle, 1902). Scale bars: 20 mm.

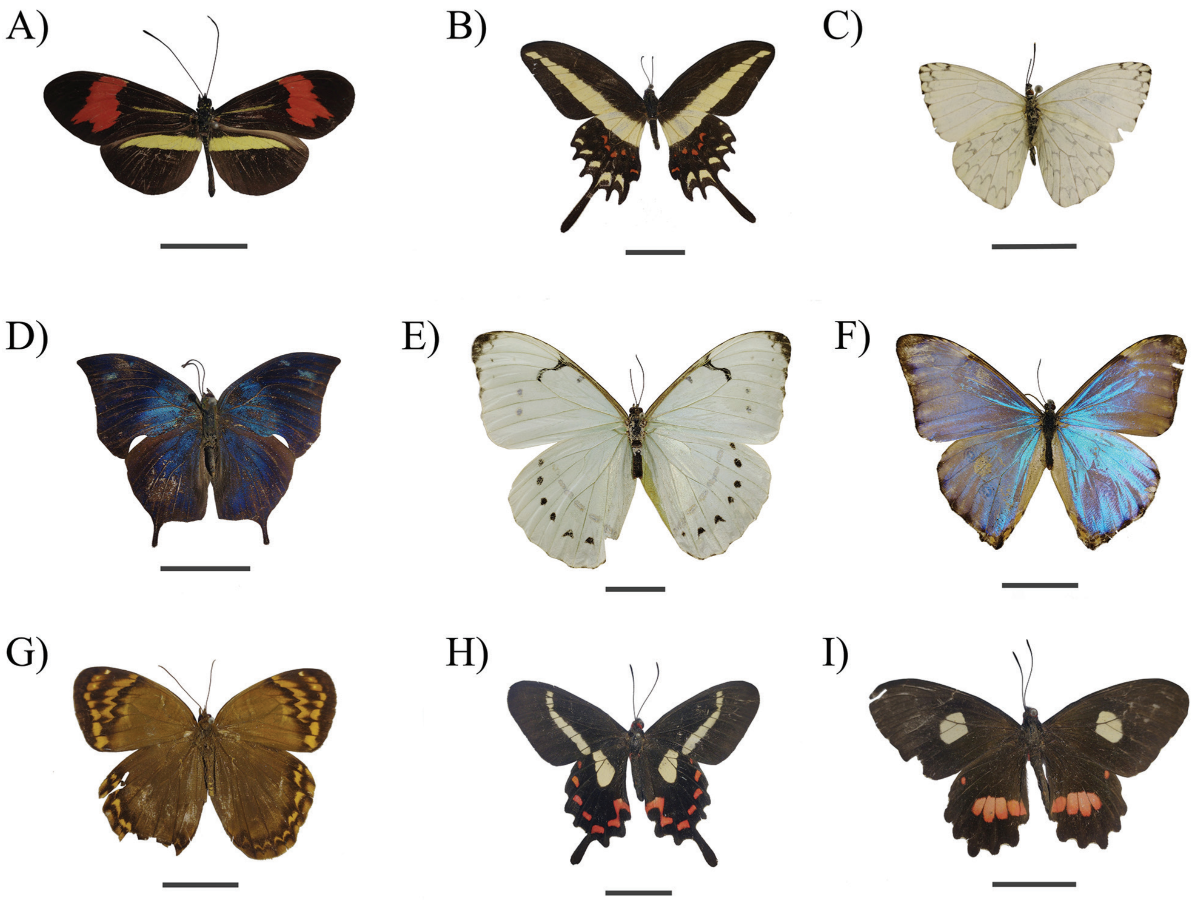


Figure 4. Butterfly species recorded in Reserva Particular do Patrimônio Natural (RPPN) Alto-Montana, Itamonte, Minas Gerais, Brazil **A** *Heliconius besckei* (Ménétriés, 1857) **B** *Heraclides hectorides* (Esper, 1794) **C** *Hesperocharis erota* (Lucas, 1852) **D** *Memphis moruus* (Fabricius, 1775) **E** *Morpho epistrophus* (Fabricius, 1796) **F** *Morpho portis* (Hübner, 1821) **G** *Opoptera syme* (Hübner, 1821) **H** *Parides agavus* (Drury, 1782) **I** *Parides anchises* (Linnaeus, 1758). Scale bars: 20 mm.

The rarefaction curve did not reveal a tendency toward stabilization (Fig. 6). However, the indices showed higher values for the 124 species sampled. The analysis performed using the Bootstrap estimator predicted a total of 143.22 species (± 10.87 SE), with 19 additional species than observed. Chao 1 predicted 153.42 (± 11.82 SE), and Jackknife 1 predicted 164.00 (± 16.29 SE) species, with 29 and 40 additional species than observed, respectively.

Discussion

Our survey showed that the butterfly community of the RPPN Alto Montana is quite diverse, especially when we take into account the size of the area in hectares. With considerable richness, our data suggested a positive potential for the RPPN Alto Montana to provide relevant information on butterfly richness for the Serra da Mantiqueira region. Although data on butterfly biodiversity is still incipient today, especially for mountainous regions (Shirai et al. 2019), works such as ours highlight the importance of fauna inventories for knowledge of communities, detection of rare and/or threatened species and identification of endemisms. We also point out the crucial role that fauna inventories can play as precursors to environmental conservation actions.

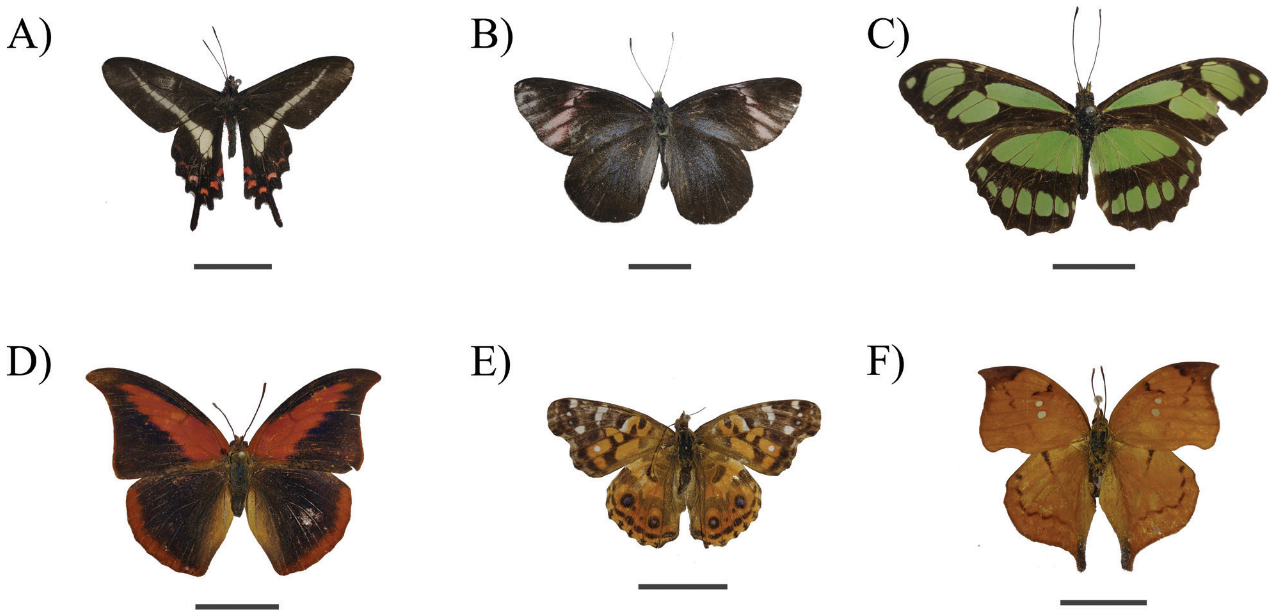


Figure 5. Butterfly species recorded in Reserva Particular do Patrimônio Natural (RPPN) Alto-Montana, Itamonte, Minas Gerais, Brazil **A** *Parides proneus* (Hübner, 1831) **B** *Pereute swainsoni* (Gray, 1832) **C** *Philaethria wernickei* (Röber, 1906) **D** *Polygrapha suprema* (Schaus, 1920) **E** *Vanessa braziliensis* (Moore, 1897) **F** *Zaretis itys* (Cramer, 1777). Scale bars: 20 mm.

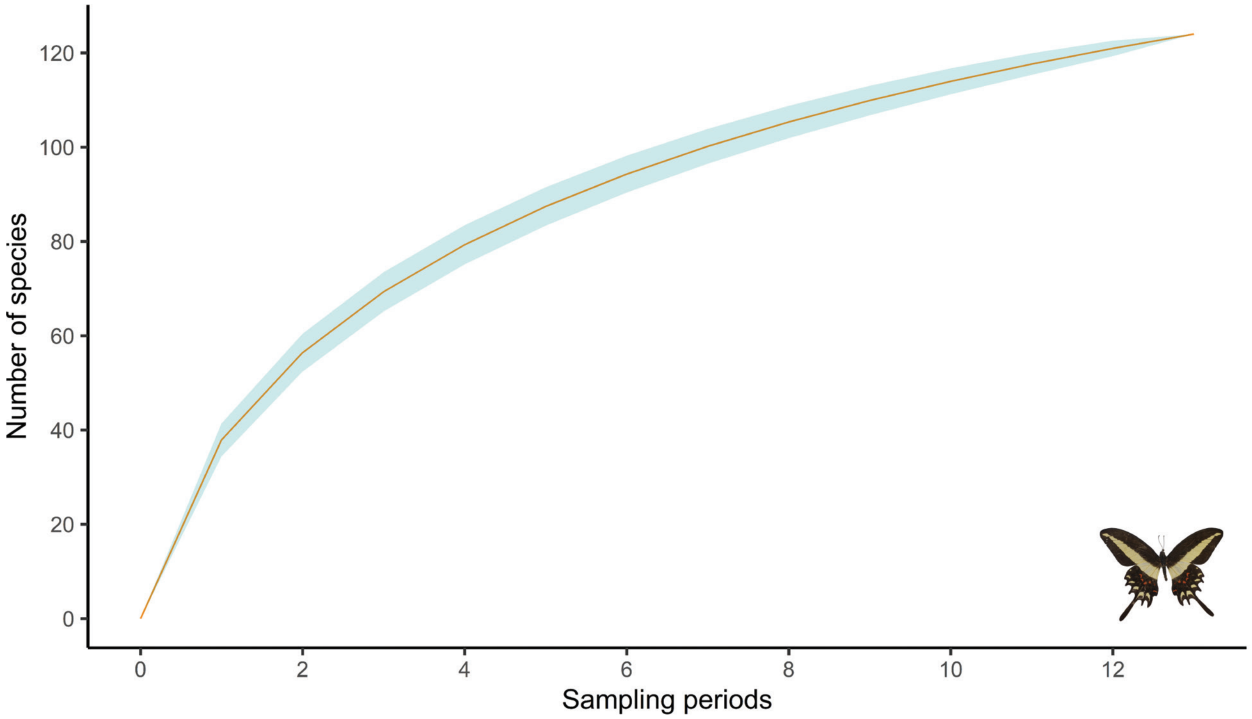


Figure 6. Rarefaction curve representing the relationship between species and sampled area. The line represents the average calculated value of the species collected at the 13 sampling periods in Reserva Particular do Patrimônio Natural (RPPN) Alto-Montana, Itamonte, Minas Gerais, Brazil. The shaded area represents the standard error ($\alpha = 0.05$).

Our results showed a high richness of butterflies for a relatively small area, when we compare the extension in hectares of the RPPN Alto Montana with other areas already studied. Thus, the richness of butterfly species found in this work was higher than that observed for other mountainous regions of Brazil

(Bordin et al. 2019; Henriques et al. 2019; Gueratto et al. 2020), even presenting a considerably smaller area of 672 ha. Gueratto et al. (2020), for comparison, in Serra do Japi, SP, recorded 69 species of butterflies in 24 months of collection, a large area (35 thousand ha) when compared to the area of the RPPN Alto Montana. Our survey is also similar to studies such as Vieira et al. (2022), who worked in the Área De Proteção Ambiental Fernão Dias, south of Minas Gerais, using the same sampling methods employed here, recording 154 butterfly species, but in a larger area. Such variations in richness may occur due to differences in methodology, collection efforts, altitudinal range, and the size of sampling sites (Santos and Fernandes 2021). However, when we consider the extension in hectares of the RPPN Alto Montana, these values suggest that this conservation unit, even small, can play an important role for the conservation of butterfly communities in Mantiqueira, with potential for new butterfly records.

Comparing our study with the species lists published by Freitas et al. (2011) and Vieira et al. (2022) – both in the Serra da Mantiqueira region – we found 45 species that are also present in the Parque Nacional do Itatiaia, RJ, and 48 of the species in Área de Proteção Ambiental (APA) Fernão Dias, MG. The number of species shared between this and the studies mentioned above was expected, since these areas are located 14 and 130 km from the study area, respectively, being located in the same geographic region (Serra da Mantiqueira). However, many species were unique to each study. This exclusivity is perhaps due to the fact that there may be a certain dependence between the species and the environment in which they live. Many butterflies are sensitive to climatic and environmental variations, which could act by restricting the occurrence of species to certain regions (Chowdhury et al. 2023). The Parque Nacional do Itatiaia is a conservation unit of integral protection. The APA Fernão Dias and the RPPN Alto Montana are conservation units for sustainable use. Considering that anthropic interference in these places is different in response to the different levels of protection that each area performs, perhaps there could be a subtle difference in climate or vegetation, for example, which could explain the exclusivity of some species of butterflies for each location. Furthermore, mountains and altitude are important factors for speciation, sometimes leading many species to be restricted to a mountain peak/ridge (Montejo-Kovacevich et al. 2022).

Being a megadiverse group, studies with insects generally do not show stabilization in their rarefaction curves (Crist and Veech 2006). The Jackknife 1 estimator showed that 75.61% of the species were sampled, a higher value but very close to that found by Vieira et al. (2022) in Mantiqueira and Henriques et al. (2019) in Serra de São José, MG. For Serra do Japi, Gueratto et al. (2020) found 82% of the species according to Jackknife 1 and 63% according to Chao 1. For the RPPN Alto Montana, Chao 1 indicated 80.82% of sample sufficiency. These values are in agreement with the studies carried out for forest regions. However, taking into account the size in hectares of the studied area, these values can be a strong indication that the community of butterflies in the region is quite diverse, with more species to be registered.

The lack of stabilization in the accumulation curve may be due to the species' rarity, since almost half of the sampling species are doubletons and singletons. The classification of species recorded in singletons and doubletons showed that the evaluated butterfly community is largely represented by rare species, 46% of the sampled richness. Rare species in the vast majority of cases are distributed in defined environmental ranges, with specific conditions (Thomson et al. 2006; Pearman and Weber 2007; Henriques et al. 2019). In the study area, replacements of tree communities along the altitudinal gradient were observed by Mariano et al. (2020). This characteristic could suggest that the occurrence of rare butterfly species in the RPPN Alto Montana would occur on microscales defined by vegetation change, restricted to small amplitude ranges. Also, the mountain climate leads to a very short window for species reproduction and activity, being a very important issue when considering sampling design (Freitas et al. 2009). For example, some Actinote species fly during short time periods, and they can be very abundant but only for one or two weeks in the summer (Freitas et al. 2009).

Nymphalidae and Hesperiidae were the most representative families in this study, as well as in similar surveys already carried out for other Atlantic Forest sampling sites in Minas Gerais (Oliveira et al. 2018; Vieira et al. 2022). This is probably due to the fact that Nymphalidae is an abundant frugivorous butterfly family found in almost all ecosystems (Brown and Freitas 2000; Bordin et al. 2019). Easily captured in attractive traps, they are generally highly representative in butterfly surveys, and is a family with high diversity in behavior and resource use variation (Bordin et al. 2019). Hesperiidae, in turn, is a large family with a predominant occurrence in open areas (Henriques et al. 2019). It has many endemic species (Warren et al. 2008), and in Brazil, its richness and abundance is concentrated mainly in high altitude areas of the Southeast region (Henriques et al. 2019). The representativeness of these families in this survey may have occurred due to some local phytophysiognomic and geographic characteristics (Mariano et al. 2020). The range of variations in resource use performed by butterflies of the Nymphalidae family, combined with the local phytophysiognomic richness (Mariano et al. 2020) may have contributed to this being the most sampled family. In the same way, active collections with sweep nets carried out in regions of borders and access trails along the altitudinal gradient or in open areas such as in Campos de Altitude, may have contributed to the capture of representatives of Hesperiidae.

Morpho portis (Hübner, 1821), *Dismorphia thermesia* (Godart, 1819), and *Foetterleia schreineri* (Foetterle, 1902) were the three most abundant species in this survey. All occur predominantly in high-altitude regions, both in reforestation areas and preserved forests (Viloria 1998; Bond-Buckup et al. 2008). *F. schreineri* is an endemic species of southeastern Brazil with known records for the Serra da Mantiqueira (Viloria 1998). *P. suprema* was the only species found in the Livro Vermelho da Fauna Brasileira Ameaçada de Extinção (Ministério do Meio Ambiente 2022) whose category is endangered (EN). Its occurrence is restricted to regions with altitudes higher than 1,500 m in Serra da Mantiqueira (ICMBio 2018) and is endemic to this region. Only five species belonging to the Papilionidae family were found on the IUCN Red List with Least Concern (LC) status (Table 1). However, little is known about the conservation of insects, including butterflies, for the entire Neotropical region (Montgomery et al. 2020), and most searches performed on the IUCN Red List for butterflies do not result in much information. Even though studies on butterflies have proliferated in recent years, this advance is still discreet (Shirai et al. 2019). This suggests that the difficulty in knowing the conservation status of most butterfly species may be a consequence of the scarcity of studies that assess local communities. The lack of data on the species conservation listed here, added to the presence of endemisms, highlights the need for greater efforts aimed at understanding the communities and distribution patterns of the species, especially for mountainous regions, where less information is available. Our results also highlight the importance of fauna inventories as tools to support more effective conservation strategies.

Conclusion

The Atlantic Forest has suffered high losses in its biodiversity (de Lima 2020), and for the state of Minas Gerais, there are few butterfly inventories in this phytogeographic Domain (Santos et al. 2018), highlighting some of them - Andrade and Teixeira (2017), Oliveira et al. (2018), Bordin et al. (2019), Gueratto et al. (2020), and Vieira et al. (2022). In recent years, new species have been described for the Serra da Mantiqueira region such as *Actinote mantiqueira* (Freitas, Francini, Paluch & Barbosa, 2018) and *Carminda surpresa* (Barbosa, Aguiar, Rosa, Zacca & Freitas, 2020). These descriptions show the potential of Serra da Mantiqueira to contribute to the discovery of new species, expanding knowledge of biodiversity for the Atlantic Domain. Our study contributes to the knowledge of the biodiversity of tropical butterflies and reveals a high species richness within the relatively small area of the RPPN Alto Montana, in addition to providing the first inventory of butterflies for the site. Our results can support additional conservation actions in Serra da Mantiqueira due to the presence of endemic and rare species observed here, in addition to a threatened species whose occurrence is known only for the region. Finally, we also suggest with this work that there may be several species still unknown, and we encourage research in this region of Brazil.

Acknowledgements

The authors would like to thank UFSJ, UFMG, UniRV, CNPq, and CAPES for their continuous support. We thank Tiago Fernandes for his help in processing the butterfly photos.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

Cássio Cardoso Pereira and Vinícius Sperandei thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brazil (CAPES) – Finance Code 001. Tatiana Cornelissen acknowledges CNPq.

Author contributions

Andrêsa Garcia Andrade: Contribution in the concept and design of the study; Contribution to data collection; Contribution to butterfly identification; Contribution to manuscript preparation. Cássio Cardoso Pereira: Contribution to data analysis and interpretation; Contribution to manuscript preparation; Contribution to critical revision. Vinícius da Fontoura Sperandei: Contribution to data collection; Contribution to butterfly identification; Contribution to manuscript preparation; Contribution to critical revision. Tatiana Cornelissen: Contribution in the concept and design of the study; Contribution to data collection; Contribution to manuscript preparation; Contribution to critical revision.

Author ORCIDs

Andrêsa G. Andrade https://orcid.org/0000-0002-7514-2324
Cássio Cardoso Pereira https://orcid.org/0000-0002-6017-4083
Vinícius F. Sperandei https://orcid.org/0000-0002-3093-706X
Tatiana Cornelissen https://orcid.org/0000-0002-2721-7010

Data availability

All of the data that support the findings of this study are available in the main text.

References

Alvares CA, Stape JL, Sentelhas PC, De Moraes Gonçalves JL, Sparovek G (2013) Köppe's climate classification map for Brazil. Meteorologische Zeitschrift (Berlin) 22(6): 711–728. https://doi.org/10.1127/0941-2948/2013/0507

Andrade DA de, Teixeira IRV (2017) Diversidade de lepidoptera em um fragmento florestal em muzambinho, minas gerais. Ciência Florestal 27: 1229–1241. https://doi.org/10.5902/1980509830311

Bond-Buckup G, Buckup L, Dreier C (2008) Biodiversidade dos campos de Cima da Serra. Libretos, Porto Alegre, 195 pp.

Bordin SMS, Monteiro M, Ferreira VW, Lutinski JA, Rodrigues ENL (2019) Frugivorous butterflies from the Atlantic Forest in Southern Brazil (Lepidoptera: Nymphalidae). Biota Neotropica 19(4): e20180722. https://doi.org/10.1590/1676-0611-bn-2018-0722

Brown KS, Freitas AVL (2000) Atlantic Forest Butterflies: Indicators for Landscape Conservation1. Biotropica 32(4): 934–956. https://doi.org/10.1111/j.1744-7429.2000.tb00631.x

Casagrande MM, Duarte M (2023) Lepidoptera. Catálogo Taxonômico da Fauna do Brasil. http://fauna.jbrj.gov.br/fauna/faunadobrasil/84

Chao A (1984) Non-parametric estimation of the number of classes in a population. Scandinavian Journal of Statistics, Theory and Applications 11(4): 265–270. https://www.jstor.org/stable/4615964

Chao A, Shen T-J (2004) Nonparametric prediction in species sampling. Journal of Agricultural Biological & Environmental Statistics 9(3): 253–269. https://doi.org/10.1198/108571104X3262

Chowdhury S, Jennions MD, Zalucki MP, Maron M, Watson JEM, Fuller RA (2023) Protected areas and the future of insect conservation. Trends in Ecology & Evolution 38(1): 85–95. https://doi.org/10.1016/j.tree.2022.09.004

Colli-Silva M, Reginato M, Cabral A, Forzza RC, Pirani JR, Vasconcelos TNDC (2020) Evaluating shortfalls and spatial accuracy of biodiversity documentation in the Atlantic Forest, the most diverse and threatened Brazilian phytogeographic domain. Taxon 69(3): 567–577. https://doi.org/10.1002/tax.12239

Colwell RK (2013) EstimateS: Statistical Estimation of Species Richness and Shared Species from Samples. Version 9.1.0. User's Guide and application. https://www.robertkcolwell.org/pages/1407-estimates

Colwell RK, Chao A, Gotelli NJ, Lin S-Y, Mao CX, Chazdon RL, Longino JT (2012) Models and estimators linking individual-based and sample-based rarefaction, extrapolation and comparison of assemblages. Journal of Plant Ecology 5(1): 3–21. https://doi.org/10.1093/jpe/rtr044

Crist TO, Veech JA (2006) Additive partitioning of rarefaction curves and species–area relationships: Unifying α-, β- and γ-diversity with sample size and habitat area. Ecology Letters 9(8): 923–932. https://doi.org/10.1111/j.1461-0248.2006.00941.x

Cruz BPD, De Castro EM, Cardoso MDG, De Souza KF, Machado SMF, Pompeu PV, Fontes MAL (2014) Comparison of leaf anatomy and essential oils from Drimys brasiliensis Miers in a montane cloud forest in Itamonte, MG, Brazil. Botanical Studies 55(41): 1–14. https://doi.org/10.1186/s40529-014-0041-y

de Lima RAF, Oliveira AA, Pitta GR, De Gasper AL, Vibrans AC, Chave J, Ter Steege H, Prado PI (2020) The erosion of biodiversity and biomass in the Atlantic Forest biodiversity hotspot. Nature Communications 11(1): 6347. https://doi.org/10.1038/s41467-020-20217-w

DeVries PJ (1987) The butterflies of Costa Rica and their natural history. Priceton University, New Jersey, 327 pp.

Dinerstein E, Olson D, Joshi A, Vynne C, Burgess ND, Wikramanayake E, Hahn N, Palminteri S, Hedao P, Noss R, Hansen M, Locke H, Ellis EC, Jones B, Barber CV, Hayes R, Kormos C, Martin V, Crist E, Sechrest W, Price L, Baillie JEM, Weeden D, Suckling K, Davis C, Sizer N, Moore R, Thau D, Birch T, Potapov P, Turubanova S, Tyukavina A, De Souza N, Pintea L, Brito JC, Llewellyn OA, Miller AG, Patzelt A, Ghazanfar SA, Timberlake J, Klöser H, Shennan-Farpón Y, Kindt R, Lillesø J-PB, Van Breugel P, Graudal L, Voge M, Al-Shammari KF, Saleem M (2017) An Ecoregion-Based Approach to Protecting Half the Terrestrial Realm. Bioscience 67(6): 534–545. https://doi.org/10.1093/biosci/bix014

Efron B (1979) Bootstrap methods: Another look at the Jackknife. Annals of Statistics 7(1): 1–26. https://doi.org/10.1214/aos/1176344552

Ferraz ACP, Gadelha BDQ, Aguiar-Coelho VM (2009) Análise faunística de Calliphoridae (Diptera) da Reserva Biológica do Tinguá, Nova Iguaçu, Rio de Janeiro. Revista Brasileira de Entomologia 53(4): 620–628. https://doi.org/10.1590/S0085-56262009000400012

Francini RB, Duarte M, Mielke OHH, Caldas A, Freitas AVL (2011) Butterflies (Lepidoptera, Papilionoidea and Hesperioidea) of the "Baixada Santista" region, coastal São Paulo, southeastern Brazil. Revista Brasileira de Entomologia 55(1): 55–68. https://doi.org/10.1590/S0085-56262011000100010

Freitas AVL, Francini RB, Souza TS (2009) Immature stages and natural history of the threatened butterfly Actinote quadra (Nymphalidae: Heliconiinae: Acraeini). Tropical Lepidoptera Research 19(2): 83–88.

Freitas AVL, Kaminski LA, Iserhard CA, Silva AK, Barbosa EDP, Araujo PF (2011) Inventário das borboletas do Parque Nacional Do Itatiaia, RJ, 1–9. http://www2.ib.unicamp.br/labor/site/wp-content/uploads/2020/08/RT-Itatiaia-RJ-Relat%C3%B3rio.pdf

Global Biodiversity Information – GBIF (2023) Free and open access to biodiversity data. https://www.gbif.org/what-is-gbif

Girardello M, Chapman A, Dennis R, Kaila L, Borges PAV, Santangeli A (2019) Gaps in butterfly inventory data: A global analysis. Biological Conservation 236: 289–295. https://doi.org/10.1016/j.biocon.2019.05.053

Gueratto PE, Carreira JYO, Santos JP, Tacioli A, Freitas AVL (2020) Effects of forest trails on the community structure of tropical butterflies. Journal of Insect Conservation 24(2): 309–319. https://doi.org/10.1007/s10841-019-00199-x

Henriques NR, Beirão MDV, Brasil E, Cornelissen T (2019) Butterflies (Lepidoptera: Papilionoidea) from the campos rupestres of Serra de São José, Minas Gerais, Brazil. Biota Neotropica 19(3): e20180655. https://doi.org/10.1590/1676-0611-bn-2018-0655

IBGE - Instituto Brasileiro de Geografia e Estatística (2023) Biomas do Brasil. https://www.ibge.gov.br

ICMBio (2018) Livro vermelho da fauna brasileira ameaçada de extinção. https://www.gov.br/icmbio/pt-br/centrais-de-conteudo/publicacoes/publicacoes-diversas/livro_vermelho_2018_vol7.pdf

International Union for Conservation of Nature – IUCN (2023) The IUCN red list of threatened species. https://www.iucnredlist.org/

Joly CA, Metzger JP, Tabarelli M (2014) Experiences from the Brazilian Atlantic Forest: Ecological findings and conservation initiatives. The New Phytologist 204(3): 459–473. https://doi.org/10.1111/nph.12989

Mariano RF, Rezende VL, Mendes CN, Santos RMD, Souza CRD, Moura ASD, Machado FS, Pompeu PV, Carvalho WAC, Fontes MAL (2020) Phylogenetic beta diversity in an upper montane Atlantic Forest along an altitudinal gradient. Plant Ecology 221(8): 671–682. https://doi.org/10.1007/s11258-020-01041-0

Marques MC, Grelle CE (2021) The Atlantic Forest: History, biodiversity, threats and opportunities of the mega-diverse forest. Springer Nature, London, 527 pp. https://doi.org/10.1007/978-3-030-55322-7

Mazza I, Rosa CAD, Souza ACD, Aximoff I, Passamani M (2018) Mamíferos de médio e grande porte registrados em florestas dominadas por *Araucaria angustifolia* na rppn alto-montana, serra da mantiqueira. Oecologia Australis 22(1): 74–88. https://doi.org/10.4257/oeco.2018.2201.07

Melo DHA, Filgueiras BKC, Iserhard CA, Iannuzzi L, Freitas AVL, Leal IR (2019) Effect of habitat loss and fragmentation on fruit-feeding butterflies in the Brazilian Atlantic Forest. Canadian Journal of Zoology 97(7): 588–596. https://doi.org/10.1139/cjz-2018-0202

Ministério do Meio Ambiente (2022) Lista oficial das espécies da fauna e flora ameaçadas de extinção. https://www.icmbio.gov.br/cepsul/images/stories/legislacao/Portaria/2020/P_mma_148_2022_altera_anexos_P_mma_443_444_445_2014_atualiza_especies_ameacadas_extincao.pdf

Montejo-Kovacevich G, Meier JI, Bacquet CN, Warren IA, Chan YF, Kucka M, Salazar C, Rueda-M N, Montgomery SH, McMillan WO, Kozak KM, Nadeau NJ, Martin SH, Jiggins CD (2022) Repeated genetic adaptation to altitude in two tropical butterflies. Nature Communications 13(1): 4676. https://doi.org/10.1038/s41467-022-32316-x

Montgomery GA, Dunn RR, Fox R, Jongejans E, Leather SR, Saunders ME, Shortall CR, Tingley MW, Wagner DL (2020) Is the insect apocalypse upon us? How to find out. Biological Conservation 241: 108327. https://doi.org/10.1016/j.biocon.2019.108327

Myers N, Mittermeier RA, Mittermeier CG, Da Fonseca GAB, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature 403(6772): 853–858. https://doi.org/10.1038/35002501

Oksanen J (2013) Vegan: ecological diversity. https://cran.r-project.org/web/packages/vegan/vignettes/diversity-vegan.pdf

Oliveira GCS, Souza MM de, Clemente MA, Vieira KM (2021) Efeito da altitude sobre riqueza de vespas sociais (Vespidae, Polistinae). Acta Biológica Catarinense 8(2): 54–61. https://doi.org/10.21726/abc.v8i2.1436

Oliveira LA, Milani LR, Souza MM (2018) Riqueza de borboletas (Lepidoptera) no Parque Estadual da Serra do Papagaio, sul de Minas Gerais, Brasil. MG. Biota 11(3): 5–21.

Pearman PB, Weber D (2007) Common species determine richness patterns in biodiversity indicator taxa. Biological Conservation 138(1–2): 109–119. https://doi.org/10.1016/j.biocon.2007.04.005

Pompeu PV, Fontes MAL, Santos RMD, Garcia PO, Batista TA, Carvalho WAC, Oliveira Filho ATD (2014) Floristic composition and structure of an upper montane cloud forest in the Serra da Mantiqueira Mountain Range of Brazil. Acta Botanica Brasílica 28(3): 456–464. https://doi.org/10.1590/0102-33062014abb3239

Quenouille MH (1956) Notes on bias in estimation. Biometrika 43(3/4): 353–360. https://doi.org/10.2307/2332914

Quintero I, Jetz W (2018) Global elevational diversity and diversification of birds. Nature 555(7695): 246–250. https://doi.org/10.1038/nature25794

R Core Team (2021) R: A language and environment for statistical computing. https://www.r-project.org/

Raven PH, Gereau RE, Phillipson PB, Chatelain C, Jenkins CN, Ulloa Ulloa C (2020) The distribution of biodiversity richness in the tropics. Science Advances 6(37): eabc6228. https://doi.org/10.1126/sciadv.abc6228

Ribeiro MC, Metzger JP, Martensen AC, Ponzoni FJ, Hirota MM (2009) The Brazilian Atlantic Forest: How much is left, and how is the remaining forest distributed? Implications for conservation. Biological Conservation 142(6): 1141–1153. https://doi.org/10.1016/j.biocon.2009.02.021

Ribeiro DB, Batista R, Prado PI, Brown Jr KS, Freitas AVL (2012) The importance of small scales to the fruit-feeding butterfly assemblages in a fragmented landscape. Biodiversity and Conservation 21(3): 811–827. https://doi.org/10.1007/s10531-011-0222-x

Ribeiro DB, Williams MR, Specht A, Freitas AVL (2016) Vertical and temporal variability in the probability of detection of fruit-feeding butterflies and moths (Lepidoptera) in tropical forest: Detection of fruit-feeding Lepidoptera. Austral Entomology 55(1): 112–120. https://doi.org/10.1111/aen.12157

Sánchez-Bayo F, Wyckhuys KAG (2019) Worldwide decline of the entomofauna: A review of its drivers. Biological Conservation 232: 8–27. https://doi.org/10.1016/j.biocon.2019.01.020

Santos JC, Fernandes GW (2021) Measuring arthropod biodiversity: a handbook of sampling methods. Springer, London, 600 pp. https://doi.org/10.1007/978-3-030-53226-0

Santos JPD, Iserhard CA, Teixeira MO, Romanowski HP (2011) Fruit-feeding butterflies guide of subtropical Atlantic Forest and Araucaria Moist Forest in State of Rio Grande do Sul, Brazil. Biota Neotropica 11(3): 253–274. https://doi.org/10.1590/S1676-06032011000300022

Santos JPD, Freitas AVL, Brown Jr KS, Carreira JYO, Gueratto PE, Rosa AHB, Lourenço GM, Accacio GM, Uehara-Prado M, Iserhard CA, Richter A, Gawlinski K, Romanowski HP, Mega NO, Teixeira MO, Moser A, Ribeiro DB, Araujo PF, Filgueiras BKC, Melo DHA, Leal IR, Beirão MDV, Ribeiro SP, Cambuí ECB, Vasconcelos RN, Cardoso MZ, Paluch M, Greve RR, Voltolini JC, Galetti M, Regolin AL, Sobral-Souza T, Ribeiro MC (2018) Atlantic butterflies: A data set of fruit-feeding butterfly communities from the Atlantic forests. Ecology 99(12): 2875–2875. https://doi.org/10.1002/ecy.2507

Shirai LT, Machado PA, Mota LL, Rosa AHB, Freitas AVL (2019) DnB, the Database of Nymphalids in Brazil, with a Checklist for Standardized Species Lists. Journal of the Lepidopterists Society 73(2): 93. https://doi.org/10.18473/lepi.73i2.a4

Smith EP, Van Belle G (1984) Nonparametric Estimation of Species Richness. Biometrics 40(1): 119. https://doi.org/10.2307/2530750

Soldati D, Silveira FAD, Silva ARM (2019) Butterfly fauna (Lepidoptera, Papilionoidea) in an ecotone between two biodiversity hotspots in Minas Gerais, Brazil. Papéis Avulsos de Zoologia 59: e20195902. https://doi.org/10.11606/1807-0205/2019.59.02

Stevens VM, Trochet A, Van Dyck H, Clobert J, Baguette M (2012) How is dispersal integrated in life histories: a quantitative analysis using butterflies: Dispersal life-history correlates. Ecology Letters 15(1): 74–86. https://doi.org/10.1111/j.1461-0248.2011.01709.x

Thomson JR, Fleishman E, Nally RM, Dobkin DS (2006) Original article: Comparison of predictor sets for species richness and the number of rare species of butterflies and birds: Comparing predictors of species richness and rare species. Journal of Biogeography 34(1): 90–101. https://doi.org/10.1111/j.1365-2699.2006.01576.x

Uehara-Prado M, Fernandes JDO, Bello ADM, Machado G, Santos AJ, Vaz-de-Mello FZ, Freitas AVL (2009) Selecting terrestrial arthropods as indicators of small-scale disturbance: A first approach in the Brazilian Atlantic Forest. Biological Conservation 142(6): 1220–1228. https://doi.org/10.1016/j.biocon.2009.01.008

Vieira LR, Henriques NR, De Souza MM (2022) Communities of Lepidoptera along an elevational gradient in the Brazilian Atlantic Forest (Lepidoptera: Papilionoidea). SHILAP Revista de Lepidopterologia 50(197): 175–189. https://doi.org/10.57065/shilap.203

Vilanova MRN (2015) Long-term rainfall trends in Serra da Mantiqueira Environmental Protection Area, southeast Brazil. Environmental Earth Sciences 73(8): 4779–4790. https://doi.org/10.1007/s12665-014-3763-y

Viloria AL (1998) Studies on the systematics and biogeography of some montane satyrid butterflies (Lepidoptera). PhD Thesis, King's College London, University of London, London, England.

Warren AD, Ogawa JR, Brower AVZ (2008) Phylogenetic relationships of subfamilies and circumscription of tribes in the family Hesperiidae (Lepidoptera: Hesperioidea). Cladistics 24(5): 642–676. https://doi.org/10.1111/j.1096-0031.2008.00218.x

Zikán JF, Zikán W (1968) Inseto-fauna do Itatiaia e da Mantiqueira. Pesquisa Agropecuária Brasileira 3(1): 45–109.