

## Short Communication

# Under the lion's paw: lion farming in South Africa and the illegal international bone trade

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## Abstract

The African lion *Panthera leo* is subject to numerous anthropogenic pressures across its natural range. In South Africa, although free ranging populations are increasing, the number of lions in captivity in private commercial facilities far outnumber those in the wild. South Africa's captive lion industry was reportedly created primarily to generate income and take pressure off wild populations through the supply of captive-bred lions for trophy hunting. However, the industry has become a highly contentious topic under ongoing international scrutiny and debate. Here, we present new information from direct interviews with workers at two closed-access lion facilities located in North West Province, on how some facilities continue to use legal activities, such as captive breeding and hunting, to facilitate their involvement in the illegal international felid bone trade. The sources also report other illegal and unethical activities including animal welfare violations, unsafe conditions for workers, potential shifts to the commercial exploitation of other felid species such as tigers *Panthera tigris* and incidents involving poaching of captive lions and tigers by non-affiliated actors. Sources described how some facilities use various tools and tactics, such as security cameras, patrols and messaging apps to avoid detection during inspections. If the South African Government is to be successful in meeting its publicly stated goal of ending the captive lion industry, a comprehensive well-managed plan to transition away from current practices is required. To aid enforcement, the industry should also be fully audited, with all facilities officially registered, a moratorium on the breeding of lions and plans put in place to prevent the stockpiling of lion bones.

**Key words:** African lion, big cat bone trade, captive lion breeding, *Panthera leo*, South Africa, wildlife trade, wildlife trafficking



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## Introduction

The African lion *Panthera leo* is one of the world's most iconic large carnivores. The species is categorised as Vulnerable on the IUCN Red List with wild populations suspected to have declined by around 43% between 1993 and 2014 (around three lion generations) (Bauer et al. 2016) to an estimated 20,000 – 25,000 individuals (Nicholson et al. 2023). Widespread habitat loss, extensive prey base depletion and indiscriminate retaliatory or pre-emptive killing due to

human-wildlife conflict are amongst the main causes of population declines (Bauer et al. 2016). Regionally, lion populations are projected to reduce by a further 50% over the next two decades in West, Central and East Africa (Bauer et al. 2015). However, in a restricted, intensively managed geographical range in southern Africa (Botswana, Namibia, South Africa and Zimbabwe), several lion populations are stable or increasing (Nicholson et al. 2023).

In South Africa, the African lion is classified as Least Concern with an increasing wild population trend (Miller et al. 2016). Isolated and fragmented subpopulations of around 3,500 free-roaming lions occur in a few large protected areas, as well as many (~ 45) small (< 1000 km<sup>2</sup>) fenced private or state-owned reserves (Miller et al. 2016). However, far more lions are currently kept in private captive facilities for commercial purposes in South Africa than exist in the wild (Chetty et al. 2024). South Africa's captive lion industry was reportedly created primarily to generate income and take pressure off wild populations through the supply of captive-bred lions for trophy hunting. Since 2008, the industry has grown exponentially, contributing an estimated R500 million (US\$42 million) annually to the country's economy (Van Der Merwe et al. 2017). The current captive African lion population in South Africa is estimated at 7,838 individuals housed across 342 facilities that actively breed or keep felids and other large predators (Chetty et al. 2024). Since the 1990s, these lions have primarily been commercially captive-bred for the captive or "canned" hunting industry (where the skins and skulls of hunted lions are exported internationally as trophies for recreational hunters), along with interactive tourism and live exports (D'Cruze and Green 2023). The international supply of bones, body parts and derivatives to be used as ingredients in traditional Chinese medicine (TCM), is mostly a by-product of the captive hunting industry (Schroeder 2018; Williams and 't Sas-Rolfes 2019; Coals et al. 2020; D'Cruze and Green 2023).

African lions are the only *Panthera* species listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) since 1977, meaning that the international commercial trade in live specimens and derivatives is permitted with a relevant CITES export permit. Captive-bred lion bones and skeletons were legally exported from South Africa to Southeast Asia between 2008 and 2018 to be used in traditional medicinal and cultural preparations (Williams et al. 2017a). Annual exports of lion skeletons from South Africa rose from 60 in 2008 to a peak of more than 1700 in 2016 (Williams et al. 2017a). Export quotas were subsequently established for South African captive-bred lions, limiting the number of annual skeleton exports to 800 in 2017 and 2018 (Coals et al. 2019a). However, in 2019, a South African High Court decision determined that the export quotas were unlawful and constitutionally invalid due to insufficient consideration of the welfare of captive-bred lions (SAFLII 2019). Subsequently, no CITES export quotas for lion skeletons have been set (Williams et al. 2021), meaning that the export of lion bones originating from lion farms in South Africa is currently illegal.

South Africa's captive lion industry is a highly contentious topic under ongoing international scrutiny and scientific, ethical and political debate (Coals et al. 2019b, 2019a; Hiller and MacMillan 2021; Chetty et al. 2024). Some argue that breeding lions may help to reduce pressure on wild populations by saturating the market with affordable farmed lions for hunting and other products (e.g. Williams and 't Sas-Rolfes 2019). Criticism has, however, been directed on the



unethical practices, negative animal welfare, human health risks and the unregulated nature of the industry (Williams et al. 2015; NSPCA 2017; Fobar 2019; Green et al. 2020, 2022; de Waal et al. 2022; Heinrich et al. 2022). Increasingly, there are also concerns that the supply of captive-origin lion bones from breeding facilities could have unintended negative impacts on lion conservation by potentially driving an increase in poaching and trafficking of wild-sourced lions and perpetuating the international market for felid bones and other wildlife products (Williams et al. 2017a; Republic of South Africa 2021). In addition, some proponents of the industry have cited captive lion breeding as a potential source for re-introduction to help bolster wild lion populations (Abell et al. 2013). However, others argue that captive lions pose little value for species restoration because they are poorly equipped for survival in the wild, are often habituated to humans and their release could pose risks of human or livestock attacks or the introduction of novel pathogens which could be catastrophic to wild populations (Hunter et al. 2013).

In May 2021, South Africa's Department of Forestry, Fisheries and the Environment (DFFE) made a public announcement of its intention to immediately halt the "domestication and exploitation of lions and to ultimately close all captive lion facilities in South Africa" due to concerns that, amongst others, the industry was a threat to the country's reputation as a leader in conservation and ecotourism (Republic of South Africa 2021). Despite ongoing controversy and the Government's public statement of its intention to end the industry, commercial captive lion breeding and keeping has continued to operate legally in South Africa under a patchwork of complex provincial and national laws and regulations (Wilson 2019; Green et al. 2021; Chetty et al. 2024). Inconsistencies, regulatory complexity, together with a lack of national databases for permits issued related to the breeding, keeping, transport, hunting, killing and trade of lions, has resulted in legal loopholes and enforcement challenges, creating opportunity for harmful and fraudulent practices to exist (de Waal et al. 2022). In particular, a lack of enforcement on the illegal export of lion bones has created a legal grey area leading to confusion and non-compliance. Consequently, there are concerns that South Africa's legal captive lion breeding and hunting industry is a detrimental conduit for illegal wildlife trade activities (de Waal et al. 2022).

Around a third of South Africa's 342 captive lion facilities are open to the public who pay to see and have direct contact with lions through activities such as "cub petting" and "walking with lions" experiences (de Waal et al. 2022; Wilson and Phillips 2023). The other two thirds are situated in remote locations and operate "off grid" and closed to the public. These facilities primarily function as breeding operations to supply lions for the legal captive hunting industry. However, because they are poorly monitored [in part because the provincial nature conservation authorities lack capacity to adequately monitor and enforce legislation (de Waal et al. 2022; Chetty et al. 2024)], little is currently known about how these facilities operate and the practices taking place related to the hunting, slaughter, preparation and trade of hunted animals.

Here, we present new information from direct interviews with workers at two closed-access lion facilities located in the North West Province, on how some facilities in South Africa use legal activities, such as captive breeding and hunting, to practise illegal and unethical activities including international felid bone trade.

## Methods

The rural North West Province in South Africa (Fig. 1) is a hotspot for the country's commercial captive lion breeding industry (Van Der Merwe et al. 2017). Around 90 facilities located in North West Province hold around a third of South Africa's total captive large felid population (de Waal et al. 2022) and approximately 80% of lion hunting in South Africa is thought to occur in the North West and Free State Provinces (Van Der Merwe et al. 2017). Lion facilities in North West Province are predominantly orientated towards hunting, but some also breed, keep and sell live lions, typically also for the hunting industry (Williams and 't Sas-Rolfes 2019).

Face-to-face interviews with anonymous sources from two closed-access lion facilities located in North West were conducted by external field researchers between August and October 2022. Transcripts from the interviews were subsequently provided to World Animal Protection in late 2022. At the time of the interviews, one of the facilities reportedly kept lions and the other kept both lions and tigers *Panthera tigris* for captive hunting and the felid bone trade. Hunting of the animals occurred in purpose-built enclosures on their premises. One of the facilities did not breed lions, but purchased them from local breeding facilities for each hunt. The other facility bred both lions and tigers and hunts were facilitated for a "largely Asian client base". The anonymous sources were employed at the facilities to transport and collect lions and tigers for trophy hunting and to skin and prepare the bones of hunted animals.



**Figure 1.** Map of the study area in North West Province, South Africa.

A set of predetermined questions that included open-ended, closed and multiple-choice were used during the interviews to gather insights into the different stages of production of lion bones for export. Key questions related to the welfare conditions of lions in captivity, the processes involved in the lion trade, such as how the animals were transported and slaughtered, how the products (e.g. skin and bones) from trophy hunts were processed and which stakeholders were involved in the different trade chain stages. In accordance with the British Sociological Association Statement of Ethical Practice (BSA 2017), informed consent was obtained verbally from the anonymous sources prior to the interviews and they were made aware of their rights to voluntarily participate or to decline. In order not to compromise the privacy and safety of the sources, no personal identifiable information was recorded and the names and/or the precise locations of the facilities are not reported.

## Results

The export of lion bones originating from captive lion facilities is currently illegal in South Africa, following the High Court declaration in 2019 in which the lion bone export quota was declared unconstitutional and no CITES export quotas for lion skeletons have subsequently been set (Williams et al. 2021). Despite this, the anonymous sources reported that, after a captive trophy hunt, the skin, paws and skull of the lions were kept as trophies by the hunters, whereas the lion's bones were cleaned and prepared for "Asian bone buyers". The stages of production of lion bones for illegal export are presented in Table 1, along with an outline of the relevant national and provincial level legislation pertaining to each stage of production and the legal irregularities noted in this study.

In some cases, entire lion carcasses were reportedly left intact and were packed into cardboard boxes ready for shipment and for "Asian bone buyers to collect". This was reportedly done to certify authenticity (that it was a lion carcass) and ensure tracking devices had not been inserted into the bones. One source stated that all tigers and lions killed on the premises in the past year were used to supply the bones and parts trade. Other actors involved reportedly assisted in obtaining permits and licences for lion hunting and bone export (Note - It is currently not possible to obtain a CITES export permit for lion/tiger bone exports from South Africa. If provinces issue a CITES permit, they are in contravention of the zero lion bones quota set by the Minister. It is unclear what permit the source was referring to in this case study.). The sources described how the facilities use various tools and tactics, such as security cameras, patrols and messaging apps to avoid detection during inspections.

Sources also stated that some farm owners appeared to be shifting operations from lions to tigers and ligers, lion-tiger hybrids, which could be in response to South Africa's recent announcement of its decision to end lion farming. One source reported that tigers and lions from various age classes were transported to the facility from other breeding facilities several times a year. Nearly twice as many tigers compared to lions were reportedly kept at the facility.

Furthermore, according to the sources, several lion facilities in the region have been experiencing pressure from organised poaching networks suspected of targeting lions and tigers for illicit wildlife trade. This has allegedly happened more frequently over the past four years, resulting in the death of more than sixty animals

**Table 1.** The stages of production of lion and tiger bones for illegal export from a case study of two closed-access commercial captive lion facilities in North West Province, the relevant national and provincial level legislation pertaining to each stage of production and legal irregularities noted in this study.

Stages	Steps	Location	Actor(s)	Product status	Example of participant's statement	All relevant legislation and permits required <sup>1</sup>	Legal irregularities specifically suggested in this study in relation to North West Province
Captive bred	Lion/tiger cub legally born and raised at a registered lion breeding facility	Lion breeding facility in North West Province	Facility owner; Facility labourer(s); Off-site veterinarian	Live lion/tiger	"One to four lions and tigers are kept per enclosure. Both lion and tiger cubs stay with their mothers in the enclosure. Cubs remain with their mother for up to six months"; "Cubs are not tagged, clipped or marked in any way for record keeping purposes"; "The enclosures are not cleaned regularly. Lions are fed once a month - on any meat available - dead beef cattle, chicken and sometimes wildlife harvested for the specific reason. Portable borehole water is available at the enclosures. No enrichment is provided. Nobody is taking care of the lions full-time"; "Veterinary care is minimal. The owners use a veterinarian from a neighbouring town to perform clinical and mainly dart work on the lion"; "The perimeter fence and gates are run down and wide gaps exist between run-over fence material. As a result, a vehicle can easily drive through openings in the perimeter fence".	National Environmental Management Biodiversity Act, 2004 (Act 10 of 2004); Threatened or Protected Species Regulations (2007) (TOPS). TOPS captivity permit required for lions and tigers. North West minimum enclosure requirements: Min. 1,500 m <sup>2</sup> up to four animals, plus 150 m <sup>2</sup> or 400 m <sup>2</sup> for each additional animal; Max. of 10 individuals per enclosure. Animals Protection Act (1962); North West Wildlife Fencing Policy (2008); Amendment to the North West Wildlife Fencing Policy (2009).	Lack of marking for cubs is non-compliant with TOPS regulations; Dirty enclosures and an inadequate feeding regime for the animals is potentially non-compliant with the APA; Fencing is potentially non-compliant with the North West Wildlife Fencing Policy (2008) and/or Amendment to the North West Wildlife Fencing Policy (2009). <sup>2</sup>
Procure	Adult lion/tiger legally captive-bred at a registered lion breeding facility is selected for the hunt; OR lion/tiger is purchased from breeding facility by facility owner or hunter(s)	Captive lion facility, lion breeding facilities in North West or other provinces (e.g. Free State and Limpopo)	Facility owner; Supplier(s); Hunter(s)	Live lion/tiger	"Lions and tigers are transported from other facilities as well as captive bred on the premises"; "The lions are brought from within the region or other provinces"; "Lions are purchased as required for each hunt or at least shortly prior to each hunt. There are multiple suppliers"; "During the hunting season, there are more lions arriving"; "Lions are collected from numerous different lion breeding facilities and deliveries of lions also take place"; "The lion is pre-selected by the hunter".	TOPS permits required for both lions and tigers [captive permit, transport permit (both within North West Province and between provinces - import and export), hunting permit]; TOPS permit required for lion hunt. Within the North West Province, only permission of the landowner is required for a tiger hunt. of the landowner is required for a tiger hunt.	No suggestion of legal irregularities related to procurement specifically referenced in this study.
Broker	Contact person who ordered the hunt; Contact Asian bone buyer/collector; Contact other actor to obtain permit for lion/tiger hunt	Captive lion facility	Facility owner; Asian bone buyer/collector; Hunter(s); Middleman (to obtain hunting permit)	Live lion/tiger	"Lions are bred at the facility for lion trophy hunting and the bone trade"; "All lions and tigers on the property last year were killed for their bones and parts"; "All lions hunted are slaughtered and some of the clients want their complete skeletons, whilst other clients do not insist on the skeletons and, in those cases, the bones are sold to a bone buyer"; "Other actors assist in obtaining permits and licences for lion hunting and bone export".	TOPS transport permit required to move the live animal or carcass within the province and/or import/export permits required between provinces; TOPS permit required for lion hunt. Within the North West Province, only permission of the landowner is required for a tiger hunt; TOPS permit required to be in possession of a lion carcass. For lions (and possibly for tigers), records need to be kept by facility owner on the origin of the carcass, such as hunting permit and professional hunter registers or veterinarian report in case of a natural death. If bones obtained through euthanasia rather than hunting, in North West, a TOPS permit is required and no euthanasia is allowed unless for medical reasons with veterinarian approval. CITES export permit required for bone export.	No legal CITES lion bone export quota since 2019. No legal CITES tiger bone export quota since 1975. Lion/tiger bone export is illegal (although domestic trade in lion/tiger bones is currently legal in South Africa).

Stages	Steps	Location	Actor(s)	Product status	Example of participant's statement	All relevant legislation and permits required <sup>1</sup>	Legal irregularities specifically suggested in this study in relation to North West Province
Transport	Lion/tiger is "darted with a tranquilliser" and loaded in a crate on a utility vehicle/pickup truck; Lion/tiger transported via a lorry from breeding facility to hunting facility by supplier; facility owner or hunter(s); OR lion/tiger transported from breeding enclosure to hunting enclosure (if on same site); Facility labourer assists with loading and offloading of lion/tiger	Road network within North West or other provinces (e.g. Free State or Limpopo); OR within the captive lion facility	Facility labourer(s); Facility owner; Hunter(s); Off-site veterinarian	Live lion/tiger	"Lions are darted and tranquillised by chemicals and loaded in the sleeping position in crates and utility vehicles"; "A local veterinarian illegally supplies sedation drugs to non-certified personnel for a fee to aid canned hunting".	Currently no national norms and standards or permits required for darting a lion/tiger and the type of tranquilliser used for transporting a lion/tiger, but this should be administered by a veterinarian or a person authorised by a veterinarian and in possession of a valid permit <sup>3</sup> . South African National Standards (SANS) codes relating to the transportation of wild animals, such as "the vehicles for the transportation of wild carnivores by road to holding pens and other facilities (SANS 1884-3:2008). In North West, lions can be bred and hunted on the same facility, but regulations vary by province. <sup>4</sup>	Non-certified person administering sedatives to captive wild animals is non-compliant with SANS 1884-3:2008.
Slaughter	Lion/tiger shot by hunter(s) in hunting enclosure	Purpose-built hunting enclosure on captive lion facility	Hunter(s); Facility labourer(s) / trackers	Whole carcass	"All lions [at the facility] are hunted"; "For most cases it [tracking the released lion] would not take more than four hours"; "About an hour after their release the lion has been hunted"; "Three lions were shot by a hunter from the back of a vehicle within an hour of release and while still partially sedated"; "No tracking of lions and tigers is done as the animals are killed in a [area less than 1 ha] hunting enclosure";	TOPS permit required for lion hunt. Within North West, only permission of the landowner is required for a tiger hunt. North West provincial regulations stipulate a required minimum captive hunting area of 1,000 ha. North West provincial regulations stipulate a minimum time of 96 hrs between the release of a captive-bred lion into a hunting camp and the hunt taking place. Hunting of sedated lions/tigers is not allowed under the TOPS Regulations. Note - specific regulations pertaining to captive hunting vary by province <sup>5</sup> .	Hunts conducted in an enclosure measuring < 1 ha is non-compliant with the North West provincial regulations pertaining to the captive lion breeding industry. Lions transported from breeding to hunting facilities drugged and hunted within an hour of release is non-compliant with the North West provincial regulations pertaining to the captive lion breeding industry. Hunting sedated lions is non-compliant with TOPS Regulations.
Transport	Hunted lion/tiger transported via a pickup truck/hunting vehicle from hunting area to slaughter/processing area on captive lion facility	Processing area on captive lion facility ("slaughterhouse or 'skinning shed")	Facility labourer(s)	Whole carcass	"[lions come] from the hunting enclosure on a hunting vehicle SUV / pickup truck";	TOPS permit required to be in possession of a lion carcass. For lions (and possibly for tigers), records need to be kept by facility owner on the origin of the carcass, such as hunting permit and professional hunter registers, or veterinarian report in case of a natural death. TOPS transport permit required to move the lion/tiger carcass within the province and/or import/export permits required between provinces.	No suggestion of legal irregularities related to the transport of lion/tiger carcasses specifically referenced in this study.



Stages	Steps	Location	Actor(s)	Product status	Example of participant's statement	All relevant legislation and permits required'	Legal irregularities specifically suggested in this study in relation to North West Province
Process	Facility labourer(s) skins hunted lion/ tiger as soon as possible after the hunt. All meat and tendons are removed from the skeleton. The skin, paws, and skull are processed and cleaned by the facility labourer(s). These parts are then sent to a taxidermist to be processed into trophies which are kept by the hunter. The hunted lion/ tiger bones / skeleton are cleaned, salted and prepared by the facility labourer(s) for illegal wildlife traffickers	Processing area on captive lion facility ("slaughterhouse or "skinning shed")	Facility labourer(s)	Bones/ skeleton without skull (cleaned); Whole carcass (cleaned)	"[bone preparation] happens as the animals are hunted throughout the day"; "Lion is gutted, skinned and all meat removed from the skeleton with a knife and left to dry. After a few days, the skeleton is submerged in coarse salt to preserve it"; "Due to the heat, all animals hunted are to be skinned as soon as possible"; "Hunted animals are skinned for the purpose of preserving the skin for the tannery or taxidermy process involved in building the trophy"; "Skins are salted and left to dry in a shaded place with no direct sunlight. Normally a skinning shed"; "Skins, paws, skull are sought after trophies by the hunter. Bones and skeleton are sold to Asian buyers"; "Once dried of all meat and washed, the bones will be stored in a secure barn"; "Records of the bones processed are not kept".	TOPS permit required to be in possession of a lion carcass. For lions (and possibly for tigers), records need to be kept by facility owner on the origin of the carcass, such as hunting permit and professional hunter registers or veterinarian report in case of a natural death. No national norms and standards on processing of lion/tiger carcasses. CITES export permit required for lion bone/carcass need stipulates that records for lion bone/carcass need to be kept on the origin of carcass, such as hunting permit and professional hunter registers or veterinary report in case of natural deaths.	No legal CITES lion bone export quota since 2019. No legal CITES tiger bone export quota since 1975. Lion/ tiger bone export is illegal (although domestic trade in lion/tiger bones is currently legal in South Africa); Lack of recording keeping of the bones processed is non-compliant with North West permit regulations.
Package	Cleaned lion/ tiger bones / skeleton, OR whole intact carcass is carefully packaged into a cardboard box by facility labourer(s) and prepared ready for shipment OR cleaned lion/ tiger bones / skeleton are packed with the skin (when requested by the client)	Captive lion facility	Facility labourer(s)	Cleaned whole carcass (packaged); OR cleaned bones/ skeleton (packaged)	"Bones are carefully packed in cardboard boxes and in other cases, packed with the skins when the clients insisted that the bones be shipped/exported with the trophies"; "Bones are not tagged or market for record keeping – everything goes to the buyer unmarked".	CITES export permit required for bone export.	No legal CITES lion bone export quota since 2019. No legal CITES tiger bone export quota since 1975. Lion/ tiger bone export is illegal (although domestic trade in lion/tiger bones is currently legal in South Africa). Pre-2019, the DFFE stipulated that all skeletons that qualify for the quota must be tagged by a provincial Environmental Management Inspector. Not marking or tagging of bones would therefore be non-compliant with TOPS regulations.
Inspect	Prepared bones are "inspected" by facility owner	Captive lion facility	Facility owner	Cleaned whole carcass (packaged); OR cleaned bones/ skeleton (packaged)	"All bones are "inspected" by the employer".	No legislation available	No suggestion of legal irregularities related to the inspection of prepared bones specifically referenced in this study.

Stages	Steps	Location	Actor(s)	Product status	Example of participant's statement	All relevant legislation and permits required <sup>1</sup>	Legal irregularities specifically suggested in this study in relation to North West Province
Sell	Lion/tiger bones/skeleton OR whole intact carcass are sold to Asian bone buyer/collector for export; Permit obtained for bone export	Captive lion facility	Facility owner; Asian bone buyer/collector; Middleman (obtains export permit); Exporter	Cleaned whole carcass (packaged); OR cleaned bones/skeleton (packaged)	"The facility owners handle the [processed] bones"; "Other actors assist in obtaining permits and licences for lion hunting and bone export".	CITES export permit required for bone export. If bones are obtained through euthanasia rather than hunting, in North West, a TOPS permit is required and euthanasia is not allowed unless for medical reasons with veterinarian approval.	No legal CITES lion bone export quota since 2019. No legal CITES tiger bone export quota since 1975. Lion/tiger bone export is illegal (although domestic trade in lion/tiger bones is currently legal in South Africa). It is currently not possible to obtain a CITES export permit for lion/tiger bone exports from South Africa. If provinces issue a CITES permit, they are in contravention of the zero lion bones quota set by the Minister. It is unclear what permit the source was referring to in this case study.
Collect	Asian bone buyer collects prepared shipment from facility owner	Captive lion facility	Facility owner; Asian bone buyer/collector	Cleaned whole carcass (packaged); OR cleaned bones/skeleton (packaged)	"Bones are collected by Asian individuals"; "[buyers are at the facility] whenever bones and other products are available"; "A person of Asian descent visits specifically to collect bones"; "All bones are collected after every Safari".	CITES export permit required for bone export. TOPS transport permit required for transporting bones.	No legal CITES lion bone export quota since 2019. No legal CITES tiger bone export quota since 1975. Lion/tiger bone export is illegal (although domestic trade in lion/tiger bones is currently legal in South Africa).
Export	Shipment is exported	International airport (South Africa)	Exporter	Cleaned whole carcass (packaged); OR cleaned bones/skeleton (packaged)	No specific reference was made by the participants in relation to international export (only "export"). However, recent lion bone seizures have been made at airports in South Africa and reported in the media. <sup>6</sup>	CITES export permit required for bone export.	No legal CITES lion bone export quota since 2019. No legal CITES tiger bone export quota since 1975. Lion/tiger bone export is illegal (although domestic trade in lion/tiger bones is currently legal in South Africa).

<sup>1</sup>The following legislation pertains to the industry and its activities overall: NW primary nature conservation and biodiversity ordinances: North West / Transvaal Nature Conservation Ordinance, 12 of 1983; Cape Nature & Environmental Conservation Ordinance, 19 of 1974; Bophuthatswana Nature Conservation Act, 3 of 1973. Policies pertaining captive lions: Cape Problem Animal Control Ordinance, 26 of 1957. <sup>2</sup>See Chetty et al. (2024) Table 6.3 for a summary of the NW fencing policy for captive lions. <sup>3</sup>Protocols have been developed by the MTT (see Chetty et al. 2024). <sup>4</sup>See Chetty et al. (2024) for an outline of provincial differences in regulations. <sup>5</sup>See Chetty et al. (2024) Tables 6.1-6.5 for an outline of provincial differences in regulations pertaining to captive lion hunting. <sup>6</sup> <https://www.news24.com/news24/southafrica/news/fur-crying-out-loud-man-en-route-to-vietnam-with-lion-bones-in-luggage-arrested-at-or-tambo-airport-20230624>

in five facilities by non-affiliated and/or criminal actors. The most common method of inducing death is reported to be through the use of poison-laced chicken and often only the heads and paws of the animals are harvested by the poachers.

Testimony also indicates that illegal hunting practices have taken place at the facilities (Table 1). First, that hunts have been conducted in an enclosure measuring < 1 ha, which would be illegal due to non-compliance with provincial regulations which stipulate a required minimum area of 1,000 ha (Chetty et al. 2024). Second, that some lions transported from breeding to hunting facilities were drugged and hunted within an hour of release, which would be in violation of the provincial legal minimum release period of 96 hours (Chetty et al. 2024). According to one source, *“a local veterinarian illegally supplies sedation drugs to non-certified personnel for a fee to aid canned hunting”*. An incident was also recalled when three lions were shot by a hunter from the back of a vehicle within an hour of release and while still partially sedated. The hunting of sedated lions is illegal in South Africa and would contravene the national Threatened or Protected Species (TOPS) Regulations of 2007 (Table 1).

The sources also reported unethical animal welfare practices taking place at the facilities. Specifically, they detailed malnutrition, lack of clean water, dirty enclosures with inadequate fencing, minimal veterinary treatment, lack of enrichment or shelter and other poor husbandry practices. One source described how lions kept on the premises were being deliberately starved throughout the low-hunting season, reportedly to save costs.

The transcripts also provide information on the unsafe working conditions for labourers at the facilities. The labourers, who reportedly earn minimum wage, have limited subsistence allowances, and do not wear protective gear when slaughtering and preparing the bodies of hunted animals. One source also stated that they may be threatened with consequences to themselves and their families if they speak out about the mismanagement of lions and their involvement in the illegal supply of lion bones for international trade.

## Discussion

Our findings corroborate pre-existing concerns that South Africa's captive lion industry is inadequately protected from criminal activity and non-compliance with regulations (Williams et al. 2015; Sharife 2022). We provide new insights into the procedures and mechanics of how some lion facilities use legal activities, such as commercial captive breeding and hunting, to practise illegal and unethical activities including international felid bone trade (Table 1, Plate 1). Our case study highlights how the complexity of the regulatory system pertaining to South Africa's captive lion industry, which currently operates under a patchwork of provincial and national laws and regulations related to the breeding, keeping, transport, hunting, killing and trade of lions (Wilson 2019; Green et al. 2021), can result in legal loopholes and enforcement challenges, creating opportunity for harmful and fraudulent practices to exist (Table 1). Corruption amongst wildlife enforcement officials and a lack of resources and proper record-keeping also make it difficult for authorities to manage the industry and ensure facilities comply with the law (de Waal et al. 2022; Heinrich et al. 2022).

Our finding that there has reportedly been an increase in the number of tigers and lion-tiger hybrids being kept and bred at some lion facilities in South Africa is



**Plate 1.** Left: Prepared lion skeleton (credit: Lord Ashcroft KCMG PC); Right: Lion at a commercial breeding facility in South Africa (credit: Roberto Vieto/World Animal Protection).

also a source for concern. The apparent shift in focus from lions to tigers could be a potential mechanism to supplement lion bone trade demand in response to the Government's announcement of its decision to close the captive lion breeding industry (Republic of South Africa 2021). A large proportion of the tigers bred in South Africa are exported as live animals and body parts to China, Vietnam and Thailand, which are renowned hotspots for demand in tiger body parts and the illegal tiger trade (Williams et al. 2015). Both tiger and lion parts (including bones, teeth and claws) are known to be traded in Southeast Asia and China (Coals et al. 2020). Lion bone is thought to be used as a substitute in tiger bone wine in Traditional Chinese Medicine (TCM) products (Coals et al. 2020; Moorhouse et al. 2021), but recent evidence also suggests an emerging demand for lion derivative products in Vietnam and China (EMS and BAT 2018; Outhwaite 2018). Given the difficulty in distinguishing lion bones from tigers (Williams et al. 2015; Dalton et al. 2020), there is a particular risk posed by having other large felids on farms in South Africa when their bones could be used as substitutes for lion bones and this situation could have detrimental compounding effects on already vulnerable populations of species, such as tiger, across their range (Nicholson et al. 2023).

Our interviews with anonymous sources also suggest that some lion facilities have been experiencing pressure from organised poaching networks suspected of targeting captive lions and tigers for illegal wildlife trade and that, often, only the heads and paws of poisoned animals are harvested by the poachers. This finding raises a particular concern that warrants further investigation and verification. Other similar anecdotal reports have been documented of the suspected poaching in South Africa of lions to supply the Asian market (Miller et al. 2014) and a rise in incidents of lion poisonings on private property in South Africa over the past decade (Williams et al. 2017b). Although the direct link between trade in farmed lion parts and poaching of wild lions is currently largely unknown (Everatt et al. 2019; Coals et al. 2020; Macdonald et al. 2021), across range states, there has been growing concern over the impact of illegal trade in lions, as well as increasing numbers of targeted lion poaching incidences, with the removal of body parts, such as skulls,



teeth and claws from lions in Mozambique, Namibia and Tanzania (Everatt et al. 2019; Coals et al. 2022; Arias et al. 2024). These incidents are thought to be linked to domestic, regional and international trade in lion body parts from illegal sources due to their proliferating value (Outhwaite 2018; Bodasing 2022; Miller et al. 2023; Arias et al. 2024). The targeted poaching of lions for their body parts was also recognised as an emerging threat to lion conservation in the most recent IUCN species assessment (Nicholson et al. 2023). Although the impact of lion farming on wild populations in South Africa is considered to be minimal (Scientific Authority of South Africa 2018), the role of farms in stimulating illegal poaching from wild populations is currently unclear (The Supreme Court of South Africa 2010; Hunter et al. 2013; Bauer et al. 2018) and warrants further investigation.

The South African government is currently on a trajectory to close the captive lion breeding industry through a consultative process initiated in 2018. A High-Level Panel of experts were appointed in 2020 to review the industry's policies, practices and management, which led them to recommend termination of the industry and all its associated commercial activities. These recommendations were subsequently approved by Cabinet, based on conclusions that, amongst others, captive lion breeding does not contribute to the conservation of wild lions, shows inherent animal welfare and public health concerns, poses concerns over the safety of workers and visiting public and presents a threat to South Africa's reputation as a responsible ecotourism destination, with associated political and economic risks (Department of Forestry, Fisheries and the Environment 2023). These concerns were corroborated in the Government's Policy Position on Conservation and Sustainable use of South Africa's Biodiversity (Department of Forestry, Fisheries and the Environment 2024) and in a report published earlier this year by the Ministerial Task Team (MTT) that was initiated to identify voluntary exit options from the captive lion breeding industry (Chetty et al. 2024).

If the South African Government is to be successful in meeting its publicly stated goal of ending the captive lion industry, a comprehensive, well-managed plan will be required to ensure a responsible transition away from current practices and recommendations have been provided in the recent MTT report (Chetty et al. 2024). To help enforcement agencies manage and ensure facilities comply with the law during an effective phase-out, the industry should be fully audited, with all facilities officially registered, a moratorium on the breeding of lions issued and suitable plans put in place to prevent the stockpiling of lion bones (Chetty et al. 2024). This decision should be mandatory and made in lock step across provinces through the streamlining of all provincial and national-level legislation pertaining to the industry. The decision should also be extended to other felid species that could potentially be used as substitutes for the illegal international bone trade (D'Cruze and Elwin 2023). For example, an estimated 492 tigers were kept in registered private facilities in South Africa in 2022 (de Waal et al. 2022) and our study highlights reports of increasing numbers of tigers held on lion farms in recent years, which is corroborated by the 626 tigers reported in 2023 (Chetty et al. 2024). More broadly, we reiterate the importance of monitoring and regulation to reduce the risk of criminal and non-compliant activity related to the commercial captive lion industry and associated illegal international bone trade, particularly during the transition to terminate the industry. To support these efforts, all information provided here on illegal wildlife trade activities has been reported to the South African Government.



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## Additional information

### Conflict of interest

None. All authors are employed by the animal welfare organisation World Animal Protection (NDC holds the position of Head of Animal Welfare and Research; AE is Wildlife Research Manager; EA is Wildlife Investigations Advisor). Our results pertaining to this study were in no way influenced by our own personal views on animal welfare.

### Ethical statement

In accordance with the British Sociological Association Statement of Ethical Practice (BSA 2017), informed consent was obtained verbally from the anonymous sources prior to the interviews, and they were made aware of their rights to voluntarily participate or to decline. In order not to compromise the privacy and safety of the sources, no personal identifiable information was recorded, and the names of and/or the precise locations of the facilities are not reported.

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### Author contributions

AE: Formal analysis, Writing – Original draft, Writing – Review and Editing; EA: Formal analysis, Writing – Review and Editing; NDC: Formal analysis, Writing – Review and Editing.

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### Data availability

The data that support the findings of this study are available on request from the corresponding author, NDC. The data are not publicly available due to their containing information that could compromise the privacy and safety of research participants.

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## Research Article

# Phylogeography and genetic population structure of the endangered bitterling *Acheilognathus tabira tabira* Jordan & Thompson, 1914 (Cyprinidae) in western Honshu, Japan, inferred from mitochondrial DNA sequences

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## Abstract

We examined the genetic population structure of the endangered freshwater cyprinid *Acheilognathus tabira tabira* in the Japanese archipelago, which has only been analyzed in limited sampling in previous studies, based on cytochrome *b* region of the mitochondrial gene. We confirmed the existence of the same three lineages determined in the previous study, the natural distribution area of Lineage I and II+III were considered to be the Seto Inland Sea and Ise Bay regions, respectively. Furthermore, the Seto Inland Sea region population was divided into five groups inhabiting neighboring water systems using the spatial analysis of molecular variance (SAMOVA). We estimated that populations in the Seto Inland Sea region migrated through a single paleowater system during the last glacial period and were then separated and genetically differentiated due to marine transgression. The Yoshino River system population was estimated to be a non-native population because it belonged to the same group as the Lake Biwa-Yodo River system, which is the only separate water system across the Seto Inland Sea. This study provides new evidence of genetic differentiation in *A. t. tabira* populations within the Seto Inland Sea region, where genetic differentiation has not been detected in previous studies, corresponding to five different groups by significantly increasing the number of individuals and sites compared with previous studies. Therefore, we propose these five groups as conservation units in the Seto Inland Sea region.

**Key words:** Artificial introduction, biogeography, conservation, Cytochrome *b*, SAMOVA

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## Introduction

The distribution of many freshwater fishes of the Japanese archipelago has been strongly influenced by geomorphic changes such as uplift of mountains (e.g., Watanabe et al. 2017). In particular, western Japan, including the Ise Bay and Seto Inland Sea regions, was significantly affected by mountainous uplift and transgression during the Pleistocene (Ota et al. 2004; Machida et al. 2006). It is known that these geomorphic changes have caused populations of freshwater fish to become fragmented and genetically differentiated within localized areas (Watanabe et al. 2017). Previous phylogeographic and genetic population structure studies of freshwater fishes in these regions suggest that geological events, such as uplift of mountains and marine transgression that influence the distribution of freshwater fishes may differ among species (Kitagawa et al. 2001; Watanabe et al. 2010, 2014; Tominaga et al. 2016, 2020; Nakagawa et al. 2016; Ito et al. 2019; Ito and Koya 2022). Identifying the phylogeographic patterns and genetic population structure of each species inhabiting the same region is an effective approach to identify the factors shaping the genetic diversity of freshwater fish (Avice 2000).

The *tabira* bitterling, *Acheilognathus tabira* Jordan & Thompson, 1914 (Cyprinidae: Acheilognathinae), is a freshwater fish endemic to Honshu, Japan. It has been classified into five subspecies, mainly due to their different nuptial color patterns (Arai et al. 2007). Each subspecies has an allopatric distribution, differs in egg shape, and is clearly distinguishable by mitochondrial DNA (mtDNA) and nuclear DNA (Arai et al. 2007; Kitamura et al. 2012). Therefore, each subspecies is thought to have followed its own evolutionary path. The white *tabira* bitterling, *Acheilognathus tabira tabira* Jordan & Thompson, 1914, is a subspecies of *A. tabira*. The natural distribution range of *A. t. tabira* is the Ise Bay waters and the Seto Inland Sea regions on the Pacific side, with some rivers flowing into the Sea of Japan in western Japan (Kitamura and Uchiyama 2020). Previous phylogeographical studies of *A. t. tabira* have identified three lineages (Kitamura et al. 2012; Umemura et al. 2012), of which two are thought to be naturally distributed in the Ise Bay region (Nobi Plain groups I and II), and one lineage is thought to occur in the Seto Inland Sea region (Kinki-Sanyo group). However, the populations of the Seto Inland Sea region analyzed in previous studies were restricted to only two river systems; therefore, knowledge of the ranges of natural distribution in each lineage of *A. t. tabira* is incomplete.

In addition, *A. t. tabira* is listed as Endangered on the Red List of Japan because its population has been decreasing owing to improvements in rivers and agricultural canals (Ministry of the Environment of Japan 2020). However, the Kinki-Sanyo group has been artificially introduced into several regions as a result of incidental introductions associated with fishery releases of *Plecoglossus altivelis altivelis* (Temminck & Schlegel, 1846) and private releases, which raises concerns about the impact of genetic introgression on the native population of *A. t. tabira* (Umemura et al. 2012; Kumagai and Hagiwara 2013; Kitamura and Uchiyama 2020; Ito et al. 2021). In the Ise Bay region, the non-native lineage known as the Kinki-Sanyo group has been artificially introduced into the Nagara, Kiso, and Kushida Rivers, which are the natural distribution areas of the Nobi Plain Groups I and II (Kitamura et al. 2012; Umemura et al. 2012; Kitamura and Uchiyama 2020). Therefore, the native population of *A. t. tabira* in the Ise Bay region may have

become extinct due to genetic introgression (Kitamura and Uchiyama 2020). In the Yoshino River system in northeastern Shikoku, located in the Seto Inland Sea region, the past freshwater fish fauna is unknown; therefore, it has been difficult to determine whether the population of *A. t. tabira* is naturally distributed or originated from artificial introduction (Kitamura and Uchiyama 2020). Phylogeographic patterns and genetic population structures can help identify whether the population is naturally distributed or originates from non-native populations (Miyake et al. 2011; Umemura et al. 2012; Matsuba et al. 2014; Uemura et al. 2018; Tominaga et al. 2020; Ito et al. 2021, 2022). Therefore, understanding the phylogeographic patterns and genetic population structure across the natural distribution of *A. t. tabira* is essential for promoting appropriate conservation activities.

In the present study, we attempted to elucidate the factors responsible for distribution patterns of *A. t. tabira* by estimating its phylogeographic and genetic population structures covering its whole distribution range using the cytochrome *b* (*cytb*) region of the mtDNA. In addition, we discuss the artificial introduction and conservation units of *A. t. tabira* based on the results obtained.

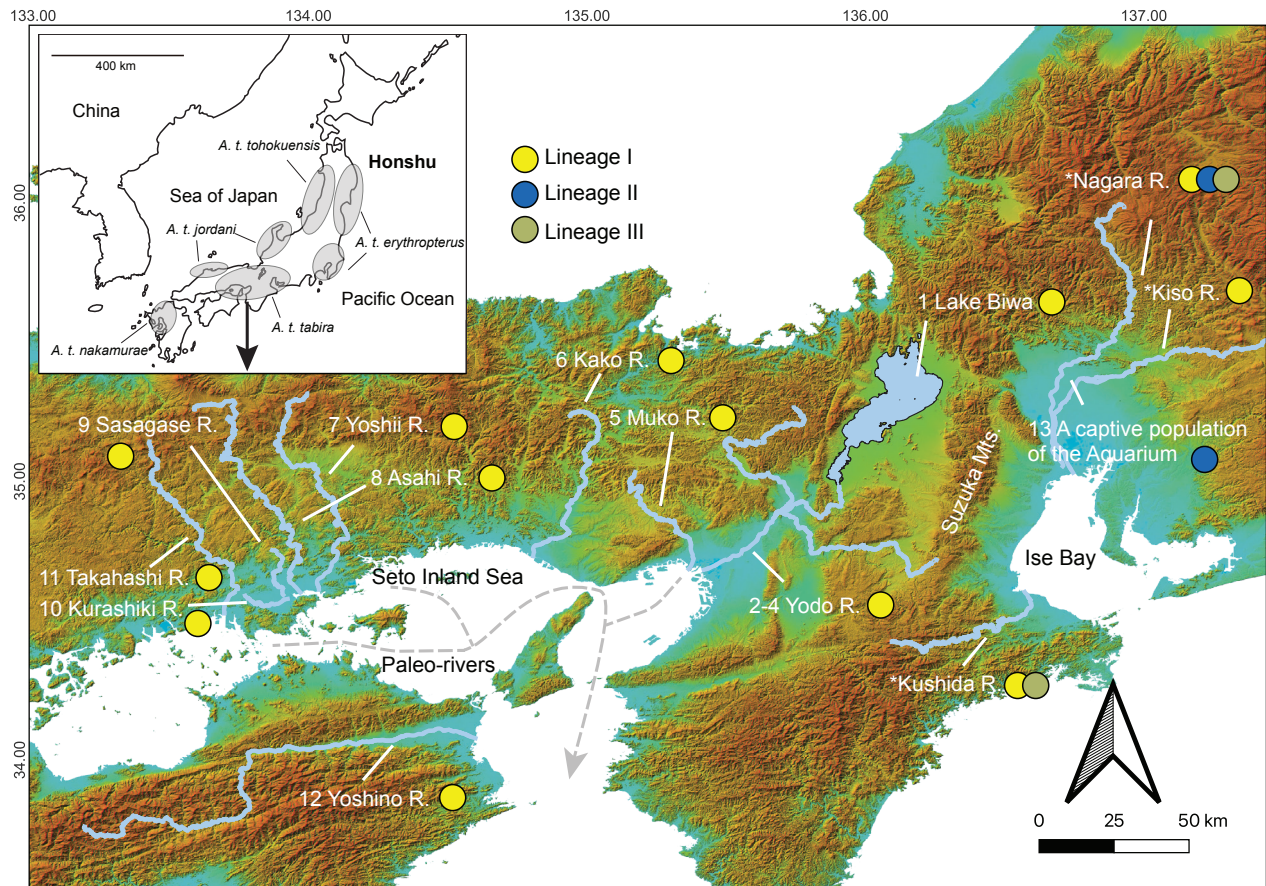
## Materials and methods

### Sample collection

In total, 140 individuals were collected from 12 localities in 10 river systems in the Seto Inland Sea and Ise Bay regions from 2015 to 2020 (Fig. 1, Suppl. material 1), covering the whole geographic range of *A. t. tabira*. The unsampled sites in this study are the Nagara, Kiso, and Kushida river systems investigated by previous studies (Kitamura et al. 2012; Umemura et al. 2012), and rivers that flow into the Sea of Japan, which are probably extinct. For the populations in the Nagara, Kiso, and Kushida River, we cited base sequence data from previous studies (Kitamura et al. 2012; Umemura et al. 2012, Table 1). Additionally, a captive population from the Gifu World Freshwater Aquarium was included in this study. This captive population is believed to have originated from a population collected and cultured by a citizen from the Nagara River system in the Ise Bay area, which was donated to the Gifu World Freshwater Aquarium in 2004 (Koki Ikeya, personal communication). The mtDNA lineage of this population is described as native to the Ise Bay region by Mukai (2019). However, it is not indicated whether it belongs to the Nobi Plain group I or II. Individual bitterling were collected using hand nets and fishing methods. Each specimen was subjected to caudal fin clipping, and the remaining specimens were fixed in 10% formalin and preserved in 70% ethanol. For habitat conservation, the number of individuals collected was limited to 20 or fewer. The fin clips were preserved in 99% ethanol and stored at  $-20^{\circ}\text{C}$ . The specimens were registered with the Gifu and Mie Prefectural Museum along with collection site information (GPM-Z-22109, MIE-Fi3500, 3506–3512, 3519–3534, 3536–3540, 3543–3579, 3581–3604, 3606–3624, 3626, 3630–3633, 3718, 4272).

### mtDNA analysis

Total genomic DNA was extracted from a portion of each caudal fin using the Kaneka Easy DNA extraction kit version 2 (Kaneka, Hyogo, Japan) or the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). Total genomic DNA was



**Figure 1.** Sampling localities of *Acheilognathus tabira tabira*. The asterisks indicate localities used by Kitamura et al. (2012), Umemura et al. (2012), and Kitamura and Uchiyama (2020). Circles show mtDNA lineages (see Fig. 2). Gray areas represent the estimated natural distribution area of the five subspecies of *A. tabira* based on Kitamura et al. (2012). This altitude map was used with permission from the Geospatial Information Authority of Japan (<https://maps.gsi.go.jp/>) and digital national and information of Ministry of Land, Infrastructure, Transport and Tourism (<https://nlftp.mlit.go.jp>).

used to amplify DNA fragments using polymerase chain reaction (PCR). For PCR, the following forward primer was used: L14690-Cb-AH, 5'-GGT CAT AAT TCT TGC TCG GA-3' (Kitanishi et al. 2016), and for the reverse primers H15913-Thr-AH, 5'-CCG ATC TTC GGA TTA CAA GAC CG-3' (Kitanishi et al. 2016), or Cytb-Rev, 5'-GAT CTT CGG ATT ACA AGA CC-3' (Hashiguchi et al. 2006). The sequencing protocol was previously described by Ito et al. (2020). All sequences were deposited in the DNA Data Bank of Japan (DDBJ), European Nucleotide Archive (EMBL), and GenBank databases under the accession numbers LC775317–LC775352.

## Sequence and phylogenetic analyses

Multiple alignments of nucleotide sequences were performed using MUSCLE (Edgar 2004). For the phylogenetic analysis, nucleotide sequence data for *A. t. tabira* were obtained from DDBJ, EMBL, and GenBank (AB620138, AB620141, AB620150, AB620159, AB759881–AB759890, and LC578851, Kitamura et al. 2012; Umemura et al. 2012; Ito et al. 2021; Table 1). In addition, we used *Acheilognathus tabira jordani* Arai, Fujikawa & Nagata, 2007, a sister group of *A. t. tabira*, as outgroups (AB620149, and AB620156, Kitamura et al. 2012).



**Table 1.** Sampling location numbers and names and GenBank accession numbers of samples.

Species name	Collection location	Accession No.	Haplotypes	Reference
<i>Acheilognathus tabira tabira</i>	Lake Biwa, Shiga, Japan	AB620138	–	Kitamura et al. 2012
<i>A.t. tabira</i>	Harai River, Mie, Japan	AB620141	–	Kitamura et al. 2012
<i>A.t. tabira</i>	Yoshii R., Okayama, Japan	AB620150	–	Kitamura et al. 2012
<i>A.t. tabira</i>	Kizu R., Kyoto, Japan	AB620159	–	Kitamura et al. 2012
<i>A.t. tabira</i>	Kiso R., Gifu, Japan	AB759881	–	Umemura et al. 2012
<i>A.t. tabira</i>	Kiso R., Gifu, Japan	AB759882	–	Umemura et al. 2012
<i>A.t. tabira</i>	Kiso R., Gifu, Japan	AB759883	–	Umemura et al. 2012
<i>A.t. tabira</i>	Kiso R., Gifu, Japan	AB759884	–	Umemura et al. 2012
<i>A.t. tabira</i>	Kiso R., Gifu, Japan	AB759885	–	Umemura et al. 2012
<i>A.t. tabira</i>	Nagara R., Gifu, Japan	AB759886	–	Umemura et al. 2012
<i>A.t. tabira</i>	Nagara R., Gifu, Japan	AB759887	–	Umemura et al. 2012
<i>A.t. tabira</i>	Nagara R., Gifu, Japan	AB759888	–	Umemura et al. 2012
<i>A.t. tabira</i>	Nagara R., Gifu, Japan	AB759889	–	Umemura et al. 2012
<i>A.t. tabira</i>	Nagara R., Gifu, Japan	AB759890	–	Umemura et al. 2012
<i>A.t. tabira</i>	Northern district, Mie, Japan	LC578851	–	Ito et al. 2021
<i>A.t. tabira</i>	Lake Biwa, Shiga, Japan	LC775317	T1	This study
<i>A.t. tabira</i>	Lake Biwa, Shiga, Japan	LC775318	T2	This study
<i>A.t. tabira</i>	Lake Biwa, Shiga, Japan, etc	LC775319	T3	This study
<i>A.t. tabira</i>	Lake Biwa, Shiga, Japan, etc	LC775320	T4	This study
<i>A.t. tabira</i>	Yodo R., Kyoto, Japan	LC775321	T5	This study
<i>A.t. tabira</i>	Yodo R., Kyoto, Japan	LC775322	T6	This study
<i>A.t. tabira</i>	Yodo R., Kyoto, Japan	LC775323	T7	This study
<i>A.t. tabira</i>	Yodo R., Kyoto, Japan	LC775324	T8	This study
<i>A.t. tabira</i>	Yodo R., Kyoto, Japan	LC775325	T9	This study
<i>A.t. tabira</i>	Yodo R., Kyoto, Japan	LC775326	T10	This study
<i>A.t. tabira</i>	Yodo R., Kyoto, Japan, etc	LC775327	T11	This study
<i>A.t. tabira</i>	Yodo R., Kyoto, Japan, etc	LC775328	T12	This study
<i>A.t. tabira</i>	Muko R., Hyogo, Japan	LC775329	T13	This study
<i>A.t. tabira</i>	Muko R., Hyogo, Japan	LC775330	T14	This study
<i>A.t. tabira</i>	Muko R., Hyogo, Japan	LC775331	T15	This study
<i>A.t. tabira</i>	Muko R., Hyogo, Japan	LC775332	T16	This study
<i>A.t. tabira</i>	Muko R., Hyogo, Japan	LC775333	T17	This study
<i>A.t. tabira</i>	Muko R., Hyogo, Japan	LC775334	T18	This study
<i>A.t. tabira</i>	Muko R., Hyogo, Japan	LC775335	T19	This study
<i>A.t. tabira</i>	Muko R., Hyogo, Japan	LC775336	T20	This study
<i>A.t. tabira</i>	Kako R., Hyogo, Japan	LC775337	T21	This study
<i>A.t. tabira</i>	Kako R., Hyogo, Japan	LC775338	T22	This study
<i>A.t. tabira</i>	Kako R., Hyogo, Japan, etc	LC775339	T23	This study
<i>A.t. tabira</i>	Yoshii R., Okayama, Japan, etc	LC775340	T24	This study
<i>A.t. tabira</i>	Yoshii R., Okayama, Japan	LC775341	T25	This study
<i>A.t. tabira</i>	Yoshii R., Okayama, Japan	LC775342	T26	This study
<i>A.t. tabira</i>	Yoshii R., Okayama, Japan, etc	LC775343	T27	This study
<i>A.t. tabira</i>	Yoshii R., Okayama, Japan	LC775344	T28	This study
<i>A.t. tabira</i>	Yoshii R., Okayama, Japan	LC775345	T29	This study
<i>A.t. tabira</i>	Asahi R., Okayama, Japan	LC775346	T30	This study
<i>A.t. tabira</i>	Sasagase R., Okayama, Japan	LC775347	T31	This study
<i>A.t. tabira</i>	Sasagase R., Okayama, Japan	LC775348	T32	This study
<i>A.t. tabira</i>	Sasagase R., Okayama, Japan	LC775349	T33	This study
<i>A.t. tabira</i>	Sasagase R., Okayama, Japan	LC775350	T34	This study
<i>A.t. tabira</i>	Yodo R., Kyoto, Japan	LC775351	T35	This study
<i>A.t. tabira</i>	Gifu R., Japan	LC775352	T36	This study
<i>A.t. jordani</i>	Oohara R., Shimane, Japan	AB620149	–	Kitamura et al. 2012
<i>A.t. jordani</i>	Kuzuryu R., Fukui, Japan	AB620156	–	Kitamura et al. 2012

Phylogenetic analyses were conducted using maximum likelihood (ML) and Bayesian inference (BI) methods. Search for the best evolutionary model each partition and ML analyses were performed using IQ-TREE 2.2.2.6 (Minh et al. 2020), with the TIM2 + F + I, K2P + I, and F81 + F models for the first, second, and third codon positions as selected by ModelFinder (Chernomor et al. 2016; Kalyaanamoorthy et al. 2017) based on the Bayesian information criterion (BIC). The reliability of each internal branch was evaluated using Shimodaira-Hasegawa-like approximate likelihood ratio test (SH-aLRT, Guindon et al. 2010) and ultrafast bootstrap (UFBoot, Hoang et al. 2018) and with 1000 replicates. In this study, according to the manual, support values of 80% or higher for SH-aLRT and 95% or higher for UFBoot were considered as high support values. BI analyses were performed using MrBayes v3.2.7a (Ronquist et al. 2012), with the HKY + I, K80 + I + G4, F81 models for the first, second, and third codon positions as selected by ModelTest-NG (Darriba et al. 2017) based on the BIC. The MCMC analyses were performed using the following settings: ngen = 10000000, sample freq = 100, and burnin = 25000. Statistical parsimony networks were constructed using TCS v1.2.1 (Clement et al. 2000). In this study, the Bayesian posterior probability (PP) of 80% or higher was considered to indicate high support values.

### Genetic structure analyses

In populations of the Kinki-Sanyo region, we calculated genetic differentiation, estimated by genetic differentiation coefficient ( $\Phi_{st}$ ; Excoffier et al. 1992) using Arlequin version 3.5 (Excoffier and Lischer 2010). Critical significance levels for multiple testing were corrected using the sequential Bonferroni procedure (Rice 1989). In addition, we identified groups of populations using the spatial analysis of molecular variance version 2.0 (SAMOVA 2.0) (Dupanloup et al. 2002). To detect the number  $K$  of groups with the largest  $F_{ct}$  value,  $K$  was user-defined between 2 and 8, and 100 independent simulated annealing processes were performed in each run. However, collection sites with two or fewer individuals (the Kurashiki and Takahashi River systems) were excluded from the  $\Phi_{st}$  and SAMOVA.

### Estimation of divergence time

The divergence times of intraspecific lineage of *Acheilognathus tabira tabira* were estimated using BEAST ver. 2.7.6 (Bouckaert et al. 2019). We used sequences of *A. t. jordani*, *A. cyanostigma* Jordan & Fowler, 1903, and *Opsariichthys platypus* (Temminck & Schlegel, 1846) as outgroups. We adopted the estimated clock rate of cytb (0.76%, Zardoya and Doadrio 1999) that has been applied to the Acheilognathinae (Tominaga et al. 2020; Miyake et al. 2021). Divergence times were calibrated using the first appearance in the fossil record of Acheilognathinae from the early Miocene (ca. 20 Mya) found in Japan (Yasuno 1984). Constraints of the fossil were specified as a log-normal distribution, ranging from 16.5–23.0 Mya in the 95% range. Estimation was carried out using an optimised relaxed clock and applied the substitution model HKY+I selected by the BIC in ModelTest-NG. MCMC chains were run for 50000000 generations and sampled every 1000 generations, with the exclusion of the first

5000000 generations as burn-in. The convergence of MCMC was checked by calculating ESS values ( $> 200$ ) using Tracer ver. 1.7.2 (Rambaut et al. 2018). TreeAnnotator ver. 2.7.6 in the BEAST package was used to obtain a maximum credibility tree with the annotation of average node ages and 95% highest posterior density (HPD) interval. The phylogenetic tree was visualized with FigTree ver. 1.4.4 (Rambaut 2018).

## Results

### Genetic structure

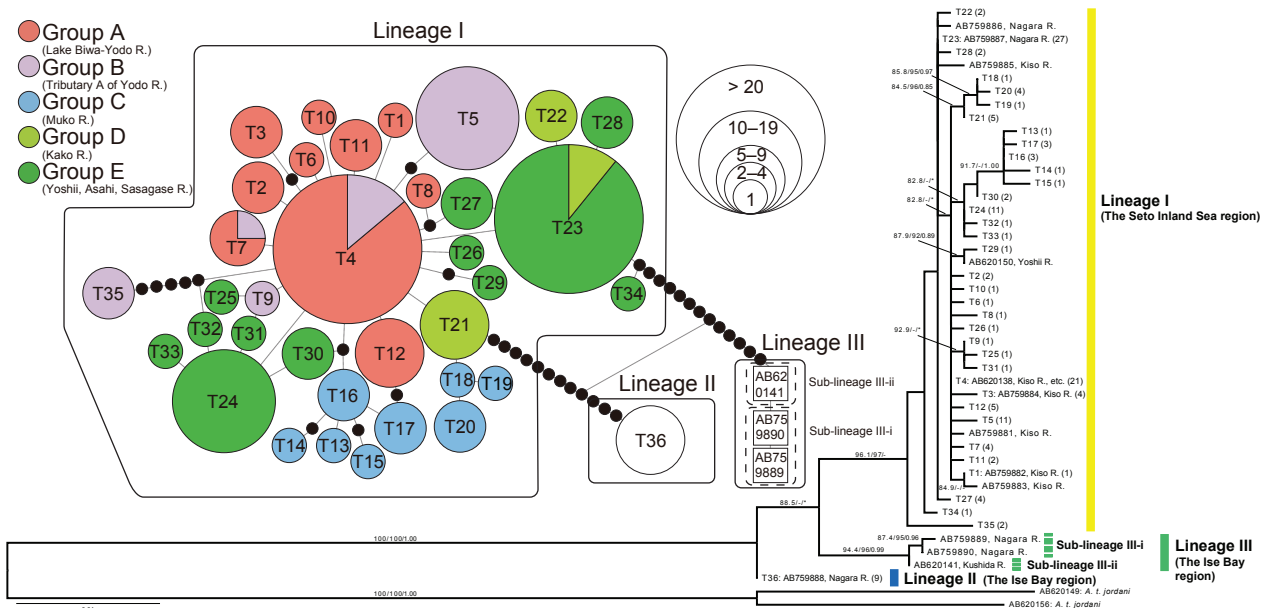
We sequenced 1069-bp mtDNA cytb gene nucleotide fragments from 140 individuals of *A. t. tabira* collected from 10 river systems and the captive population. As a result, 36 haplotypes were detected (T1–36), 5 of which [T1: AB759882 (Kiso River system), T3: AB759884 (Kiso R.), T4: AB620138, AB620159, and LC578851 (Lake Biwa, Kizu R., Kiso R., and northern Mie Prefecture), T23: AB759887 (Nagara R.), and T36: AB759888 (Nagara R.)] had been detected in previous studies (Kitamura et al. 2012; Umemura et al. 2012; Ito et al. 2021).

The topologies of the ML and BI phylogenetic trees were partially different (Fig. 2, Suppl. material 2). In the ML tree, Lineage I and III were supported as monophyletic with high values, respectively (SH-aLRT  $> 94\%$ , UFBoot  $> 96\%$ ). The monophyletic clade of Lineage I and III was supported with high values (88.5%) by SH-aLRT, however with slightly lower value (88%) by UFBoot. In the BI tree, the monophyletic clade of Lineage I was supported by PP with a low value (0.54), while Lineage III was supported with a high value (0.99). The monophyletic clade of Lineage I and II was not supported, whereas the monophyletic clade of Lineage II and III was supported, albeit with a lower value (0.69). In addition, Lineage III was further subdivided into two sub-lineages: Sub-lineages III-i and ii in the ML and BI trees (SH-aLRT = 87.4%, UFBoot = 95%, and PP = 0.96, Fig. 2).

In Lineage I, Haplotypes T1–12 and T35 were mainly detected in Lake Biwa and the Yodo River system (Loc. 1–4), four of which (T3, T4, T11, and T12) were detected in the Yoshino River system (Loc. 12). Haplotypes T13–20 were detected only in the Muko River system (Loc. 5). Haplotypes T21 and T22 were only detected in the Kako River system (Loc. 6). Haplotype T23 was detected in the Kako, Yoshii Asahi, Sasagase, and Takahashi River systems (Loc. 6–9, and 11). Haplotypes T24–34 were detected in the Yoshii Asahi, Sasagase, Kurashiki, and Takahashi River systems (Loc. 7–11). Haplotype T36 in Lineage II was detected only in a captive Gifu World Freshwater Aquarium population collected from an unknown river system in Gifu Prefecture (Loc. 13).

In the statistical parsimony network, *A. t. tabira* exhibits a bottleneck pattern (Fig. 2). Lineage I was represented by a star-like pattern centered on the ancestral haplotype T4 (Fig. 2).

The results of the pairwise  $\Phi_{st}$  among the local populations are shown in Table 2. The  $\Phi_{st}$  values of Yodo1 and Muko populations showed significant genetic differentiation between populations, excluding Yodo3 and Biwa populations, respectively ( $\Phi_{st}$  0.235–392, 0.269–0.415;  $P < 0.05$ ). The  $\Phi_{st}$  value of the Yoshino population also showed significant genetic differentiation between Kako, Yoshii, Asahi, and Sasagase populations ( $\Phi_{st}$  0.191–0.301;  $P < 0.05$ ).



**Figure 2.** Maximum likelihood (ML) tree of the 1069-bp cytochrome *b* gene sequences of *Acheilognathus tabira tabira* individuals from the Seto Inland Sea and Ise Bay regions. Numbers at nodes indicate Shimodaira-Hasegawa-like approximate likelihood ratio test values (left), ultrafast bootstrap values (middle) in the ML tree, and Bayesian posterior probabilities (right) in Bayesian inference tree. Each value is indicated when it exceeds 80%, 95%, and 0.80. Numbers in parentheses indicate the number of specimens. The parentheses after each Lineage name indicate the natural distribution area. The statistical parsimony network of *A. t. tabira* is shown to the left of the tree. Pie charts of Lineage I indicate the relative frequencies of haplotypes of the five groups defined by SAMOVA.

**Table 2.** Pairwise  $\Phi_{st}$  among local populations of *Acheilognathus tabira tabira* collected from the Seto Inland Sea region.

Site no.	Collection site	Group	1	2	3	4	5	6	7	8	9	12
1	Lake Biwa	A										
2	Yodo R. 1	B	0.235*									
3	Yodo R. 2	A	0.042	0.237*								
4	Yodo R. 3	A	0.019	0.236	-0.004							
5	Muko R.	C	0.266	0.358**	0.280*	0.261**						
6	Kako R.	D	0.271	0.339**	0.298	0.256**	0.281*					
7	Yoshii R.	E	0.159	0.301**	0.145	0.164	0.330**	0.135				
8	Asahi R.	E	0.282*	0.359**	0.297*	0.269**	0.349**	0.241	-0.006			
9	Sasagase R.	E	0.331**	0.392**	0.340**	0.321**	0.415**	0.186	0.018	0.072		
12	Yoshino R.	A	0.033	0.261**	0.093	-0.047	0.269**	0.289**	0.191*	0.301**	0.349**	

\* $P < 0.05$ ; \*\* $P < 0.001$

In addition, the  $\Phi_{st}$  values of the Asahi and Sasagase populations showed significant genetic differentiation between Biwa, Yodo2, and Yodo3 populations ( $\Phi_{st}$  0.269–0.392;  $P < 0.05$ ), and Kako population showed significant genetic differences from Yodo3 population ( $\Phi_{st}$  0.281–0.339,  $P < 0.05$ ).

The results of the population group estimation using SAMOVA are shown in Table 3 and Suppl. material 3. The highest  $F_{ct}$  value (0.32166;  $P < 0.001$ ) was obtained when the 10 populations were divided into  $K = 5$  groups: Group A (Lake Biwa-Yodo River and Yoshino River: Loc. 1, 3, 4, and 12), Group B (tributary A of Yodo River: Loc. 2), Group C (Muko R. Loc. 5), Group D (Kako R.: Loc. 6) and Group E

**Table 3.** Fixation indicating corresponding groups of populations inferred by spatial analysis of molecular variance (SAMOVA).

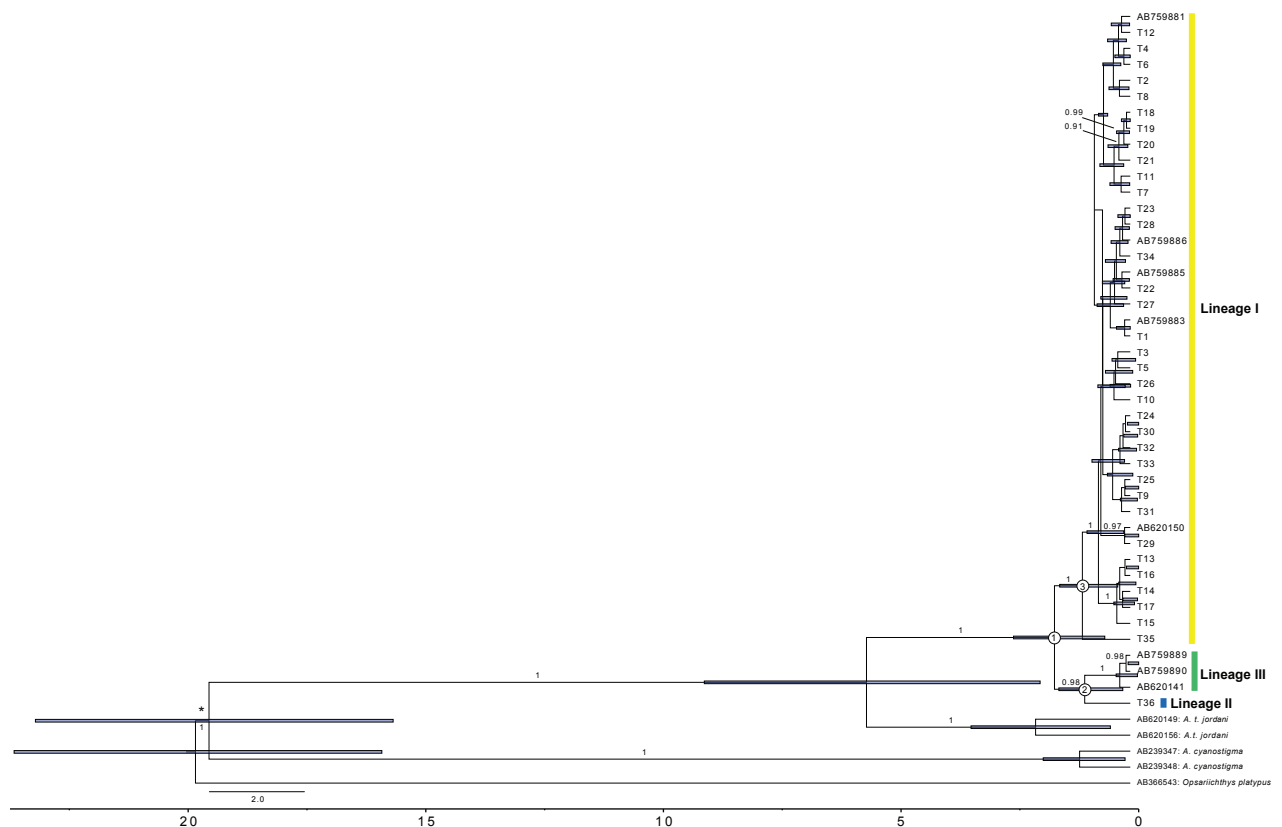
Number of groups (K)	Group composition	$F_{sc}$	$F_{st}$	$F_{ct}$
2	"Biwa"+"Yodo1"+"Yodo2"+"Yodo3"+"Kako"+"Yoshii"+"Asahi"+"Aasagase"+"Yoshino" "Muko"	0.21769***	0.42786***	0.26865**
3	"Biwa"+"Yodo2"+"Yodo3"+"Kako"+"Yoshii"+"Asahi"+"Sasagase"+"Yoshino" "Muko" "Yodo1"	0.14052***	0.38805***	0.288*
4	"Biwa"+"Yodo2"+"Yodo3"+"Yoshino" "Kako"+"Yoshii"+"Asahi"+"Sasagase" "Muko" "Yodo1"	0.0344**	0.33127***	0.30745***
5	"Biwa"+"Yodo2"+"Yodo3"+"Yoshino" "Yoshii"+"Asahi"+"Sasagase" "Muko" "Yodo1" "Kako"	-0.00438	0.31869***	0.32166***
6	"Biwa"+"Yodo2"+"Yodo3"+"Yoshino" "Yoshii"+"Asahi" "Muko" "Yodo1" "Kako" "Sasagase"	-0.02137	0.30139***	0.31601***
7	"Yodo2"+"Yodo3"+"Yoshino" "Yoshii"+"Asahi" "Muko" "Yodo1" "Kako" "Sasagase" "Biwa"	-0.02246	0.29394***	0.30945**
8	"Yodo2"+"Yodo3"+"Yoshino" "Muko" "Yodo1" "Yoshii" "Kako" "Sasagase" "Biwa" "Asahi"	-0.03234	0.2872***	0.30953*

$P < 0.05^*$ ,  $P < 0.01^{**}$ ,  $P < 0.001^{***}$

(Yoshii, Asahi, and Sasagase Rs: Loc. 7–9), and explained 32.17% of the variation among groups ( $P < 0.001$ ), -0.3% of the variation among populations within groups ( $P > 0.1$ ), and 68.13% of the variation within populations ( $P < 0.001$ ).

### Divergence time

We showed the divergence times of the three lineages of *A. t. tabira* in Fig. 3. In this tree, the exclusivity of Lineage I was supported (1.00), similar to the ML and BI trees described above, whereas the exclusivity of Lineages II and



**Figure 3.** Divergence time estimation by Bayesian inference tree of the 1069-bp cytochrome *b* gene sequences of *Acheilognathus tabira tabira* and outgroups. The blue rectangular bars on the nodes indicate the 95% highest probability density. Bayesian posterior probabilities are indicated at nodes, with values exceeding 0.90 shown. The node marked with an asterisk indicates the calibration point based on fossil record for the Acheilognathinae. Nodes with circled numbers are referenced in the text.

III was supported (0.98), similar to the topology of the BI tree. The estimated divergence time between Lineage I and II+III was approximately 1.53 Mya (95% HPD, 0.71–2.64 Mya; Fig. 3, node 1), while that between Lineage II and III was approximately 0.91 Mya (0.34–1.68 Mya; node 2). The time of the most recent common ancestor (tMRCA) of Lineage I was estimated approximately 0.96 Mya (0.44–1.66 Mya; Fig. 3, node 3).

## Discussion

### Phylogeographic and genetic population structure patterns

We estimated the phylogenetic tree of *A. t. tabira* based on the sequence of the cytochrome *b* region of the mtDNA, and in the samples used in the present study, three lineages (Lineages I, II, and III) were identified primarily based on the ML tree. The results were similar to those of a previous study (Umemura et al. 2012), where Lineage I to III were referred to as the Kinki-Sanyo Group, Nobi Plain Group I, and Nobi Plain Group II, respectively. The populations newly analyzed in the present study (Muko, Kako, Sasagase, Takahashi, Kurashiki, and Asahikawa River systems) were all included in Lineage I, and the captive Gifu World Freshwater Aquarium population collected from Gifu Prefecture was included in Lineage II. In a previous study, a haplotype of Lineage II was detected



only in an individual collected from a tributary of the Nagara River system in the previous study (Umemura et al. 2012). Lineages I (non-native lineage) and III (native lineage) were identified in this tributary; therefore, it was unclear whether Lineage II was native to the Ise Bay region or non-native to the Seto Inland Sea region (Umemura et al. 2012). In the present study, the haplotype belonging to Lineage II was not identified in any water system in the Seto Inland Sea region; therefore, the natural distribution area of Lineage II was considered to be the Ise Bay region.

The populations of many freshwater fishes [e.g., *Sarcocheilichthys variegatus variegatus* (Temminck & Schlegel, 1846) and *Opsariichthys platypus*] in the Ise Bay region are thought to have been divided from the populations of the Seto Inland Sea region by the uplift of the Suzuka Mountains approximately one million years ago (Mya) (Watanabe et al. 2017). The genetic lineages of populations in many freshwater fishes in each region of Ise Bay and the Seto Inland Sea are generally exclusive (e.g., *S. v. variegatus*, Komiya et al. 2014; *O. platypus*, Kitanishi et al. 2016; *Pseudogobio esocinus* (Temminck & Schlegel, 1846) and *P. agathonectris* Tominaga & Kawase, 2019, Tominaga et al. 2016; *Tanakia lanceolata* (Temminck & Schlegel, 1846), Tominaga et al. 2020). However, in the case of *A. t. tabira*, the exclusivity of the two native lineages in the Ise Bay region (lineages II and III) was not supported in the ML tree and was supported with low values in the BI tree. In the ML tree, these two lineages were shown as paraphyletic groups, and each branch was highly supported. Furthermore, the results of the statistical parsimony network also indicated that lineages II and III were closely related to different haplotypes of Lineage I. On the other hand, the BI tree does not have the same topology as the ML tree. Divergence time estimates suggest that the supported topology is similar to that of the BI tree, with Lineages II and III diverging from Lineage I approximately 1.53 Mya, and Lineages II and III diverging approximately 0.91 Mya. However, due to the varying exclusivity of Lineages II and III across different phylogenetic trees, accepting this divergence time estimation as it is would be risky. And more, the original distribution range of Lineage II and III is unclear, as *A. t. tabira* in the Ise Bay region has already become extinct in many river systems (Mukai 2019). If DNA analysis of specimens collected decades ago and stored in museums becomes possible, it will be possible to verify this issue. To consider the divergence order among the three lineages, further studies using longer sequences, such as mitogenomes, including sequence data from historical specimens, are necessary to re-estimate divergence times.

In Lineage I, which was detected only in the Seto Inland Sea region, genetic differentiation has not been recognized in previous studies because of the small number of sampling sites and individuals (Kitamura et al. 2012). In the present study, we greatly increased the number of sampling sites and individuals; as a result, five genetic groups (A–E) were distinguished within Lineage I using SAMOVA. These five groups comprised adjacent river systems, indicating that the genetic population of *A. t. tabira* within the Seto Inland Sea region was genetically differentiated into narrow regions. The river systems flowing into the Seto Inland Sea are thought to have connected as a single paleo-river system during the glacial periods of the Pleistocene (ca. 0.01–2.5 Mya) and were isolated during the interglacial periods (Kuwashiro 1959; Ota et al. 2004). Additionally, the uplift of the mountains areas surrounding the Seto Inland

Sea is thought to have become active since the Pleistocene (Ota et al. 2004), making the migration of freshwater fish between river systems difficult during this period. The tMRCA of Lineage I is estimated to be 0.96 Mya (95% HPD, 0.44–1.66 Mya), overlapping with the periods of connection and isolation of the paleo-river systems and the uplift of mountains around the Seto Inland Sea. Therefore, the isolation factors between the regional groups are suggested to be related to the paleo-river systems and active uplift of mountains that occurred during the Pleistocene. Genetic differentiation in the same period due to similar factors in the Seto Inland Sea region has also been suggested in *P. esocinus* (Tominaga et al. 2016).

A unique genetic group (Group D) was identified in the Kako River system. However,  $\Phi_{st}$  showed no significant genetic differentiation ( $P > 0.05$ ) between the Kako River system and the other three river systems (Yoshii, Asahi, and Sasagase) included in Group E. SAMOVA results indicated that most of the genetic variation in this subspecies was within populations (68.13%) and that differentiation among groups was relatively small (32.17%). Genome-wide analysis of nuclear DNA may be useful for more detailed elucidation of the genetic population structure of *A. t. tabira*.

### Artificially introduced populations

Populations collected from the Lake Biwa-Yodo and Yoshino River systems were included in Group A. In addition, four haplotypes detected in the Yoshino River system were similar to those in the Lake Biwa-Yodo River system. This study demonstrates that populations in the Seto Inland Sea region are genetically differentiated by localized areas. The reason for this is thought to be the same as with other species: the disappearance of the paleo-river system and isolation due to the uplift of mountains. Therefore, it is unlikely that the population in the Yoshino River system has the same haplotype as the population in the Lake Biwa-Yodo River system, which is across the Seto Inland Sea. In the Yoshino River system, non-native freshwater fishes [e.g., *Acheilognathus cyanostigma* and *Acheilognathus rhombeus* (Temminck & Schlegel, 1846)] were estimated to have been artificially introduced from Lake Biwa (Hosoya 2019; Miyake et al. 2021). Ministry of the Environment of Japan (2015) also indicated that *A. t. tabira* populations were artificially introduced into the Yoshino River system. Therefore, the finding that only haplotypes of the Yoshino River system are common to those of the Lake Biwa-Yodo River system supports the hypothesis that the Yoshino River system population was artificially introduced from the Lake Biwa-Yodo River system.

### Conservation

The captive population of the Gifu World Freshwater Aquarium was identified as Lineage II, which is thought to be native to the Ise Bay region. Non-native populations belonging to Lineage I have been artificially introduced into all habitats of native populations in the Ise Bay region (Umemura et al. 2012; Kitamura and Uchiyama 2020). Therefore, the captive population of the Gifu World Freshwater Aquarium may be a native population of the Ise Bay region that has not undergone genetic introgression. However, to confirm that the population

is not genetically introgressed, examining the possibility of hybridization with Lineage I using nuclear DNA is necessary.

Conservation units need to focus on levels below species (Moritz 1994, Frankham et al. 2010). Evolutionarily significant units (ESUs) refer to phylogenetically unique intraspecific population groups, established by factors such as mitochondrial DNA monophyly (Moritz 1994). In the case of *A. t. tabira*, the exclusivity of Lineages I–III was supported; thus, we propose to designate them as ESUs.

Furthermore, Management Units (MUs) are established based on allele frequencies among populations (Moritz 1994, Frankham et al. 2010). In the case of *A. t. tabira*, Groups A–E found within Lineage I were significantly genetically differentiated by SAMOVA. Therefore, we propose that these five groups be conserved as MUs of *A. t. tabira* in the Seto Inland Sea region. However, it has been proposed that adaptive traits should also be taken into account in the establishment of ESUs and MUs (Crandall et al. 2000). In the future, in order to establish better conservation units, it is necessary to study adaptive traits among populations of *A. t. tabira*.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

Gen Ito: Conceptualization, Data curation, Formal Analysis, Funding acquisition, and Writing – original draft. Naoto Koyama, Ryota Noguchi, Ryoichi Tabata, Seigo Kawase, and Jyun-ichi Kitamura: Investigation, Resources, and Writing – review & editing. Yasunori Koya: Supervision, Funding acquisition, and Writing – review & editing.

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### Data availability

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

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## Supplementary material 1

### List of collection sites for *Acheilognathus tabira tabira* and distribution of each haplotype across the 13 collection sites

Authors: Gen Ito, Naoto Koyama, Ryota Noguchi, Ryoichi Tabata, Seigo Kawase, Jun-ichi Kitamura, Yasunori Koya

Data type: xlsx

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Link: <https://doi.org/10.3897/natureconservation.56.111745.suppl1>

## Supplementary material 2

### **Bayesian inference (BI) tree of the 1069-bp cytochrome *b* gene sequences of *Acheilognathus tabira tabira* individuals from the Seto Inland Sea and Ise Bay regions**

Authors: Gen Ito, Naoto Koyama, Ryota Noguchi, Ryoichi Tabata, Seigo Kawase, Jyun-ichi Kitamura, Yasunori Koya

Data type: pdf

Explanation note: Numbers at nodes indicate Bayesian posterior probabilities; the value is indicated when it exceeds 0.80.

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Link: <https://doi.org/10.3897/natureconservation.56.111745.suppl2>

## Supplementary material 3

### **The distribution map of Groups estimation using SAMOVA of *Acheilognathus tabira tabira***

Authors: Gen Ito, Naoto Koyama, Ryota Noguchi, Ryoichi Tabata, Seigo Kawase, Jyun-ichi Kitamura, Yasunori Koya

Data type: pdf

Explanation note: Circles show groups (see Fig. 2). Gray areas are the estimated natural distribution area of *A. tabira* 5 subspecies based on Kitamura et al. (2012). This altitude map was used with permission from the Geospatial Information Authority of Japan (<https://maps.gsi.go.jp/>) and digital national and information of Ministry of Land, Infrastructure, Transport and Tourism (<https://nlftp.mlit.go.jp>). The paleo-river system follows Kuwashiro (1959).

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## Research Article

# Amphibians and reptiles of the Transvolcanic Belt biogeographic province of Mexico: diversity, similarities, and conservation

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## Abstract

The Transvolcanic Belt (TVB) of Mexico is a biogeographic province of significant biodiversity, acting as a transition zone between eastern and western Mexico. Using available literature, we collected species lists for amphibians and reptiles in Mexican states within the TVB biogeographic province, updating them with additional literature. The TVB is home to 427 native species of amphibians and reptiles, 154 amphibians and 273 reptiles, which represent 30.5% of the species of amphibians and reptiles in Mexico. The TVB also houses 50 endemic species, with 84 species listed by the IUCN. Threats include habitat destruction and pollution. The TVB shares a significant portion of its amphibian and reptile species with neighboring provinces, particularly the Sierra Madre Oriental and the Sierra Madre del Sur, suggesting a mixture of species from both eastern and western Mexico. Cluster analyses based on species composition reveal distinct groupings of provinces, with the TVB forming a cluster with the Sierra Madre Oriental, Veracruz, and Sierra Madre del Sur for both amphibians and reptiles. Conservation assessments indicate that a significant proportion of the amphibian and reptile species in the TVB are at risk, primarily due to habitat loss from urbanization, agriculture, and pollution. Urgent conservation actions are needed to protect the unique herpetofauna of the TVB from further decline.

**Key words:** Amphibians, biogeographic province, conservation, herpetofauna, reptiles, species richness, Transvolcanic Belt



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## Introduction

The Transvolcanic Belt (TVB) is the highest mountain range in Mexico, extending from west-central Veracruz, through Tlaxcala, Puebla, Morelos, Mexico City, state of Mexico, northern Guerrero, Michoacán, Colima, Jalisco and central Nayarit (Ferrari et al. 2012). The TVB divides Mexico into a northern and southern half, and is home to an impressive biodiversity due to its contact with the biogeographic provinces of eastern and western Mexico: Veracruz and Sierra Madre Oriental in the east and Pacific Lowlands and Sierra Madre Occidental in the west. Likewise, the TVB acts as a barrier preventing the exchange of species from northern provinces, such as the Chihuahuan Desert, and southern provinces, such as the Balsas Basin and Sierra Madre del Sur (Morrone 2019). The

location of the TVB adjacent to provinces that differ in their biotas results in a diverse mix of species with Neotropical and Nearctic affinities. The TVB is therefore considered a transitional biogeographic province (Morrone et al. 2017) that connects three other transition provinces, the Sierra Madre Occidental, Sierra Madre Oriental, and Sierra Madre del Sur, representing the core of the Mexican Transition Zone (Espinosa and Ocegueda 2007; Halffter and Morrone 2017). In addition, the rugged topography of the TVB, which houses the highest mountains in Mexico with several surpassing 3,000 m, provides the TVB with unique alpine environments, absent from other Mexican biogeographic provinces, and providing it with a unique assortment of species. Several of the volcanoes found in the TVB are of recent origin, the youngest being the Parícutín which originated on 20 February 1943, and many are still active, resulting in this province being commonly known as the Neovolcanic Axis (Yarza De la Torre 2003).

The intense volcanic and orogenic activity in the TVB has resulted in the formation of many fluvial deposits; with soils in this biogeographic province able to retain water (Pangea 2023). Thus, forests act as rain traps, contributing to the refilling of underground aquifers (Pangea 2023). The climate is mostly temperate, and humidity levels vary according to altitude. Pine forests in the TVB grow at elevations of 2,275–2,600 masl, pine-oak forests at 2,470–2,600 masl, pine-cedar forests at more than 2,700 masl, and pine-fir forests above 3,000 masl (Pangea 2023). This province is important for its high biodiversity and number of endemic species, because its mountains probably served as a refuge for Nearctic species that were forced to migrate to temperate areas when the Chihuahuan Desert became drier and hotter during the Pleistocene (Pangea 2023). The diversity of terrestrial vertebrates that inhabit the TVB is notable including 703 bird species (Lepage 2023), and 152 species of mammals (Gámez et al. 2012).

The TVB is also home to several of the country's largest cities, including Mexico City, which together with the metropolitan area found in the state of Mexico forms the most populated region of Mexico. Very close to this large city, to the southwest, south, and north-northeast, are the cities of Toluca, Cuernavaca, and Pachuca. Together with the metropolitan area of Mexico City, these cities are continuing to grow and expand with devastating consequences for the natural resources of the region, especially flora and fauna of the central part of the TVB, including amphibians and reptiles (Lemos-Espinal and Smith 2020b). The expansion of these urban areas has caused the loss of habitat that is considered one of the main threats facing the herpetofauna of the region (IUCN 2022). Indeed, there are species in Mexico City that are known only from their original records (e.g., *Geophis bicolor* and *Geophis petersi*), or whose conservation status is quite tenuous (e.g., *Eleutherodactylus grandis*, *Rana tlaloci*, and *Ambystoma mexicanum*) (Lemos-Espinal and Smith 2020b).

Here we provide a list of the species of amphibians and reptiles found in the TVB, as well as summarizing the conservation status and their similarity with neighboring biogeographic provinces.

## Physiographic characteristics

The TVB comprises a total area of 82,840 km<sup>2</sup>, a perimeter of 5,750 km, a length of approximately 930 km, and an average width of 180 km. The TVB is located between 18.30605744° and 21.83918068° latitude and -96.70389784° and

-105.1007273° longitude. The TVB contains the highest mountains in Mexico, including Citlaltépetl (5,610 m), Popocatepetl (5,400 m), Iztaccíhuatl (5,215 m), Nevado de Toluca (4,680 m), Malinche (4,420 m), Nevado de Colima (4,260 m), and Ajusco (3,930 m). To the north, the TVB is bordered by the Chihuahuan Desert (border length = 1,488 km), the Sierra Madre Oriental (border length = 307 km), and the Veracruz (border length = 281 km), the Balsas Basin (border length = 1,968 km) and the Sierra Madre del Sur (border length = 434 km) to the south, and the Pacific Lowlands (border length = 1,272 km) to the west (Fig. 1). Although the main characteristic of this province is its high mountains and volcanoes, they are separated by large flat areas in the form of intermontane valleys and plains, through which rivers, fed by the montane runoff, run and form closed basins leading to lakes, some of which are located in the cones of extinct volcanoes, such as those in the region of Alchichica, Puebla, and the Lago de la Luna, in the Nevado de Toluca.

One of the most important valleys in this province is the Valley of Mexico, where the metropolitan area of the state of Mexico and Mexico City is located, surrounded by the Sierra Nevada to the east, the Sierra del Ajusco in the south, the Sierra de las Cruces in the west, and the Sierra de Guadalupe in the north. Other important valleys are home to large cities such as the valleys of Puebla and Toluca. The geological history of the TVB, its topography and varied biotic connections with other biogeographic provinces makes it one of the most complex and heterogeneous provinces in Mexico (Gámez et al. 2012).

The climate of the TVB is varied and depends on its location with respect to the coasts and the height of the relief. In coastal regions, at altitudes < 800 masl, where oceanic humidity has a direct influence, the climate is semi-warm with heavy precipitation rate, and with an average annual temperature between 18 and 22 °C (García 2004). From 800 masl to approximately 2,000 masl, the climate within the TVB changes to temperate-humid, with an average annual temperature lower than 18 °C and annual precipitation reaching up to 4,500 mm, but the average annual precipitation in most of this altitudinal range is 2,500 mm. At altitudes > 2,000 masl, the climate is temperate-semi-cold with an average annual temperature of 8 °C and an average annual precipitation commonly < 1,200 mm. The cold tundra climate occurs between 4,000 and 5,100 m and with temperatures ranging between -2 and 5 °C. The coldest climate, with perpetual snow, is located at approximately 5,100 masl, with an average annual temperature below -2 °C. On the other hand, in the high plains in central of the TVB and far from oceanic influence, the climate is mostly temperate with an average annual temperature of 18 to 22 °C, with less precipitation compared to the mountain areas. In the central plateaus there are also large areas that have dry steppe climates; in part due to the katabatic winds that give rise to the Föhn effect in regions adjacent to mountain areas (Fig. 2; Soto Molina et al. 2021).

The topographic and climatic variety of the TVB determines the presence of a wide variety of vegetation types which, like climate, are related to the altitude at which they are found. The semi-warm climates near the Gulf coast promote the presence of mountain cloud forest and deciduous forest, and on the Pacific coast, tropical deciduous forest. These forests generally occur between 800 and 2,000 masl. The largest proportion of the central part of the TVB above 2,000 masl is occupied by oak and coniferous forests, with the pine-oak association



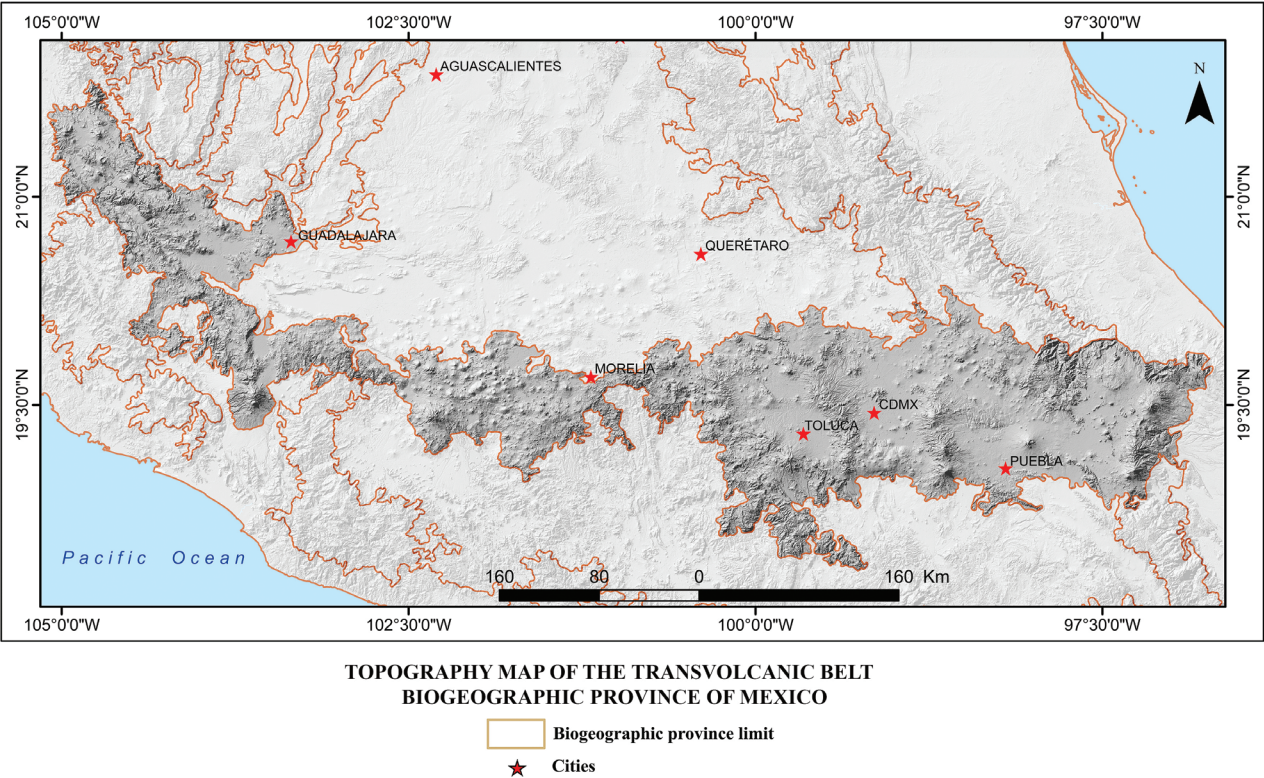


Figure 1. Topography map of the TVB biogeographic province of Mexico. (ASTER GDEM2 2011).

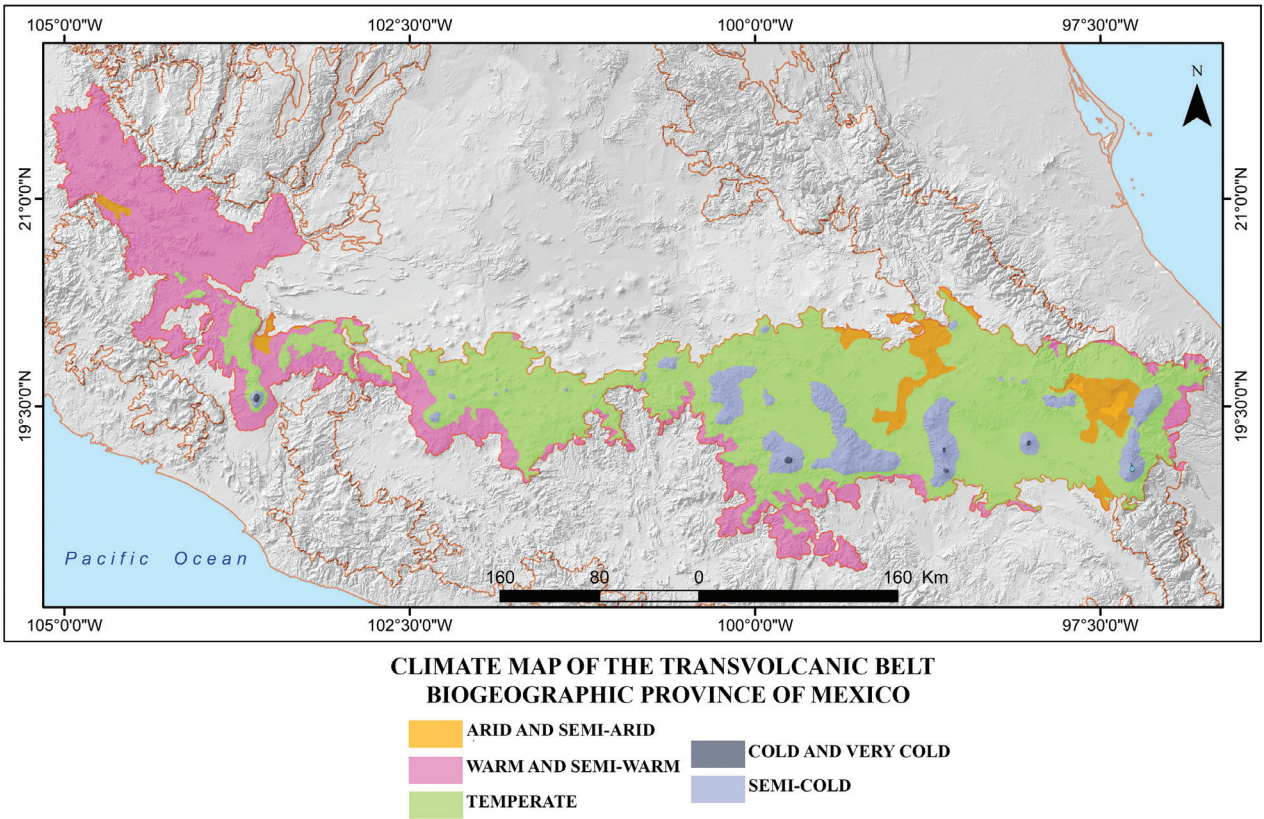
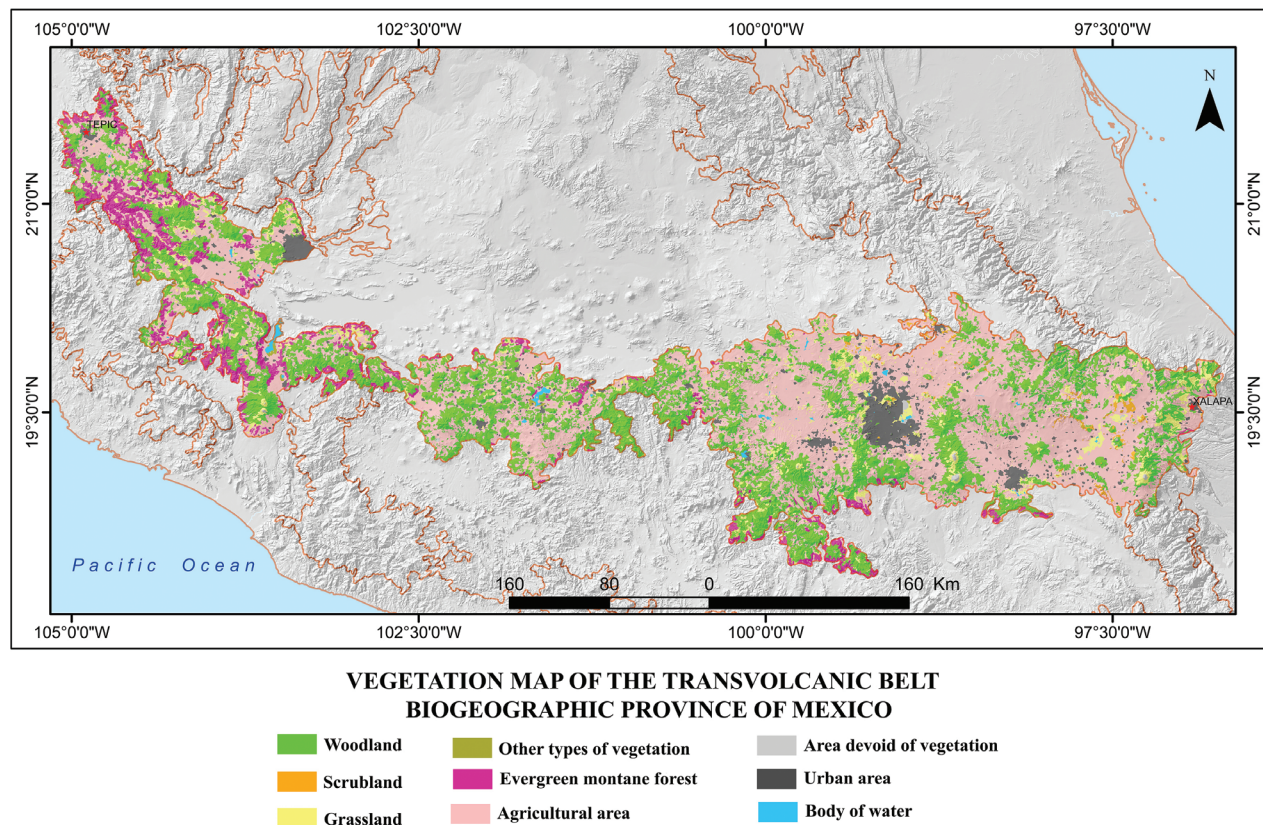


Figure 2. Climate map of the TVB biogeographic province of Mexico. (García 1998).





**Figure 3.** Vegetation map of the TVB biogeographic province of Mexico. (INEGI 2016).

predominating. Oak forests dominate below 2,200 masl, above this altitude pine trees start to appear, and between 2,200 and 2,600 masl are mixed with oaks and higher with cedar, and above 3,000 masl there are pine-fir forests (Pangea 2023). Above 3,700 masl there are forests dominated by *Pinus* with continuous forests up to 4,019 masl and discontinuous forests up to 4,072 masl (Soto Molina et al. 2021). At a higher elevation there is a strip of alpine grassland composed of grasses of the genera *Agrostis*, *Bromus*, *Calamagrostis*, *Festuca*, and *Mühlenbergia* that reaches up to 4,800 masl (Fig. 3) (Rzedowski 1978).

The central part of the TVB is the most densely populated region of Mexico, and home to around 30 million people. Cities such as Mexico City, Toluca, and Cuernavaca make up a large megalopolis, which is located in a high mountainous area that houses a total of 36 federal protected natural areas and more than one hundred state protected natural areas (Deutsche Gesellschaft für Internationale Zusammenarbeit 2023). Furthermore, east of the Valley of Mexico, several cities of the state of Puebla are rapidly growing, threatening important federal protected areas such as the Iztaccíhuatl – Popocatepetl National Park, which is part of the Sierra Nevada Mountain range to the east of the Valley of Mexico.

## Methods

Using the available literature, we collected species lists of the amphibians and reptiles found in all of the Mexican states included in the Transvolcanic Belt (TVB) biogeographic province, that we updated using additional literature

(see Appendix 1). For the most part, we follow Frost (2024) and AmphibiaWeb (2024) (<http://amphibiaweb.org>) for amphibian names and Uetz et al. (2023) for reptile names. We defined the TVB biogeographic province according to Morrone (2005, 2006, 2019), and Morrone et al. (2017). 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 biogeographic provinces (TVB, Sierra Madre Oriental, Sierra Madre del Sur, Veracruz, Pacific Lowlands, Balsas Basin, and Chihuahuan Desert) for amphibians and reptiles separately. We identified clusters and sub-clusters by visually examining the resulting cluster tree and grouping biogeographic provinces that shared common nodes, taking into account the Jaccard distances. We used the species lists to calculate pair-wise Jaccard distances for the seven neighboring biogeographic provinces for amphibians and reptiles, separately. In addition, we obtained four geospatial estimates using the map of biotic provinces of Mexico by Morrone et al. (2017) on a Lambert Conformal Conic projection in Datum WGS84 in ArcGIS 10.8.1 (Environmental Systems Research Institute, Inc, Redlands, CA): 1) the length of shared borders between the biogeographic provinces using the Polygon Neighbors Tool; 2) the straight-line distance between the centroids of the biogeographic provinces using the Feature to Point Tool and Point Distance; 3) the territorial area using the Calculate Geometry Tool; and 4) the perimeter of each biogeographic province also using the Calculate Geometry Tool. We also determined the latitudinal and longitudinal extremes of each biogeographic province, using the layer properties option. We used non-parametric Spearman's  $r$  tests to examine correlations among Jaccard distance estimates, the length of shared borders, and the distance between the centroids of the biogeographic provinces. 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

### Species richness

The TVB is home to 427 native species of amphibians and reptiles, 154 amphibians and 273 reptiles, representing 40 families, 13 of amphibians (10 anurans, two salamanders, and one caeciliid) and 27 reptiles (15 lizards, nine snakes, and three turtles), and 123 genera (35 amphibians and 88 reptiles) (Tables 1, 2). According to Lemos-Espinal and Smith (unpublished data), the total number of native amphibian and reptile species in Mexico is 1,399 (435 amphibians and 964 reptiles), included in 55 families (16 amphibians and 39 reptiles) and 210 genera (55 amphibians and 155 reptiles), which are similar to those reported by Ramírez-Bautista et al. (2023). Therefore, the TVB is home to 72.7% (40/55) of the families, 79.4% (123/155) of the genera, and 30.5% (427/1,399) of the species of amphibians and reptiles present in the country. For amphibians, the TVB is home to 81.3% (13/16) of the families, 70.9% (39/55) of the genera, and 35.4% (154/435) of the species that inhabit Mexico, and for reptiles 69.2% (27/39), 56.8% (88/155), and 28.3% (273/964) of the species in Mexico.

**Table 1.** Amphibians and reptiles of Transvolcanic Belt (TVB) biogeographic province of Mexico with distributional and conservation status. IUCN Status: (DD = Data Deficient; LC = Least Concern; NT = Near Threatened; VU = Vulnerable; EN = Endangered; CR = Critically Endangered; NE = not Evaluated) according to the IUCN Red List (IUCN 2022-2); Environmental Vulnerability Score: (EVS – the higher the score the greater the vulnerability: low (L) vulnerability species (EVS of 3–9); medium (M) vulnerability species (EVS of 10–13); and high (H) vulnerability species (EVS of 14–20) (Wilson et al. 2013a,b; Johnson et al. 2015); Mx refers to conservation status in Mexico according to SEMARNAT (2019): (P = in danger of extinction, A = threatened, Pr = subject to special protection, NL – not listed). GI refers to Global Distribution: 0 = Endemic to the TVB; 1 = Endemic to Mexico; 2 = Shared between the US and Mexico; 3 = widely distributed from Mexico to Central or South America; 4 = widely distributed from the US to Central or South America; Introduced = Introduced to the TVB. Tot: total number of Mexican biogeographic provinces in which the species has been recorded; EN = endemic to the TVB.

	IUCN	EVS	Mx	GI	Tot
<b>Class Amphibia</b>					
<b>Order Anura</b>					
<b>Bufonidae</b>					
<i>Anaxyrus compactilis</i> (Wiegmann, 1833)	LC (?)	H (14)	NL	1	6
<i>Anaxyrus punctatus</i> (Baird & Girard, 1852)	LC (–)	L (5)	NL	2	10
<i>Incilius cristatus</i> (Wiegmann, 1833)	EN (↓)	H (14)	Pr	1	3
<i>Incilius marmoreus</i> (Wiegmann, 1833)	LC (=)	M (11)	NL	1	8
<i>Incilius mazatlanensis</i> (Taylor, 1940)	LC (=)	M (12)	NL	1	5
<i>Incilius nebulifer</i> (Girard, 1854)	LC (=)	L (6)	NL	2	4
<i>Incilius occidentalis</i> (Camerano, 1879)	LC (=)	M (11)	NL	1	7
<i>Incilius valliceps</i> (Wiegmann, 1833)	LC (=)	L (6)	NL	3	9
<i>Rhinella horribilis</i> (Wiegmann, 1833)	NE	L (3)	NL	4	12
<b>Centrolenidae</b>					
<i>Hyalinobatrachium viridissimum</i> (Taylor, 1942)	NE	M (10)	NL	3	6
<b>Craugastoridae</b>					
<i>Craugastor alfredi</i> (Boulenger, 1898)	LC (↓)	M (11)	NL	3	4
<i>Craugastor augusti</i> (Dugès, 1879)	LC (=)	L (8)	NL	2	9
<i>Craugastor berkenbuschii</i> (Peters, 1870)	LC (?)	H (14)	Pr	1	4
<i>Craugastor cueyatl</i> Jameson, Streicher, Manuelli, Head & Smith, 2022	NE	NE	NL	0	EN
<i>Craugastor decoratus</i> (Taylor, 1942)	LC (=)	H (15)	NL	1	4
<i>Craugastor hobartsmithi</i> (Taylor, 1937)	LC (=)	H (15)	NL	1	5
<i>Craugastor loki</i> (Shannon & Werler, 1955)	LC (=)	M (10)	NL	3	7
<i>Craugastor mexicanus</i> (Brocchi, 1877)	LC (=)	H (16)	NL	1	5
<i>Craugastor occidentalis</i> (Taylor, 1941)	LC (=)	M (13)	NL	1	6
<i>Craugastor pygmaeus</i> (Taylor, 1937)	LC (?)	L (9)	NL	3	7
<i>Craugastor rhodopis</i> (Cope, 1867)	LC (=)	H (14)	NL	1	4
<i>Craugastor spatulatus</i> (Smith, 1939)	EN (↓)	H (16)	Pr	1	3
<i>Craugastor vocalis</i> (Taylor, 1940)	LC (↓)	M (13)	NL	1	5
<b>Eleutherodactylidae</b>					
<i>Eleutherodactylus angustidigitorum</i> (Taylor, 1940)	LC (=)	H (17)	Pr	1	2
<i>Eleutherodactylus cystignathoides</i> (Cope, 1877)	LC (=)	M (12)	NL	1	4
<i>Eleutherodactylus erendirae</i> Grünwald, Reyes-Velasco, Franz-Chávez, Morales-Flores, Ahumada-Carrillo, Jones & Boissinot, 2018	EN (?)	NE	NL	0	EN
<i>Eleutherodactylus floresvillelai</i> Grünwald, Reyes-Velasco, Franz-Chávez, Morales-Flores, Ahumada-Carrillo, Jones & Boissinot, 2018	VU (?)	NE	NL	0	EN
<i>Eleutherodactylus franzi</i> Grünwald, Montañó-Ruvalcaba, Jones, Ahumada-Carrillo, Grünwald, Zheng, Strickland & Reyes-Velasco, 2023	NE	NE	NL	0	EN
<i>Eleutherodactylus grandis</i> (Dixon, 1957)	EN (↓)	H (18)	Pr	0	EN

	IUCN	EVS	Mx	GI	Tot
<i>Eleutherodactylus guttillatus</i> (Cope, 1879)	LC (?)	M (11)	NL	2	4
<i>Eleutherodactylus humboldti</i> Devitt, Tseng, Taylor-Adair, Koganti, Timugura & Cannatella, 2023	NE	NE	NL	0	EN
<i>Eleutherodactylus jamesdixoni</i> Devitt, Tseng, Taylor-Adair, Koganti, Timugura & Cannatella, 2023	NE	NE	NL	1	4
<i>Eleutherodactylus leprus</i> (Cope, 1879)	LC (=)	M (12)	NL	3	5
<i>Eleutherodactylus longipes</i> (Baird, 1869)	LC (?)	H (15)	NL	1	4
<i>Eleutherodactylus maurus</i> Hedges, 1989	VU (?)	H (17)	Pr	1	2
<i>Eleutherodactylus modestus</i> (Taylor, 1942)	LC (=)	H (16)	Pr	1	3
<i>Eleutherodactylus nitidus</i> (Peters, 1870)	LC (=)	M (12)	NL	1	5
<i>Eleutherodactylus pallidus</i> (Duellman, 1958)	LC (=)	H (17)	Pr	1	3
<i>Eleutherodactylus planirostris</i> (Cope, 1862)				IN	
<i>Eleutherodactylus rufescens</i> (Duellman & Dixon, 1959)	VU (↓)	H (17)	Pr	1	2
<i>Eleutherodactylus teretistes</i> (Duellman, 1958)	VU (?)	H (16)	NL	1	3
<i>Eleutherodactylus verrucipes</i> (Cope, 1885)	LC (=)	H (16)	Pr	1	5
<i>Eleutherodactylus verruculatus</i> (Peters, 1870)	DD (?)	H (18)	NL	1	2
<b>Hylidae</b>					
<i>Bromeliahyla dendroscarta</i> (Taylor, 1940)	EN (↓)	H (17)	Pr	1	4
<i>Charadrahyla taeniopus</i> (Günther, 1901)	VU (↓)	M (13)	A	1	2
<i>Dendropsophus microcephalus</i> (Cope, 1886)	LC (↑)	L (7)	NL	3	6
<i>Dryophytes arenicolor</i> (Cope, 1886)	LC (=)	L (7)	NL	2	8
<i>Dryophytes euphorbiaceus</i> (Günther, 1858)	LC (=)	M (13)	NL	1	4
<i>Dryophytes eximius</i> (Baird, 1854)	LC (=)	M (10)	NL	1	7
<i>Dryophytes plicatus</i> (Broccoli, 1877)	LC (=)	M (11)	A	1	3
<i>Exerodonta smaragdina</i> (Taylor, 1940)	LC (↓)	M (12)	Pr	1	6
<i>Exerodonta sumichrasti</i> Broccoli, 1879	LC (↓)	L (9)	NL	1	4
<i>Megastomahyla mixomaculata</i> (Taylor, 1950)	EN (↓)	H (14)	A	1	3
<i>Megastomahyla nubicola</i> (Duellman, 1964)	CR (?)	H (14)	A	1	2
<i>Ptychohyla zophodes</i> Campbell & Duellman, 2000	VU (↓)	M (13)	NL	1	3
<i>Rheohyla miotympanum</i> (Cope, 1863)	LC (=)	L (9)	NL	1	7
<i>Sarcohyla arborescendens</i> (Taylor, 1939)	NT (↓)	M (11)	Pr	1	3
<i>Sarcohyla bistincta</i> (Cope, 1877)	LC (↓)	L (9)	Pr	1	5
<i>Sarcohyla floresi</i> Kaplan, Heimes & Aguilar, 2020	VU (?)	NE	NL	1	2
<i>Sarcohyla hapsa</i> Campbell, Brodie, Caviedes-Solis, Nieto-Montes de Oca, Luja, Flores-Villela, García-Vázquez, Sarker & Wostl, 2018	LC (?)	NE	NL	1	5
<i>Sarcohyla robertsorum</i> (Taylor, 1940)	VU (↓)	M (13)	A	1	3
<i>Sarcohyla siopela</i> (Duellman, 1968)	CR (↓)	H (15)	NL	1	3
<i>Scinax staufferi</i> (Cope, 1865)	LC (=)	L (4)	NL	3	9
<i>Smilisca baudinii</i> (Duméril & Bibron, 1841)	LC (=)	L (3)	NL	4	11
<i>Smilisca cyanosticta</i> (Smith, 1953)	LC (=)	M (12)	NL	3	5
<i>Smilisca fodiens</i> (Boulenger, 1882)	LC (=)	L (8)	NL	2	7
<i>Tlalocohyla godmani</i> (Günther, 1901)	VU (↓)	M (13)	A	1	3
<i>Tlalocohyla loquax</i> (Gage & Stuart, 1934)	LC (=)	L (7)	NL	3	6
<i>Tlalocohyla picta</i> (Günther, 1901)	LC (↑)	L (8)	NL	3	5
<i>Tlalocohyla smithii</i> (Boulenger, 1902)	LC (=)	M (11)	NL	1	6
<i>Trachycephalus vermiculatus</i> (Cope, 1877)	NE	L (4)	NL	3	8
<i>Tripriion spinosus</i> (Steindachner, 1864)	NT (↓)	H (14)	NL	3	5
<i>Tripriion spatulatus</i> Günther, 1882	LC (=)	M (13)	NL	1	5



	IUCN	EVS	Mx	GI	Tot
<b>Leptodactylidae</b>					
<i>Leptodactylus fragilis</i> (Brocchi, 1877)	LC (=)	L (5)	NL	4	10
<i>Leptodactylus melanonotus</i> (Hallowell, 1861)	LC (=)	L (6)	NL	3	11
<b>Microhylidae</b>					
<i>Hypopachus ustus</i> (Cope, 1866)	LC (=)	L (7)	Pr	3	8
<i>Hypopachus variolosus</i> (Cope, 1866)	LC (=)	L (4)	NL	4	11
<b>Phyllomedusidae</b>					
<i>Agalychnis dacnicolor</i> (Cope, 1864)	LC (↓)	M (11)	NL	1	5
<i>Agalychnis moreletii</i> (Duméril, 1853)	LC (↓)	M (13)	NL	1	6
<b>Ranidae</b>					
<i>Rana berlandieri</i> Baird, 1854	LC (=)	L (7)	Pr	2	9
<i>Rana catesbeiana</i> Shaw, 1802				IN	
<i>Rana chichicuahutla</i> Cuellar, Méndez de la Cruz & Villagrán-Santa Cruz, 1996	CR (↓)	H (15)	NL	0	EN
<i>Rana dunni</i> Zweifel, 1957	EN (↓)	H (14)	Pr	0	EN
<i>Rana johni</i> Blair, 1965	VU (↓)	H (14)	P	1	3
<i>Rana magnaocularis</i> Frost & Bagnara, 1976	LC (?)	M (12)	NL	1	6
<i>Rana megapoda</i> Taylor, 1942	NT (↓)	H (14)	Pr	1	5
<i>Rana montezumae</i> Baird, 1854	LC (↓)	M (13)	Pr	1	6
<i>Rana neovolcanica</i> Hillis & Frost, 1985	LC (=)	M (13)	A	1	6
<i>Rana psilonota</i> Webb, 2001	LC (?)	H (14)	NL	1	4
<i>Rana spectabilis</i> Hillis & Frost, 1985	LC (↓)	M (12)	NL	1	5
<i>Rana tlaloci</i> Hillis & Frost, 1985	CR (?)	H (15)	P	0	EN
<i>Rana vaillanti</i> Brocchi, 1877	LC (=)	L (9)	NL	3	6
<i>Rana zweifeli</i> Hillis, Frost & Webb, 1984	LC (=)	M (11)	NL	1	3
<i>Rana forreri</i> Boulenger, 1883	LC (=)	L (3)	Pr	3	8
<i>Rana pustulosa</i> Boulenger, 1883	LC (=)	L (9)	Pr	1	5
<b>Scaphiropodidae</b>					
<i>Scaphiopus couchi</i> Baird, 1854	LC (=)	L (3)	NL	2	10
<i>Spea multiplicata</i> (Cope, 1863)	LC (=)	L (6)	NL	2	9
<b>Order Caudata</b>					
<b>Ambystomatidae</b>					
<i>Ambystoma altamirani</i> Dugès, 1895	EN (↓)	M (13)	A	0	EN
<i>Ambystoma amblycephalum</i> Taylor, 1940	CR (↓)	M (13)	Pr	0	EN
<i>Ambystoma andersoni</i> Krebs & Brandon, 1984	CR (↓)	H (15)	Pr	0	EN
<i>Ambystoma bombypellum</i> Taylor, 1940	DD (?)	H (15)	Pr	0	EN
<i>Ambystoma dumerilii</i> (Dugès, 1870)	CR (↓)	H (15)	Pr	0	EN
<i>Ambystoma granulosum</i> Taylor, 1944	EN (↓)	H (14)	Pr	0	EN
<i>Ambystoma leorae</i> (Taylor, 1943)	CR (↓)	H (15)	A	0	EN
<i>Ambystoma lermaense</i> (Taylor, 1940)	EN (↓)	H (15)	Pr	0	EN
<i>Ambystoma mexicanum</i> (Shaw & Nodder, 1798)	CR (↓)	H (15)	P	0	EN
<i>Ambystoma ordinarium</i> Taylor, 1940	EN (↓)	M (13)	Pr	0	EN
<i>Ambystoma rivulare</i> (Taylor, 1940)	EN (↓)	M (13)	A	0	EN
<i>Ambystoma taylori</i> Brandon, Maruska & Rumph, 1982	CR (?)	H (15)	Pr	0	EN
<i>Ambystoma velasci</i> (Dugès, 1888)	LC (?)	M (10)	Pr	1	6
<b>Plethodontidae</b>					
<i>Aquiloerycea cafetalera</i> (Parra-Olea, Rovito, Márquez-Valdelmar, Cruz, Murrieta-Galindo & Wake, 2010)	VU (=)	H (14)	Pr	1	2

	IUCN	EVS	Mx	Gl	Tot
<i>Aquiloerycea cephalica</i> (Cope, 1865)	LC (↓)	H (17)	NL	1	3
<i>Aquiloerycea praecellens</i> (Rabb, 1955)	CR (↓)	H (18)	A	1	2
<i>Aquiloerycea quetzalanensis</i> (Parra-Olea, Canseco-Márquez & García-París, 2004)	CR (↓)	H (17)	NL	1	2
<i>Bolitoglossa mexicana</i> Duméril, Bibron & Duméril, 1854	LC (↓)	M (11)	Pr	3	5
<i>Bolitoglossa platydactyla</i> (Gray, 1831)	LC (=)	H (15)	Pr	1	4
<i>Bolitoglossa rufescens</i> (Cope, 1869)	LC (=)	L (9)	Pr	3	5
<i>Chiropterotriton arboreus</i> (Taylor, 1941)	CR (↓)	H (18)	Pr	1	2
<i>Chiropterotriton casasi</i> Parra-Olea, García-Castillo, Rovito, Maisano, Hanken & Wake, 2020	CR (↓)	NE	NL	0	EN
<i>Chiropterotriton ceronorum</i> Parra-Olea, García-Castillo, Rovito, Maisano, Hanken & Wake, 2020	CR (↓)	NE	NL	0	EN
<i>Chiropterotriton chico</i> García-Castillo, Rovito, Wake & Parra-Olea, 2017	VU (=)	NE	NL	0	EN
<i>Chiropterotriton chiropterus</i> (Cope, 1863)	CR (↓)	H (16)	Pr	1	2
<i>Chiropterotriton chondrostega</i> (Taylor, 1941)	EN (↓)	H (17)	Pr	1	3
<i>Chiropterotriton cracens</i> Rabb, 1958	VU (=)	H (17)	NL	1	2
<i>Chiropterotriton laeae</i> (Taylor, 1942)	CR (↓)	H (18)	Pr	0	EN
<i>Chiropterotriton magnipes</i> Raab, 1965	EN (↓)	H (16)	Pr	1	2
<i>Chiropterotriton mosaueri</i> (Woodall, 1941)	CR (?)	H (18)	Pr	1	3
<i>Chiropterotriton multidentatus</i> (Taylor, 1939)	EN (=)	H (15)	Pr	1	3
<i>Chiropterotriton nubilus</i> García-Castillo, Soto-Pozos, Aguilar-López, Pineda-Arredondo & Parra-Olea, 2018	CR (?)	NE	NL	0	EN
<i>Chiropterotriton orculus</i> (Cope, 1865)	VU (↓)	H (18)	NL	1	2
<i>Chiropterotriton perotensis</i> Parra-Olea, García-Castillo, Rovito, Maisano, Hanken & Wake, 2020	CR (↓)	NE	NL	0	EN
<i>Chiropterotriton terrestris</i> (Taylor, 1941)	CR (?)	H (18)	NL	1	2
<i>Chiropterotriton totonacus</i> Parra-Olea, García-Castillo, Rovito, Maisano, Hanken & Wake, 2020	CR (↓)	NE	NL	0	EN
<i>Isthmura belli</i> (Gray, 1850)	LC (?)	M (12)	A	1	5
<i>Isthmura corrugata</i> Sandoval-Comte, Pineda-Arredondo, Rovito & Luría-Manzano, 2017	CR (?)	NE	NL	0	EN
<i>Isthmura gigantea</i> (Taylor, 1939)	EN (↓)	H (16)	NL	1	2
<i>Isthmura naucampatepetl</i> (Parra-Olea, Papenfuss & Wake, 2001)	CR (↓)	H (17)	NL	1	2
<i>Parvimolge townsendi</i> (Dunn, 1922)	VU (↓)	H (16)	A	1	2
<i>Pseudoeurycea altamontana</i> (Taylor, 1939)	EN (↓)	H (17)	Pr	0	EN
<i>Pseudoeurycea firscheini</i> Shannon & Werler, 1955	EN (↓)	H (18)	Pr	1	2
<i>Pseudoeurycea gadovii</i> (Dunn, 1926)	VU (↓)	M (13)	Pr	1	2
<i>Pseudoeurycea granitum</i> García-Bañuelos, Aguilar-López, Kelly-Hernandez, Vásquez-Cruz, Pineda-Arredondo & Rovito, 2020	NE	NE	NL	1	2
<i>Pseudoeurycea leprosa</i> (Cope, 1869)	LC (↓)	H (16)	A	1	3
<i>Pseudoeurycea lineola</i> (Cope, 1865)	EN (↓)	H (14)	Pr	1	3
<i>Pseudoeurycea longicauda</i> Lynch, Wake & Yang, 1983	EN (↓)	H (17)	Pr	0	EN
<i>Pseudoeurycea lynchi</i> Parra-Olea, Papenfuss & Wake, 2001	EN (↓)	H (17)	NL	1	2
<i>Pseudoeurycea melanomolga</i> (Taylor, 1941)	EN (↓)	H (16)	Pr	1	2
<i>Pseudoeurycea nigromaculata</i> (Taylor, 1941)	EN (↓)	H (17)	Pr	1	3
<i>Pseudoeurycea robertsi</i> (Taylor, 1939)	CR (↓)	H (18)	A	0	EN
<i>Pseudoeurycea tillicxitl</i> Lara-Góngora, 2003	EN (?)	H (17)	NL	0	EN
<i>Thorius dubitus</i> Taylor, 1941	CR (↓)	H (16)	Pr	1	2
<i>Thorius pennatululus</i> Cope, 1869	EN (↓)	H (15)	Pr	1	3

	IUCN	EVS	Mx	GI	Tot
<i>Thorius spilogaster</i> Hanken & Wake, 1998	CR (↓)	H (17)	NL	0	EN
<i>Thorius troglodytes</i> Taylor, 1941	EN (↓)	H (16)	Pr	1	2
<b>Order Gymnophiona</b>					
<b>Dermophiidae</b>					
<i>Dermophis mexicanus</i> (Duméril & Bibron, 1841)	LC (↓)	M (11)	Pr	3	4
<i>Dermophis oaxacae</i> (Mertens, 1930)	LC (=)	M (12)	Pr	1	4
<b>Class Reptilia</b>					
<b>Order Squamata</b>					
<b>Suborder Lacertilia</b>					
<b>Anguidae</b>					
<i>Abronia antaues</i> (Cope, 1866)	NE	H (16)	Pr	1	2
<i>Abronia deppii</i> (Wiegmann, 1828)	EN (↓)	H (16)	A	0	EN
<i>Abronia graminea</i> (Cope, 1864)	EN (↓)	H (15)	A	1	3
<i>Abronia taeniata</i> (Wiegmann, 1828)	VU (↓)	H (15)	Pr	1	4
<i>Barisia herrerae</i> Zaldivar-Riverón & Nieto Montes de Oca, 2002	EN (↓)	H (15)	NL	0	EN
<i>Barisia rudicollis</i> (Wiegmann, 1828)	EN (=)	H (15)	P	0	EN
<i>Barisia imbricata</i> (Wiegmann, 1828)	LC (?)	H (14)	Pr	1	5
<i>Elgaria kingii</i> Gray, 1838	LC (=)	M (10)	Pr	2	5
<i>Gerrhonotus infernalis</i> Baird, 1859	LC (=)	M (13)	NL	2	4
<i>Gerrhonotus ophiurus</i> Cope, 1867	LC (?)	M (12)	NL	1	4
<i>Gerrhonotus liocephalus</i> Wiegmann, 1828	LC (=)	L (6)	Pr	4	9
<i>Ophisaurus incomptus</i> McConkey, 1955	NE	H (15)	P	1	3
<b>Anolidae</b>					
<i>Anolis carlliebi</i> Köhler, Trejo-Pérez, Petersen & Méndez de la Cruz, 2014	NE	H (15)	NL	1	4
<i>Anolis cymbops</i> Cope, 1864	LC (=)	H (17)	A	1	2
<i>Anolis laevis</i> (Wiegmann, 1834)	LC (=)	L (9)	NL	3	5
<i>Anolis lemurinus</i> Cope, 1861	LC (=)	L (8)	NL	3	5
<i>Anolis nebulosus</i> (Wiegmann, 1834)	LC (=)	M (13)	NL	1	6
<i>Anolis petersii</i> Bocourt, 1873	NT (↓)	L (9)	NL	3	5
<i>Anolis schiedii</i> (Wiegmann, 1834)	DD (?)	H (16)	Pr	1	2
<i>Anolis sericeus</i> Hallowell, 1856	LC (=)	L (8)	NL	3	6
<i>Anolis tropidonotus</i> Peters, 1863	LC (=)	L (9)	NL	3	6
<b>Corytophanidae</b>					
<i>Basiliscus vittatus</i> Wiegmann, 1828	LC (=)	L (7)	NL	3	8
<i>Corytophanes hernandesii</i> (Wiegmann, 1831)	LC (=)	M (13)	NL	3	5
<i>Laemactes longipes</i> Wiegmann, 1834	LC (?)	L (9)	Pr	3	6
<b>Dibamidae</b>					
<i>Anelytropsis papillosus</i> Cope, 1885	LC (↓)	M (10)	A	1	4
<b>Diploglossidae</b>					
<i>Celestus ennegrammus</i> (Cope, 1861)	LC (=)	H (14)	Pr	1	5
<i>Celestus legnotus</i> Campbell & Camarillo, 1994	LC (=)	H (14)	NL	1	2
<b>Eublepharidae</b>					
<i>Coleonyx elegans</i> Gray, 1845	LC (=)	L (9)	A	3	8
<b>Gekkonidae</b>					
<i>Gehyra mutilata</i> (Wiegmann, 1834)				IN	
<i>Hemidactylus mabouia</i> (Moreau De Jonnés, 1818)				IN	

	IUCN	EVS	Mx	GI	Tot
<b>Helodermatidae</b>					
<i>Heloderma horridum</i> (Wiegmann, 1829)	LC (↓)	M (11)	A	3	6
<b>Iguanidae</b>					
<i>Ctenosaura acanthura</i> (Shaw, 1802)	LC (↓)	M (12)	Pr	3	7
<i>Ctenosaura pectinata</i> (Wiegmann, 1834)	LC (↓)	H (15)	A	1	7
<i>Iguana iguana</i> (Linnaeus, 1758)	LC (?)	M (12)	Pr	3	9
<b>Phrynosomatidae</b>					
<i>Phrynosoma braconneri</i> Duméril & Bocourt, 1870	LC (=)	H (15)	Pr	1	4
<i>Phrynosoma orbiculare</i> (Linnaeus, 1766)	LC (=)	M (12)	A	1	6
<i>Phrynosoma asio</i> Cope, 1864	LC (=)	M (11)	Pr	3	5
<i>Sceloporus aeneus</i> Wiegmann, 1828	LC (=)	M (13)	NL	1	4
<i>Sceloporus albiventris</i> Smith, 1939	NE	H (16)	NL	1	3
<i>Sceloporus anahuacus</i> Lara-Góngora, 1983	LC (=)	H (15)	NL	0	EN
<i>Sceloporus asper</i> Boulenger, 1897	LC (↓)	H (14)	Pr	1	4
<i>Sceloporus aureolus</i> Smith, 1942	NE	H (15)	NL	1	4
<i>Sceloporus bicanthalis</i> Smith, 1937	LC (=)	M (13)	NL	1	3
<i>Sceloporus bulleri</i> Boulenger, 1894	LC (=)	H (15)	NL	1	4
<i>Sceloporus clarkii</i> Baird & Girard, 1852	LC (=)	M (10)	NL	2	5
<i>Sceloporus dixonii</i> Bryson & Grummer, 2021	NE	NE	NL	0	EN
<i>Sceloporus formosus</i> Wiegmann, 1834	LC (=)	H (15)	NL	1	4
<i>Sceloporus grammicus</i> Wiegmann, 1828	LC (=)	L (9)	Pr	2	8
<i>Sceloporus heterolepis</i> Boulenger, 1895	LC (?)	H (14)	NL	1	5
<i>Sceloporus huichol</i> Flores-Villela, Smith, Campillo-García, Martínez-Méndez & Campbell, 2022	NE	NE	NL	1	2
<i>Sceloporus insignis</i> Webb, 1967	LC (=)	H (16)	Pr	1	2
<i>Sceloporus internasalis</i> Smith & Bumzahem, 1955	LC (↓)	M (11)	NL	3	4
<i>Sceloporus jalapae</i> Günther, 1890	LC (=)	M (13)	NL	1	4
<i>Sceloporus megalepidurus</i> Smith, 1934	VU (↓)	H (14)	Pr	1	5
<i>Sceloporus melanogaster</i> Cope, 1885	NE	NE	NL	1	3
<i>Sceloporus melanorhinus</i> Bocourt, 1876	LC (=)	L (9)	NL	3	6
<i>Sceloporus minor</i> Cope, 1885	LC (=)	H (14)	NL	1	3
<i>Sceloporus mucronatus</i> Cope, 1885	LC (=)	M (13)	NL	1	3
<i>Sceloporus nelsoni</i> Cochran, 1923	LC (=)	M (13)	NL	1	4
<i>Sceloporus ochoteranae</i> Smith, 1934	LC (=)	M (12)	NL	1	3
<i>Sceloporus palaciosi</i> Lara-Góngora, 1983	LC (=)	H (15)	NL	0	EN
<i>Sceloporus salvini</i> Günther, 1890	DD (?)	H (15)	A	1	4
<i>Sceloporus scalaris</i> Wiegmann, 1828	LC (=)	M (12)	NL	1	6
<i>Sceloporus serrifer</i> Cope, 1866	LC (=)	L (6)	NL	3	5
<i>Sceloporus spinosus</i> Weigmann, 1828	LC (=)	M (12)	NL	1	7
<i>Sceloporus subniger</i> Poglayen & Smith, 1958	NE	NE	NL	0	EN
<i>Sceloporus teapensis</i> Günther, 1890	LC (=)	M (13)	NL	3	5
<i>Sceloporus torquatus</i> Wiegmann, 1828	LC (=)	M (11)	NL	1	4
<i>Sceloporus unicanthalis</i> Smith, 1937	NE	H (16)	NL	1	4
<i>Sceloporus variabilis</i> Wiegmann, 1834	LC (=)	L (5)	NL	2	7
<i>Sceloporus dugesii</i> Bocourt, 1874	LC (=)	M (13)	NL	1	4
<i>Sceloporus horridus</i> Wiegmann, 1834	LC (=)	M (11)	NL	1	6
<i>Sceloporus pyrocephalus</i> Cope, 1864	LC (=)	M (12)	NL	1	4

	IUCN	EVS	Mx	GI	Tot
<i>Sceloporus sugillatus</i> Smith, 1942	LC (=)	H (16)	NL	0	EN
<i>Sceloporus utiformis</i> Cope, 1864	LC (=)	H (15)	NL	1	6
<i>Urosaurus bicarinatus</i> (Duméril, 1856)	LC (=)	M (12)	NL	1	7
<b>Phyllodactylidae</b>					
<i>Phyllodactylus bordai</i> Taylor, 1942	LC (=)	M (13)	Pr	1	3
<i>Phyllodactylus davisii</i> Dixon, 1964	LC (=)	H (16)	A	1	3
<i>Phyllodactylus lanei</i> Smith, 1935	LC (=)	H (15)	NL	1	5
<b>Scincidae</b>					
<i>Marisora aquilonaria</i> McCraine, Matthews & Hedges, 2020	NE	NE	NL	1	7
<i>Marisora lineola</i> McCranie, Matthews & Hedges, 2020	NE	NE	NL	3	6
<i>Plestiodon brevirostris</i> (Günther, 1860)	LC (=)	M (11)	NL	1	4
<i>Plestiodon callicephalus</i> (Bocourt, 1879)	LC (=)	M (12)	NL	2	4
<i>Plestiodon colimensis</i> (Taylor, 1935)	DD (?)	H (14)	Pr	1	3
<i>Plestiodon copei</i> (Taylor, 1933)	LC (=)	H (14)	Pr	1	3
<i>Plestiodon dugesii</i> (Thomiot, 1883)	VU (↓)	H (16)	Pr	1	2
<i>Plestiodon indubitus</i> (Taylor, 1933)	NE	H (15)	NL	1	2
<i>Plestiodon lynxe</i> (Wiegmann, 1834)	LC (=)	M (10)	Pr	1	6
<i>Plestiodon parvulus</i> (Taylor, 1933)	DD (?)	H (15)	NL	1	4
<i>Plestiodon sumichrasti</i> (Cope, 1867)	LC (↓)	M (12)	NL	3	6
<i>Scincella assata</i> (Cope, 1864)	LC (=)	L (7)	NL	3	6
<i>Scincella gemmingeri</i> (Cope, 1864)	LC (=)	M (11)	Pr	1	7
<i>Scincella silvicola</i> (Taylor, 1937)	LC (=)	M (12)	A	1	5
<b>Sphaerodactylidae</b>					
<i>Sphaerodactylus glaucus</i> Cope, 1866	LC (=)	M (12)	Pr	3	6
<b>Teiidae</b>					
<i>Aspidoscelis communis</i> (Cope, 1878)	LC (=)	H (14)	Pr	1	4
<i>Aspidoscelis costatus</i> (Cope, 1878)	LC (=)	M (11)	Pr	1	8
<i>Aspidoscelis deppii</i> (Wiegmann, 1834)	LC (=)	L (8)	NL	3	7
<i>Aspidoscelis gularis</i> (Baird & Girard, 1852)	LC (=)	L (9)	NL	2	6
<i>Aspidoscelis guttatus</i> (Wiegmann, 1834)	LC (=)	M (12)	NL	1	7
<i>Aspidoscelis lineattissimus</i> (Cope, 1878)	LC (=)	H (14)	Pr	1	5
<i>Holcosus amphigrammus</i> (Smith & Lafe, 1945)	NE	M (11)	NL	1	7
<i>Holcosus sinister</i> (Wiegmann, 1834)	NE	M (13)	NL	1	4
<b>Xantusiidae</b>					
<i>Lepidophyma gaigeae</i> Mosauer, 1936	VU (↓)	M (13)	Pr	1	4
<i>Lepidophyma sylvaticum</i> Taylor, 1939	LC (↓)	M (11)	Pr	1	3
<i>Lepidophyma zongolica</i> García-Vázquez, Canseco-Márquez & Aguilar-López, 2010	DD (?)	H (16)	NL	1	3
<i>Xantusia sanchezi</i> Bezy & Flores-Villela, 1999	LC (?)	H (16)	P	1	2
<b>Xenosauridae</b>					
<i>Xenosaurus grandis</i> (Gray, 1856)	VU (↓)	L (9)	Pr	1	5
<b>Order Squamata</b>					
<b>Suborder Serpentes</b>					
<b>Boidae</b>					
<i>Boa imperator</i> Daudin, 1803	LC (=)	M (10)	NL	3	9
<i>Boa sigma</i> (Smith, 1943)	NE	M (10)	NL	1	6



	IUCN	EVS	Mx	GI	Tot
<b>Colubridae</b>					
<i>Conopsis acuta</i> (Cope, 1886)	NE	H (14)	NL	1	5
<i>Conopsis lineata</i> (Kennicott, 1859)	LC (=)	M (13)	NL	1	5
<i>Conopsis nasus</i> (Günther, 1858)	LC (=)	M (11)	NL	1	5
<i>Conopsis biserialis</i> (Taylor & Smith, 1942)	LC (=)	M (13)	A	1	3
<i>Drymarchon melanurus</i> (Duméril, Bibron & Duméril, 1854)	LC (=)	L (6)	NL	4	12
<i>Drymobius margaritiferus</i> (Schlegel, 1837)	LC (=)	L (6)	NL	4	10
<i>Ficimia olivacea</i> Gray, 1849	LC (?)	L (9)	NL	1	3
<i>Gyalopion canum</i> Cope, 1861	LC (=)	L (9)	NL	2	4
<i>Lampropeltis abnorma</i> (Bocourt, 1886)	LC (=)	L (9)	NL	3	7
<i>Lampropeltis mexicana</i> (Garman, 1884)	LC (=)	H (15)	A	1	5
<i>Lampropeltis ruthveni</i> Blanchard, 1920	NT (↓)	H (16)	A	1	3
<i>Lampropeltis polyzona</i> Cope, 1860	LC (?)	M (11)	NL	1	9
<i>Leptophis diplotropis</i> (Günther, 1872)	LC (=)	H (14)	A	1	8
<i>Leptophis mexicanus</i> Duméril, Bibron & Duméril, 1854	LC (=)	L (6)	A	3	8
<i>Masticophis bilineatus</i> Jan, 1863	LC (=)	M (11)	NL	2	6
<i>Masticophis flagellum</i> Shaw, 1802	LC (=)	L (8)	A	2	9
<i>Masticophis mentovarius</i> (Duméril, Bibron & Duméril, 1854)	LC (?)	L (6)	A	3	11
<i>Masticophis schotti</i> Baird & Girard, 1853	LC (=)	M (13)	NL	2	5
<i>Masticophis taeniatus</i> (Hallowell, 1852)	LC (=)	M (10)	NL	2	3
<i>Mastigodryas cliftoni</i> (Hardy, 1964)	DD (?)	H (14)	NL	1	4
<i>Mastigodryas melanolomus</i> (Cope 1868)	LC (=)	L (6)	NL	3	8
<i>Oxybelis fulgidus</i> (Daudin, 1803)	LC (=)	L (9)	NL	3	6
<i>Oxybelis microphthalmus</i> Barbour & Amaral, 1926	NE	NE	NL	2	9
<i>Pituophis deppei</i> (Duméril, 1853)	LC (=)	H (14)	A	1	7
<i>Pituophis lineaticollis</i> (Cope, 1861)	LC (=)	L (8)	NL	3	5
<i>Pseudoficimia frontalis</i> (Cope, 1864)	LC (=)	M (13)	NL	1	7
<i>Salvadora bairdii</i> Jan & Sordelli, 1860	LC (=)	H (15)	Pr	1	8
<i>Salvadora grahamiae</i> Baird & Girard, 1853	LC (=)	M (10)	NL	2	6
<i>Salvadora mexicana</i> (Duméril, Bibron & Duméril, 1854)	LC (=)	H (15)	Pr	1	5
<i>Scaphiodontophis annulatus</i> (Duméril, Bibron & Duméril, 1854)	LC (=)	M (11)	NL	3	6
<i>Senticolis triaspis</i> (Cope, 1866)	LC (=)	L (6)	NL	4	11
<i>Sonora michoacanensi</i> (Dugès, 1884)	LC (=)	H (14)	NL	1	4
<i>Sonora mutabilis</i> Stickel, 1943	LC (?)	H (14)	NL	1	5
<i>Spilotes pullatus</i> (Linnaeus, 1758)	LC (=)	L (6)	NL	3	7
<i>Stenorrhina degenhardtii</i> (Berthold, 1846)	LC (=)	L (9)	NL	3	5
<i>Stenorrhina freminvillei</i> (Duméril, Bibron & Duméril, 1854)	LC (=)	L (7)	NL	3	7
<i>Sympholis lippiens</i> Cope, 1862	DD (?)	H (14)	NL	1	3
<i>Tantilla bocourti</i> (Günther, 1895)	LC (?)	L (9)	NL	1	8
<i>Tantilla cascadeae</i> Wilson & Meyer, 1981	DD (?)	H (16)	A	1	2
<i>Tantilla ceboruca</i> Canseco-Márquez, Smith, Ponce-Campos, Flores-Villela & Campbell, 2007	NE	H (16)	NL	1	1
<i>Tantilla deppei</i> (Bocourt, 1883)	LC (?)	M (13)	A	1	3
<i>Tantilla rubra</i> Cope, 1875	LC (?)	L (5)	Pr	3	8
<i>Tantilla schistosa</i> (Bocourt, 1883)	LC (?)	L (8)	NL	3	7
<i>Tantilla yaquia</i> Smith, 1942	LC (=)	M (10)	NL	2	4
<i>Tantilla calamarina</i> Cope, 1866	LC (=)	M (12)	Pr	1	4

	IUCN	EVS	Mx	GI	Tot
<i>Tantillita lintoni</i> (Smith, 1940)	LC (=)	M (12)	Pr	3	5
<i>Trimorphodon paucimaculatus</i> Taylor, 1936	NE	H (15)	NL	1	5
<i>Trimorphodon tau</i> Cope, 1870	LC (=)	M (13)	NL	1	8
<i>Trimorphodon biscutatus</i> (Duméril, Bibron & Duméril, 1854)	LC (=)	L (7)	NL	3	6
<b>Dipsadidae</b>					
<i>Adelphicos quadrivirgatum</i> Jan, 1862	LC (?)	M (10)	Pr	3	5
<i>Amastridium sapperi</i> Werner, 1903	LC (=)	M (10)	NL	3	5
<i>Chersodromus liebmanni</i> Reinhardt, 1861	LC (=)	M (12)	Pr	1	5
<i>Clelia scytalina</i> (Cope, 1867)	LC (=)	M (13)	NL	3	6
<i>Coniophanes bipunctatus</i> (Günther, 1858)	LC (=)	M (10)	NL	3	5
<i>Coniophanes fissidens</i> (Günther 1858)	LC (=)	L (7)	NL	3	7
<i>Coniophanes imperialis</i> (Baird & Girard, 1859)	LC (=)	M (8)	NL	4	8
<i>Coniophanes taeniata</i> (Peters, 1870)	NE	NE	NL	1	4
<i>Coniophanes lateritius</i> Cope, 1862	DD (?)	M (13)	NL	1	5
<i>Conophis lineatus</i> (Duméril Bribon & Duméril, 1854)	LC (=)	L (9)	NL	3	7
<i>Conophis morai</i> Pérez-Higareda, López-Luna & Smith, 2002	DD (?)	H (17)	NL	1	2
<i>Conophis vittatus</i> Peters, 1860	LC (=)	M (11)	NL	3	6
<i>Diadophis punctatus</i> (Linnaeus, 1766)	LC (=)	L (4)	NL	2	7
<i>Enulius oligostichus</i> Smith, Arndt & Sherbrook, 1967	DD (?)	H (15)	Pr	1	2
<i>Geophis annuliferus</i> (Boulenger, 1894)	LC (=)	M (13)	Pr	1	5
<i>Geophis berillus</i> Barragán-Reséndiz, Pavón-Vázquez, Cervantes-Burgos, Trujano-Ortega, Canseco-Márquez & García-Vázquez, 2022	NE	NE	NL	0	EN
<i>Geophis bicolor</i> Günther, 1868	DD (?)	H (15)	Pr	1	2
<i>Geophis blanchardi</i> Taylor & Smith, 1939	DD (?)	H (15)	Pr	1	3
<i>Geophis dugesii</i> Bocourt, 1883	LC (?)	M (13)	NL	1	3
<i>Geophis juliai</i> Pérez-Higareda, Smith & López-Luna, 2001	VU (↓)	M (13)	NL	1	2
<i>Geophis lorancai</i> Canseco-Márquez, Pavón-Vázquez, López-Luna & Nieto Montes de Oca, 2016	NE	H (14)	NL	1	2
<i>Geophis maculiferus</i> Taylor, 1941	DD (?)	H (16)	Pr	0	EN
<i>Geophis mutitorques</i> (Cope, 1865)	LC (=)	M (13)	Pr	1	3
<i>Geophis petersii</i> Boulenger, 1894	DD (?)	H (15)	Pr	1	3
<i>Geophis sartorii</i> (Cope, 1863)	LC (=)	L (9)	Pr	3	8
<i>Geophis semidoliatus</i> (Duméril, Bibron & Duméril, 1854)	LC (=)	M (13)	NL	1	5
<i>Geophis sieboldi</i> (Jan, 1862)	DD (?)	M (13)	Pr	1	3
<i>Geophis tarascae</i> Hartweg, 1959	DD (?)	H (15)	Pr	0	EN
<i>Hypsiglena affinis</i> Boulenger, 1894	NE	H (14)	Pr	1	3
<i>Hypsiglena jani</i> Dugès, 1866	LC (=)	L (6)	Pr	2	6
<i>Hypsiglena tanzeri</i> Dixon & Lieb, 1972	DD (?)	H (15)	NL	1	3
<i>Hypsiglena torquata</i> (Günther, 1860)	LC (=)	L (8)	Pr	1	5
<i>Imantodes cenchoa</i> (Linnaeus, 1758)	LC (=)	L (6)	Pr	3	7
<i>Imantodes gemmistratus</i> (Cope, 1861)	LC (=)	L (6)	Pr	3	8
<i>Leptodeira punctata</i> (Peters, 1866)	LC (?)	H (17)	NL	1	4
<i>Leptodeira septentrionalis</i> (Kennicott, 1859)	LC (=)	L (8)	NL	4	10
<i>Leptodeira splendida</i> Günther, 1895	LC (?)	H (14)	NL	1	6
<i>Leptodeira maculata</i> (Hallowell, 1861)	LC (=)	L (7)	Pr	1	9
<i>Manolepis putnami</i> (Jan, 1863)	LC (=)	M (13)	NL	1	6
<i>Ninia diademata</i> Baird & Girard, 1853	LC (=)	L (9)	NL	3	7

	IUCN	EVS	Mx	GI	Tot
<i>Ninia sebae</i> (Duméril, Bibron & Duméril, 1854)	LC (=)	L (5)	NL	3	6
<i>Pliocercus elapoides</i> Cope, 1860	LC (=)	M (10)	NL	3	7
<i>Rhadinaea cuneata</i> Myers, 1974	DD (?)	H (15)	Pr	1	4
<i>Rhadinaea decorata</i> (Günther, 1858)	LC (=)	L (9)	NL	3	5
<i>Rhadinaea fulvivittis</i> Cope, 1875	VU (↓)	M (11)	NL	1	4
<i>Rhadinaea gaigeae</i> Bailey, 1937	DD (?)	M (12)	NL	1	3
<i>Rhadinaea hesperia</i> Bailey, 1940	LC (=)	M (10)	Pr	1	7
<i>Rhadinaea laureata</i> (Günther, 1868)	LC (?)	M (12)	NL	1	3
<i>Rhadinaea quinquelineata</i> Cope, 1886	DD (?)	H (15)	Pr	1	4
<i>Rhadinaea taeniata</i> (Peters, 1863)	DD (?)	M (13)	NL	1	6
<i>Sibon nebulatus</i> (Linnaeus, 1758)	LC (=)	L (5)	NL	3	7
<i>Tropidodipsas philippii</i> (Jan, 1863)	LC (=)	H (14)	Pr	1	3
<b>Elapidae</b>					
<i>Micruroides euryxanthus</i> (Kennicott, 1860)	LC (=)	H (15)	A	2	3
<i>Micrurus browni</i> Schmidt & Smith, 1943	LC (=)	L (8)	Pr	3	6
<i>Micrurus diastema</i> (Duméril, Bibron & Duméril, 1854)	LC (=)	L (8)	Pr	3	6
<i>Micrurus distans</i> (Kennicott, 1860)	LC (=)	H (14)	Pr	1	7
<i>Micrurus elegans</i> Jan, 1858	LC (?)	M (13)	Pr	3	5
<i>Micrurus laticollaris</i> Peters, 1870	LC (=)	H (14)	Pr	1	4
<i>Micrurus proximans</i> Smith & Chrapliwy, 1958	LC (?)	H (18)	Pr	1	4
<i>Micrurus tener</i> (Baird & Girard, 1953)	LC (=)	M (11)	NL	2	5
<b>Leptotyphlopidae</b>					
<i>Epictia bakewelli</i> (Oliver, 1937)	NE	M (11)	NL	1	4
<i>Rena bressoni</i> (Taylor, 1939)	DD (?)	H (14)	Pr	1	2
<i>Rena dulcis</i> (Baird & Girard, 1853)	LC (?)	M (13)	NL	2	5
<i>Rena humilis</i> Baird & Girard, 1853	LC (=)	L (8)	NL	2	9
<i>Rena maxima</i> (Loveridge, 1932)	LC (=)	M (11)	NL	1	4
<b>Loxocemidae</b>					
<i>Loxocemus bicolor</i> Cope, 1861	LC (?)	M (10)	Pr	3	6
<b>Natricidae</b>					
<i>Nerodia rhombifer</i> (Hallowell, 1852)	LC (=)	M (10)	NL	4	6
<i>Storeria dekayi</i> (Holbrook, 1836)	LC (=)	L (7)	NL	4	5
<i>Storeria storerioides</i> (Cope, 1865)	LC (=)	M (11)	NL	1	6
<i>Thamnophis chrysocephalus</i> (Cope, 1885)	LC (=)	H (14)	A	1	4
<i>Thamnophis copei</i> Dugès, 1879	VU (↓)	H (15)	Pr	1	2
<i>Thamnophis cyrtopsis</i> (Kennicott, 1860)	LC (=)	L (7)	A	4	10
<i>Thamnophis eques</i> (Reuss, 1834)	LC (=)	L (8)	A	2	7
<i>Thamnophis godmani</i> (Günther, 1894)	LC (↓)	H (14)	A	1	4
<i>Thamnophis marcianus</i> (Baird & Girard, 1853)	LC (?)	M (10)	A	4	9
<i>Thamnophis melanogaster</i> (Peters, 1864)	EN (↓)	H (15)	A	1	5
<i>Thamnophis proximus</i> (Say, 1823)	LC (=)	L (7)	A	4	8
<i>Thamnophis pulchrilatus</i> (Cope, 1885)	LC (?)	H (15)	NL	1	6
<i>Thamnophis rossmani</i> Conant, 2000	DD (?)	H (18)	NL	0	EN
<i>Thamnophis scalaris</i> Cope, 1861	LC (=)	H (14)	A	1	3
<i>Thamnophis scaliger</i> (Jan, 1863)	VU (↓)	H (15)	A	1	4
<i>Thamnophis sumichrasti</i> (Cope, 1866)	LC (?)	H (15)	A	1	4
<i>Thamnophis validus</i> (Kennicott, 1860)	LC (=)	M (12)	NL	1	5

	IUCN	EVS	Mx	GI	Tot
<b>Typhlopidae</b>					
<i>Amerotyphlops tenuis</i> (Salvin, 1860)	LC (?)	M (11)	NL	3	4
<i>Indotyphlops braminus</i> (Daudin, 1803)				IN	
<b>Viperidae</b>					
<i>Agkistrodon bilineatus</i> (Günther, 1863)	NT (↓)	M (11)	Pr	3	6
<i>Bothrops asper</i> (Garman, 1883)	NE	M (12)	NL	3	6
<i>Cerrophidion petlalcalensis</i> López-Luna, Vogt & Torre-Loranca, 1999	DD (=)	H (18)	NL	1	3
<i>Crotalus aquilus</i> Klauber, 1952	LC (↓)	H (16)	Pr	1	4
<i>Crotalus armstrongi</i> Campbell, 1979	NE	H (18)	NL	0	EN
<i>Crotalus atrox</i> Baird & Girard, 1853	LC (=)	M (9)	Pr	2	9
<i>Crotalus basiliscus</i> (Cope, 1864)	LC (=)	H (16)	Pr	1	6
<i>Crotalus campbelli</i> Bryson, Linkem, Dorcas, Lathrop, Jones, Alvarado-Díaz, Grünwald & Murphy, 2014	NE	H (17)	NL	1	2
<i>Crotalus culminatus</i> Klauber, 1952	NE	H (15)	NL	1	4
<i>Crotalus intermedius</i> Troschel, 1865	LC (=)	H (15)	A	1	5
<i>Crotalus iannomi</i> Tanner, 1966	DD (?)	H (19)	A	1	3
<i>Crotalus lepidus</i> (Kennicott, 1861)	LC (=)	M (12)	Pr	2	4
<i>Crotalus mictlantecuhtli</i> Carbajal-Márquez, Cedeño-Vázquez, Martínez-Arce, Neri-Castro & Machkour-M' Rabet, 2020	NE	NE	NL	1	2
<i>Crotalus molossus</i> Baird & Girard, 1853	LC (=)	L (8)	Pr	2	8
<i>Crotalus polystictus</i> (Cope, 1865)	LC (↓)	H (16)	Pr	1	4
<i>Crotalus pusillus</i> Klauber, 1952	EN (?)	H (18)	A	1	2
<i>Crotalus ravus</i> Cope, 1865	LC (=)	H (14)	A	1	5
<i>Crotalus scutulatus</i> (Kennicott, 1861)	LC (=)	M (11)	Pr	2	7
<i>Crotalus tancitarensis</i> Alvarado-Díaz & Campbell, 2004	DD (?)	H (19)	NL	0	EN
<i>Crotalus tlaloci</i> Bryson, Linkem, Dorcas, Lathrop, Jones, Alvarado-Díaz, Grünwald & Murphy, 2014	NE	H (16)	NL	1	2
<i>Crotalus totonacus</i> Gloyd & Kauffeld, 1940	LC (=)	H (17)	NL	1	4
<i>Crotalus transversus</i> Taylor, 1944	LC (=)	H (17)	P	0	EN
<i>Crotalus triseriatus</i> (Wagler, 1830)	LC (=)	H (16)	NL	1	3
<i>Metlapilcoatlus nummifer</i> (Rüppell, 1845)	NE	M (13)	A	1	5
<i>Ophryacus smaragdinus</i> Grünwald, Jones, Franz-Chávez & Ahumada-Carrillo, 2015	NE	H (14)	NL	1	3
<b>Order Testudines</b>					
<b>Emydidae</b>					
<i>Terrapene nelsoni</i> Stejneger, 1925	DD	H (18)	Pr	1	3
<i>Trachemys scripta</i> (Thunberg, 1792)				IN	
<i>Trachemys venusta</i> (Gray, 1855)	NE	M (13)	NL	3	6
<b>Geoemydidae</b>					
<i>Rhinoclemmys pulcherrima</i> (Gray, 1855)	NE	L (8)	A	3	5
<i>Rhinoclemmys rubida</i> (Cope, 1870)	NT (↓)	H (14)	Pr	1	6
<b>Kinosternidae</b>					
<i>Kinosternon herrerae</i> Stejneger, 1925	NT (↓)	H (14)	Pr	1	4
<i>Kinosternon hirtipes</i> (Wagler, 1830)	LC (↓)	M (10)	Pr	2	6
<i>Kinosternon integrum</i> LeConte, 1854	LC (=)	M (11)	Pr	1	9
<i>Kinosternon scorpioides</i> (Linnaeus, 1766)	NE	M (10)	Pr	3	9
<b>Trionychidae</b>					
<i>Apalone spinifera</i> (Le Sueur, 1827)				IN	

Fifty of the 427 native species (34 amphibians and 16 reptiles) are endemic to the TVB province (Table 1). Eighty-four species, 69 amphibians and 15 reptiles, are IUCN listed (i.e., Vulnerable, Endangered, or Critically Endangered), 60 (18 amphibians and 42 reptiles) are placed in a protected category by Secretaría del Medio Ambiente y Recursos Naturales of Mexico (SEMARNAT), and 173 (69 amphibians and 104 reptiles) are categorized as high risk by the Environmental Vulnerability Score (EVS). Sixty-one of the 84 species listed with a protected category by IUCN are categorized as high risk by the EVS, and only 20 of them are listed with a threatened category by SEMARNAT. Most of the species listed in a category of conservation concern status by IUCN are facing the destruction of their habitat due to urbanization and the transformation to agricultural land, especially those species found in cloud forest. Pollution of streams is another threat, mainly for amphibians.

In addition, seven species have been introduced to the TVB: *Eleutherodactylus planirostris* from the West Indies, *Rana catesbeiana* from northeastern Mexico, *Gehyra mutilata* from southeast Asia, *Hemidactylus mabouia* from sub-Saharan Africa, Nossi Be, Madagascar, and the Seychelles Archipelago, *Indotyphlops braminus* from India and parts of Asia, *Trachemys scripta* from northeastern Mexico, and *Apalone spinifera* from northern Mexico.

## General distribution

Of the 154 species of amphibians, 121 are endemic to Mexico, and 34 of these are endemic to the TVB (nine anurans and 25 salamanders). Most of the 34 amphibians endemic to the TVB are limited to one or two states of this province, only three of these 34 species live in three states and one lives in four states. Most of the species endemic to the TVB have a limited distribution. For example, *Ambystoma altamirani* inhabits the Sierra de las Cruces that runs on a thin strip from the northwestern state of Mexico southward through the western edge of Mexico City to the northwestern edge of Morelos (Woolrich-Piña et al. 2017b); and *Ambystoma rivulare* is found from the western end of Michoacán, southward through the middle part of the state of Mexico to the northern tip of Guerrero (Woolrich-Piña et al. 2017b). The nine anurans endemic to the TVB have a very restricted distribution either to the central part of the TVB in the states of Morelos, Mexico, and Mexico City, or to the western part of this province in the states of Guerrero, Jalisco, or Michoacán (Lemos-Espinal and Smith 2020a,b,c). The reduced dispersal capacity of salamanders, which are especially diverse in the TVB, makes them susceptible to being endemic to this region. Twelve of the 17 Mexican species of the genus *Ambystoma* are endemic to the TVB. These salamanders are mostly present in the central and western part of the TVB, in the states of Morelos, Mexico, Mexico City, and Michoacán, the exception being *Ambystoma taylori* which is limited to the Crater Lakes of the state of Puebla (IUCN 2022; AmphibiaWeb 2024). Likewise, the four species of *Pseudoeurycea* endemic to the TVB are restricted to the central part of this province. The other nine plethodontid salamanders of the genera *Chiropterotriton*, *Isthmura*, and *Thorius*, are restricted to the east of the TVB mainly in the state of Veracruz, but also parts of Puebla and Hidalgo (Table 2).

Twenty-six of the remaining 87 species that are endemic to Mexico and that inhabit the TVB have a distribution that is limited to only two biogeographic provinces, the TVB and another. Twenty-two of these 26 are shared between the TVB and the Sierra Madre Oriental and are restricted to eastern Mexico.



**Table 2.** Amphibian species endemic to the TVB. States recorded refers to the state of the TVB in which the species has been recorded. Total refers to the total number of states of the TVB in which the species has been recorded. State abbreviations are: Jal (Jalisco); Mich (Michoacán); Gro (Guerrero); Mor (Morelos); Mx (State of Mexico); MxC (Mexico City); Ver (Veracruz); Hgo (Hidalgo); Pue (Puebla).

State Recorded	Jal	Mich	Gro	Mor	Mx	MxC	Ver	Hgo	Pue	Total
<b>Class Amphibia</b>										
<b>Order Anura</b>										
<b>Craugastoridae</b>										
<i>Craugastor cueyatl</i>				1		1				2
<b>Eleutherodactylidae</b>										
<i>Eleutherodactylus erendirae</i>	1	1								2
<i>Eleutherodactylus floresvillelai</i>		1								1
<i>Eleutherodactylus franzi</i>			1							1
<i>Eleutherodactylus grandis</i>						1				1
<i>Eleutherodactylus humboldti</i>					1					1
<b>Ranidae</b>										
<i>Rana chichicuahutla</i>									1	1
<i>Rana dunni</i>		1								1
<i>Rana tlaloci</i>					1	1				2
<b>Order Caudata</b>										
<b>Ambystomatidae</b>										
<i>Ambystoma altamirani</i>				1	1	1				3
<i>Ambystoma amblycephalum</i>		1								1
<i>Ambystoma andersoni</i>		1								1
<i>Ambystoma bombypellum</i>					1					1
<i>Ambystoma dumerillii</i>		1								1
<i>Ambystoma granulosum</i>					1					1
<i>Ambystoma leorae</i>					1				1	2
<i>Ambystoma lermaense</i>					1					1
<i>Ambystoma mexicanum</i>						1				1
<i>Ambystoma ordinarium</i>		1			1					2
<i>Ambystoma rivulare</i>		1	1		1					3
<i>Ambystoma taylori</i>									1	1
<b>Plethodontidae</b>										
<i>Chiropterotriton casasi</i>							1			1
<i>Chiropterotriton ceronorum</i>							1			1
<i>Chiropterotriton chico</i>								1		1
<i>Chiropterotriton lavae</i>							1			1
<i>Chiropterotriton nubilus</i>							1			1
<i>Chiropterotriton perotensis</i>							1			1
<i>Chiropterotriton totonacus</i>							1			1
<i>Isthmura corrugata</i>							1			1
<i>Pseudoeurycea altamontana</i>				1	1	1		1		4
<i>Pseudoeurycea longicauda</i>		1			1					2
<i>Pseudoeurycea robertsi</i>					1					1
<i>Pseudoeurycea tlilicxiti</i>				1	1	1				3
<i>Thorius spilogaster</i>							1			1

Three other species are found in the TVB and parts of western Mexico. The other 19 amphibians that inhabit only two provinces are salamanders of the plethodontidae family, 18 of them are shared between the TVB and the Sierra Madre Oriental, and one is shared with the Chihuahuan Desert. Another 22 species are shared between the TVB and two other provinces, 18 of these 22 with the Sierra Madre Oriental and another province, all of them restricted to eastern Mexico. Eight of these 18 are shared with the eastern end of the Chihuahuan Desert. Seven are shared with the Veracruz. Four are shared with the Sierra Madre del Sur. Four species that are shared between three provinces, including the TVB, are species from western Mexico, two of them are shared between the TVB, Sierra Madre Occidental and Pacific Lowlands, and two between the TVB, Sierra Madre del Sur and Pacific Lowlands or Balsas Basin. The other 39 species endemic to Mexico that inhabit the TVB are distributed between four and eight biogeographic provinces. 12 are in four provinces, 14 in five provinces, nine in six provinces, three in seven provinces, and one in eight provinces.

The 33 native species of amphibians that are not endemic to Mexico have a wide distribution. Nine are shared with the US, 20 are found from Mexico to Central or South America, and four are distributed from extreme southeastern US to Central or South America.

Like amphibians, the native species of reptiles that inhabit the TVB are mostly endemic to Mexico; however, their dispersal ability and their ability to occupy arid habitats means that individual reptile species are found in more biogeographic provinces and are distributed further north and south within Mexico (Pianka and Vitt 2003; Buckley and Jetz 2007; Titon and Gomes 2015). One hundred and seventy-three (63.1%) of the 273 species of reptiles found in the TVB are endemic to Mexico of which 16 are endemic to the TVB (Table 1). The other 157 species of reptiles endemic to Mexico are shared between the TVB and at least one of the other thirteen biogeographic provinces, except with California. The largest number of species are shared with the Sierra Madre del Sur (103), followed by the Sierra Madre Oriental (84), the Balsas Basin (72), the Chihuahuan Desert (66), the Sierra Madre Occidental (60), the Pacific Lowlands (60), and the Veracruz (42). The rest of the provinces share relatively few species with the TVB: Chiapas Highlands (14), Sonoran Desert (5), Tamaulipas (4), Baja California and Yucatan Peninsula (1). Furthermore, 22 of these 157 species are found only in the TVB and another biogeographic province; 34 occupy three provinces including the TVB; 43 inhabit four provinces; 25 inhabit five provinces; 14 are distributed in six provinces; 11 in seven provinces; five in eight provinces; and three in nine provinces.

Of the 100 species of reptiles in the TVB that are not endemic to Mexico, 27 are shared with the US, 62 are distributed from Mexico to Central or South America, and 11 range from the US to Central or South America. These numbers and distributions show that the TVB is home to a diversity of reptile species that are a mix of species from southern and northern, as well as eastern and western Mexico (Table 1).

### Comparison with neighboring provinces

The TVB shares >50% of its amphibian and reptile species with the Sierra Madre Oriental 58.8% (253 shared species) and the Sierra Madre del Sur 54.1% (231) (Table 3). For amphibians, the TVB shares 60.6% of its species with the Sierra

Madre Oriental: 65.6% anurans and 54.4% salamanders. The TVB shares 39.4% of its amphibian species: 61.1% of anurans, 3.5% of salamanders, and 50% of Gymnophiona with the Sierra Madre del Sur. The TVB also shares a relatively high percentage of its amphibian species with the Veracruz province: 47.4% of anurans, 10.5% of salamanders, and 50% of caecilians. The percentages of amphibian species shared with the other four neighboring provinces are much lower: Pacific Lowlands 27.1%; Balsas Basin 26.5%; Chihuahuan Desert 24.5%; and Sierra Madre Occidental 23.2%. These results show that most of the amphibian species of the TVB are characteristic of the humid tropics of eastern Mexico. This is most noticeable in the Hylidae and Plethodontidae. The TVB shares 76.7% (23/30) of its hylid species with the Sierra Madre Oriental and 73.3% (22/30) with the Sierra Madre del Sur, and shares 68.2% (30/44) of its plethodontid species with the Sierra Madre Oriental. Thirteen of the 14 species of plethodontids that are not shared between the TVB and the Sierra Madre Oriental are endemic to the TVB. Likewise, the ambystomatid salamanders of the TVB are practically exclusive to the TVB, 12 of its 13 species are endemic to the TVB, most of them with a distribution in the western half of the TVB. However, the TVB is also home to a large number of amphibian species characteristic of the western half, such as those of the genus *Ambystoma*.

The number of reptile species shared between the TVB and its neighboring provinces was highest with the Sierra Madre del Sur (62.3% = 170 /273) followed by the Sierra Madre Oriental (58.2% = 159/273). For the Phrynosomatidae, the most diverse family of lizards in the TVB, 27 of 42 species (64.3%) are shared with the Sierra Madre del Sur, and 18 of 42 species (42.9%) are shared with the Sierra Madre Oriental. For the two most diverse families of snakes, Colubridae and Dipsadidae, the majority of species are shared with the Sierra Madre del Sur (Colubridae: 36 of 49 species, 73.4%; Dipsadidae: 32 of 52 species, 61.5%), and the Sierra Madre Oriental (Colubridae: 29 of 49 species, 59.2%; Dipsadidae: 32 of 52 species, 61.5%). The percentages and numbers of species shared with the other five neighboring provinces are lower: Veracruz 44.3% (121/273); Pacific Lowlands 43.6% (119/273); Balsas Basin 40.3% (110/273); Chihuahuan Desert 37.7% (103/273); and Sierra Madre Occidental 34.4% (94/273) (Table 3). Although these numbers show a slight tendency for reptile species characteristic of eastern Mexico to inhabit the TVB due to the number of species shared between the TVB and the Sierra Madre Oriental, they also show that the TVB is home to a mixture of reptile species from both the east and west (e.g., the percentages of shared species with the Veracruz [44.3%] and Pacific Lowlands [43.6%] are similar), and the south and north (e.g., the percentages of species shared with the Balsas Basin [40.3%] and the Chihuahuan Desert [37.7%] are similar). Likewise, the number of reptile species endemic to the TVB is relatively low 16 of 273 species (5.9%), suggesting the TVB does not house a particularly unique assortment of reptile species. Total surface area, the length of the shared border with the TVB, and the distance between geographic centroids of each of the neighboring provinces of the TVB do not seem to influence the number of species shared between the TVB and its neighboring provinces (Table 4; see below). However, the total number of amphibian and reptile species in each neighboring province significantly influences the number of species shared between the TVB and its neighboring provinces (Table 4; see below).

**Table 3.** Summary of the number of species shared between the TVB and neighboring biogeographic provinces (not including introduced species). The percent of the TVB shared by neighboring provinces are given in parentheses. Total refers to the number of species found in the TVB and seven neighboring provinces (i.e., regional species pool) and the number in parentheses in this column is the percent of the regional species pool found in the TVB. – indicates either the TVB or their neighboring province has no species in the taxonomic group, or none of that specific taxon is shared between the provinces, thus no value for shared species is provided. Abbreviations of the Biogeographic Provinces are: TVB (Transvolcanic Belt); SMOri (Sierra Madre Oriental); SMS (Sierra Madre del Sur); Ver (Veracruz); Pacific (Pacific Lowlands); Balsas (Balsas Basin); CD (Chihuahuan Desert), and SMOcc (Sierra Madre Occidental). Even though the Sierra Madre Occidental does not contact the TVB (according to the map and shape file in Morrone et al. 2017), we included it as a neighboring province for the TVB.

	TVB	SMOri	SMS	Ver	Pacific	Balsas	CD	SMOcc	Total
<b>Amphibia</b>	<b>155</b>	<b>94 (60.6)</b>	<b>61 (39.4)</b>	<b>52 (33.5)</b>	<b>42 (27.1)</b>	<b>41 (26.5)</b>	<b>38 (24.5)</b>	<b>36 (23.2)</b>	<b>371 (41.8)</b>
<b>Anura</b>	<b>95</b>	<b>63 (65.6)</b>	<b>58 (61.1)</b>	<b>45 (47.4)</b>	<b>40 (42.1)</b>	<b>40 (41.7)</b>	<b>30 (31.6)</b>	<b>34 (35.8)</b>	<b>230 (41.3)</b>
Bufonidae	9	8 (88.9)	4 (44.4)	6 (66.7)	6 (66.7)	5 (55.6)	5 (55.6)	6 (66.7)	28 (32.1)
Centrolenidae	1	1 (100)	1 (100)	1 (100)	1 (100)	–	–	–	1 (100)
Craugastoridae	13	9 (69.2)	9 (69.2)	8 (61.5)	6 (46.2)	6 (46.2)	2 (15.4)	4 (30.8)	35 (37.1)
Eleutherodactylidae	19	8 (42.1)	7 (0.37)	5 (26.3)	5 (26.3)	2 (10.5)	2 (10.5)	3 (15.8)	41 (46.3)
Hylidae	30	23 (76.7)	22 (73.3)	14 (46.7)	9 (30)	12 (40)	10 (33.3)	7 (23.3)	82 (36.6)
Leptodactylidae	2	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	1 (50)	3 (66.7)
Microhylidae	2	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)	1 (50)	2 (100)	5 (40)
Phyllomedusidae	2	1 (50)	2 (100)	1 (50)	2 (100)	1 (50)	–	1 (33.3)	3 (67)
Ranidae	15	6 (40)	8 (53.3)	4 (26.7)	6 (40)	9 (60)	6 (40)	8 (53.3)	28 (53.6)
Rhinophrynidae	–	–	–	–	–	–	–	–	1 (0)
Scaphiropodidae	2	2 (100)	1 (50)	2 (100)	1 (50)	1 (50)	2 (100)	2 (100)	3 (66.7)
<b>Caudata</b>	<b>57</b>	<b>31 (54.4)</b>	<b>2 (3.5)</b>	<b>6 (10.5)</b>	<b>–</b>	<b>1 (1.8)</b>	<b>8 (14)</b>	<b>2 (3.5)</b>	<b>138 (41.3)</b>
Ambystomatidae	13	1 (7.7)	1 (7.7)	–	–	1 (7.7)	1 (7.7)	1 (7.7)	17 (76.5)
Plethodontidae	44	30 (68.2)	1 (2.3)	6 (13.6)	–	–	7 (15.9)	1 (2.3)	119 (37)
Salamandridae	–	–	–	–	–	–	–	–	1 (0)
Sirenidae	–	–	–	–	–	–	–	–	1 (0)
<b>Gymnophiona</b>	<b>2</b>	<b>–</b>	<b>1 (50)</b>	<b>1 (50)</b>	<b>2 (100)</b>	<b>–</b>	<b>–</b>	<b>–</b>	<b>3 (66.7)</b>
Dermophiidae	2	–	1 (50)	1 (50)	2 (100)	–	–	–	3 (66.7)
<b>Reptilia</b>	<b>273</b>	<b>159 (58.2)</b>	<b>170 (62.3)</b>	<b>121 (44.3)</b>	<b>119 (43.6)</b>	<b>110 (40.3)</b>	<b>103 (37.7)</b>	<b>94 (34.4)</b>	<b>749 (36.4)</b>
<b>Crocodylia</b>	<b>–</b>	<b>–</b>	<b>–</b>	<b>–</b>	<b>–</b>	<b>–</b>	<b>–</b>	<b>–</b>	<b>3 (33.3)</b>
Alligatoridae	–	–	–	–	–	–	–	–	1 (0)
Crocodylidae	–	–	–	–	–	–	–	–	2 (50)
<b>Squamata</b>	<b>265</b>	<b>154 (57.9)</b>	<b>166 (62.6)</b>	<b>116 (43.8)</b>	<b>113 (42.6)</b>	<b>106 (40)</b>	<b>101 (38.1)</b>	<b>90 (34)</b>	<b>699 (37.9)</b>
<b>Lacertilia</b>	<b>105</b>	<b>58 (54.7)</b>	<b>66 (62.9)</b>	<b>40 (38.1)</b>	<b>39 (37.1)</b>	<b>40 (38.1)</b>	<b>35 (33.3)</b>	<b>32 (30.5)</b>	<b>348 (30.2)</b>
Anguidae	12	8 (66.7)	4 (33.3)	4 (33.3)	2 (16.7)	2 (16.7)	6 (50)	3 (25)	34 (35.3)
Anolidae	9	8 (88.9)	6 (66.7)	5 (55.6)	1 (11.1)	2 (22.2)	1 (11.1)	1 (11.1)	47 (19.1)
Bipedidae	–	–	–	–	–	–	–	–	2 (0)
Corytophanidae	3	2 (66.7)	2 (66.7)	3 (100)	2 (66.7)	1 (33.3)	–	–	5 (60)
Crotaphytidae	–	–	–	–	–	–	–	–	4 (0)
Dibamidae	1	1 (100)	–	1 (100)	–	–	1 (100)	–	1 (100)
Diploglossidae	2	2 (100)	1 (50)	1 (50)	–	–	–	–	4 (50)
Eublepharidae	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	–	–	5 (20)
Gymnophthalmidae	–	–	–	–	–	–	–	–	1 (0)
Helodermatidae	1	–	1 (100)	–	1 (100)	1 (100)	–	1 (100)	4 (25)

	TVB	SMOri	SMS	Ver	Pacific	Balsas	CD	SMOcc	Total
Iguanidae	3	2 (66.7)	3 (100)	2 (66.7)	2 (66.7)	3 (100)	1 (33.3)	1 (33.3)	9 (33.3)
Phrynosomatidae	42	18 (42.9)	27 (64.3)	6 (14.3)	12 (28.6)	17 (40.5)	18 (42.9)	18 (42.9)	113 (37.2)
Phyllodactylidae	3	–	3 (100)	–	2 (66.7)	2 (66.7)		1 (33.3)	15 (20)
Scincidae	14	7 (50)	9 (64.3)	8 (57.1)	8 (57.1)	5 (35.7)	4 (28.6)	3 (21.4)	32 (43.8)
Sphaerodactylidae	1	1 (100)	1 (100)	1 (100)	1 (100)	–	–	–	3 (33.3)
Teiidae	8	4 (50)	7 (87.5)	4 (50)	6 (75)	6 (75)	3 (37.5)	3 (37.5)	34 (23.5)
Xantusidae	4	3 (75)	–	3 (75)	–	–	1 (25)	1 (25)	22 (18.2)
Xenosauridae	1	1 (100)	1 (100)	1 (100)	1 (100)	–	–	–	13 (7.7)
<b>Serpentes</b>	<b>160</b>	<b>96 (60)</b>	<b>100 (62.5)</b>	<b>76 (47.5)</b>	<b>74 (46.3)</b>	<b>66 (41.3)</b>	<b>66 (41.3)</b>	<b>58 (36.3)</b>	<b>351 (45.6)</b>
Boidae	2	1 (50)	2 (100)	1 (50)	2 (100)	2 (100)		1 (50)	4 (50)
Colubridae	49	29 (59.2)	36 (73.4)	28 (57.1)	30 (61.2)	29 (59.2)	22 (44.9)	25 (51)	115 (42.6)
Dipsadidae	52	32 (62.7)	32 (61.5)	26 (50)	24 (46.2)	15 (28.8)	19 (36.5)	12 (23.1)	119 (43.7)
Elapidae	8	4 (44.4)	6 (75)	4 (50)	5 (62.5)	2 (25)	3 (37.5)	3 (37.5)	16 (50)
Leptotyphlopidae	5	2 (40)	3 (60)	1 (20)	2 (40)	4 (80)	2 (40)	1 (20)	13 (38.5)
Loxocemidae	1	–	1 (100)	1 (100)	1 (100)	1 (100)	–	–	1 (100)
Natricidae	17	14 (82.4)	7 (41.2)	7 (41.2)	4 (23.5)	5 (29.4)	12 (70.6)	8 (47.1)	31 (54.8)
Typhlopidae	1	1 (100)	–	1 (100)	–	–	–	–	1 (100)
Viperidae	25	13 (52)	13 (52)	7 (28)	6 (24)	8 (32)	8 (32)	8 (32)	51 (41)
<b>Testudines</b>	<b>8</b>	<b>5 (62.5)</b>	<b>4 (50)</b>	<b>5 (62.5)</b>	<b>6 (75)</b>	<b>4 (50)</b>	<b>2 (25)</b>	<b>4 (50)</b>	<b>47 (17)</b>
Cheloniidae	–	–	–	–	–	–	–	–	5 (0)
Chelydridae	–	–	–	–	–	–	–	–	1 (0)
Dermatemydidae	–	–	–	–	–	–	–	–	1 (0)
Dermochelyidae	–	–	–	–	–	–	–	–	1 (0)
Emydidae	2	1 (50)	–	1 (50)	1 (50)	–	–	1 (50)	15 (13.3)
Geoemydidae	2		2 (100)	1 (50)	2 (100)	1 (50)	–	1 (50)	3 (66.7)
Kinosternidae	4	4 (100)	2 (50)	3 (75)	3 (75)	3 (75)	2 (50)	2 (50)	17 (11.8)
Testudinidae	–	–	–	–	–	–	–	–	3 (0)
Trionychidae	–	–	–	–	–	–	–	–	1 (0)
<b>Total</b>	<b>427</b>	<b>253 (58.8)</b>	<b>231 (54.1)</b>	<b>173 (40.5)</b>	<b>161 (37.7)</b>	<b>151 (35.4)</b>	<b>141 (33)</b>	<b>130 (30.4)</b>	<b>1120 (38.1)</b>

**Table 4.** Surface area in km<sup>2</sup> of the TVB and each of its seven neighboring biogeographic provinces, Sierra Madre Occidental included even though it does not contact the TVB; contact area in km between the TVB and each of its seven neighboring biogeographic provinces; distances between the centroid in km between the TVB and each of its seven neighboring biogeographic provinces (does not apply for Sierra Madre Occidental); Shared Species between the TVB and each of its seven neighboring biogeographic provinces in number of species, number in parentheses represents the percentage of the number of shared species between the neighboring province and the TVB; and Number of Species of each of the neighboring provinces.

Neighboring Provinces	Surface Area (km <sup>2</sup> )	Surface Contact Area (km)	Distance between centroids (km)	Shared Species	Number of Species
Transvolcanic Belt	82,840.00	–	–	–	427
Sierra Madre del Sur	93,606.90	434	227.7	231	517
Sierra Madre Oriental	51,897.30	307	314.4	253	382
Veracruz	191,451.10	281	465.1	173	340
Pacific Lowlands	187,112.90	1272	526.3	161	325
Chihuahuan Desert	578,001.50	1488	781.9	141	262
Sierra Madre Occidental	171,195.10	NA	890.2	130	217
Balsas Basin	76,135.80	1968	172.7	151	206



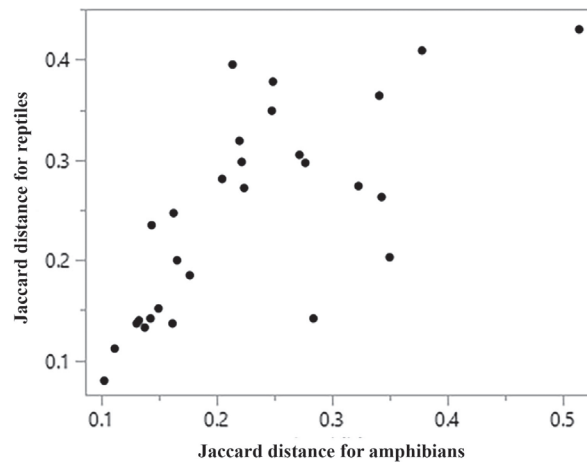
This is a reaffirmation of the fact that the TVB is a transition province inhabited by species from a variety of neighboring provinces. Furthermore, the topographical, climatic, and vegetation characteristics of the TVB mean that this province has the necessary conditions to host species of amphibians and reptiles with both Neotropical and Nearctic affinities.

## Similarities

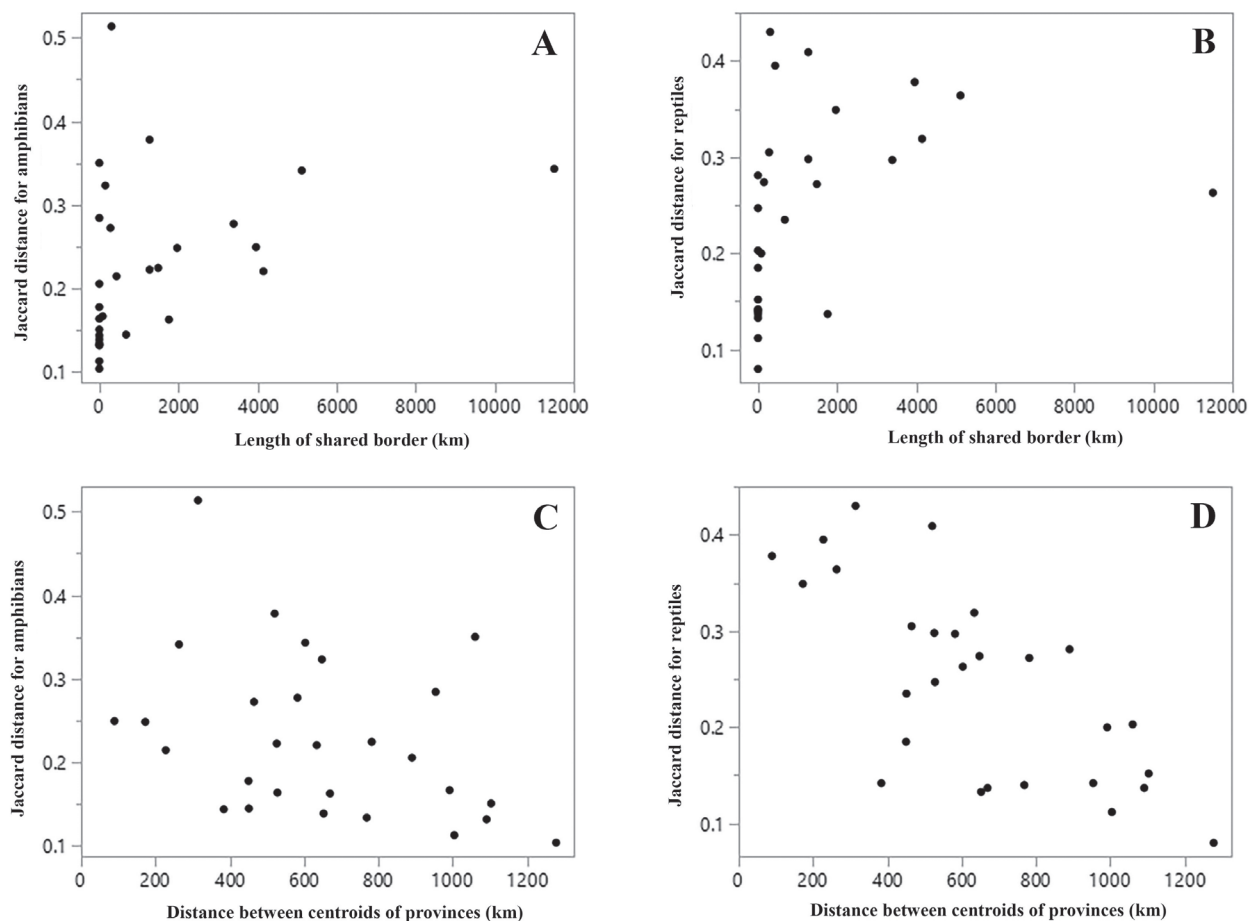
The Jaccard distances between pairs of provinces for amphibians and reptiles were highly positively correlated (Fig. 4;  $n = 28$ , Spearman's  $\rho = 0.7446$ ,  $p < 0.0001$ ). Jaccard distances between pairs of provinces for amphibians were positively correlated with the length of the shared border (Fig. 5A;  $n = 28$ , Spearman's  $\rho = 0.5342$ ,  $p < 0.005$ ) and negatively correlated with the distance between their geographic centroids (Fig. 5C;  $n = 28$ , Spearman's  $\rho = -0.3957$ ,  $p < 0.05$ ). Similarly, r Jaccard distances between pairs of provinces for reptiles were positively correlated with the length of the shared border (Fig. 5B;  $n = 28$ , Spearman's  $\rho = 0.6531$ ,  $p < 0.0005$ ) and negatively correlated with the distance between their geographic centroids (Fig. 5D;  $n = 28$ , Spearman's  $\rho = -0.7002$ ,  $p < 0.0001$ ). Taken together, our results suggest that many of the similarities and differences in the herpetofauna among Mexican biogeographic provinces are a consequence of their proximity.

On the other hand, the numbers of amphibian and reptile species in a province were not correlated with its surface area (amphibians:  $n = 8$ , Spearman's  $\rho = -0.26$ ,  $p = 0.53$ ; reptiles:  $n = 8$ , Spearman's  $\rho = -0.20$ ,  $p = 0.63$ ) or the longitude of its geographic centroid (amphibians:  $n = 8$ , Spearman's  $\rho = 0.26$ ,  $p = 0.53$ ; reptiles:  $n = 8$ , Spearman's  $\rho = 0.24$ ,  $p = 0.57$ ). Likewise, the numbers of amphibian and reptile species in a province were not correlated with the latitude of its geographic centroid (amphibians:  $n = 8$ , Spearman's  $\rho = -0.36$ ,  $p = 0.38$ ; reptiles:  $n = 8$ , Spearman's  $\rho = -0.28$ ,  $p = 0.51$ ).

The cluster analysis for amphibians revealed two well-differentiated clusters (Fig. 6A). The first is formed by the TVB, the Sierra Madre Oriental, and the Veracruz, and the second is comprised of the Sierra Madre Occidental, the Balsas Basin, the Pacific Lowlands, and the Chihuahuan Desert. The Sierra Madre del Sur then joins these two clusters. The first cluster exhibits a close relationship with the proximity of the TVB to the Sierra Madre Oriental (314 km) and Veracruz (465 km), two of the closest provinces to the TVB. Additionally, there is a high percentage of amphibian species shared with these two neighboring provinces (Sierra Madre Oriental (60.6%), and the Veracruz (34.2%)) (Table 3). The region in which these three provinces are closest to each other is a region in which a high number of endemic hylid and plethodontid species is found, some of which are shared between the three provinces. These factors likely contribute to the formation of Cluster AI. Cluster AII is composed of provinces with a predominance of amphibian species from western Mexico: Chihuahuan Desert (53), Sierra Madre Occidental (57), and Balsas Basin (51). Although the Sierra Madre del Sur shares a relatively high percentage of amphibian species with the TVB (39.4%) and is the closest province to the TVB (228 km), it is isolated from these two clusters. This is due to the high species richness of amphibians in the Sierra Madre del Sur (186) and the high percentage of species endemic to it (52.7%). In addition, the Sierra Madre del Sur itself does not appear to be a sin-

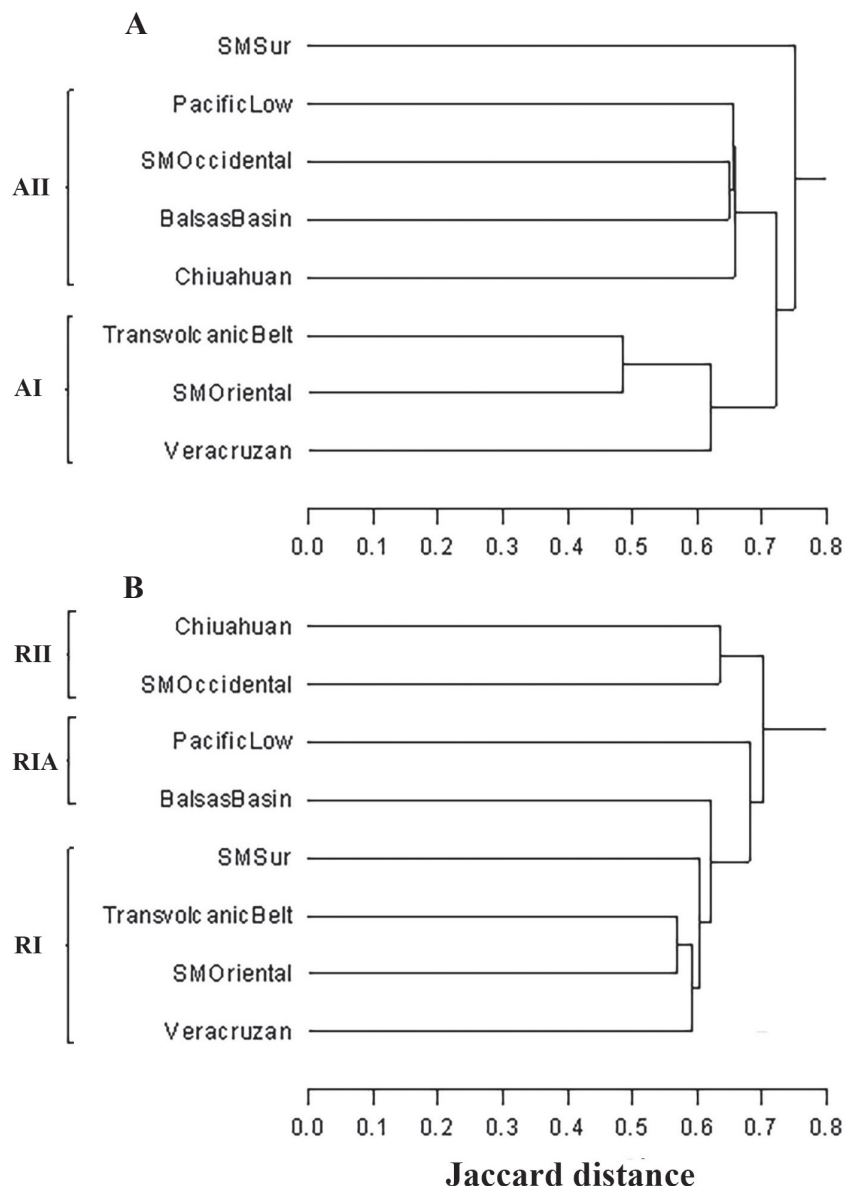


**Figure 4.** The correlation between the Jaccard distance of amphibians and reptiles among the TVB and its neighboring biogeographic provinces of Mexico.



**Figure 5.** The relationships between Jaccard distances for length of shared border and distance between centroids of the TVB and its neighboring biogeographic provinces and the Jaccard distances of amphibians (**A, C** respectively) and reptiles (**B, D** respectively).

gle, homogeneous biogeographic province with respect to its herpetofauna and other taxa (Luna-Vega et al. 1999; Santiago-Alvarado et al. 2016). This gives the Sierra Madre del Sur a unique identity, different from the other provinces, even though it shares a significant number of species with them.



**Figure 6.** Cluster trees for amphibians **A** of the TVB and its neighboring biogeographic provinces and **B** Cluster trees for reptiles of TVB and its neighboring biogeographic provinces. Clusters are identified with Roman numerals.

The cluster analysis for reptiles is somewhat similar to that for amphibians (Fig. 6B). As with the amphibians, there are two well-differentiated clusters for the reptiles, the first formed by four provinces with marked geographical proximity; the TVB, the Sierra Madre Oriental, and the Veracruz as in amphibians, but the Sierra Madre del Sur also joins this cluster. This may result from the greater mobility that reptiles show compared to amphibians, being independent of water for their reproduction and tolerating drought conditions. Unlike in the amphibians, the Balsas Basin and Pacific Lowlands connect with the Sierra Madre del Sur to form the subcluster RIA. The RII cluster is formed by the Sierra Madre Occidental and the Chihuahuan Desert which are very closely related geographically with a short distance between their geographic centroids (263 km) and an extensive shared border (5,120 km).

## Conservation status

Eighty-four (21.9% = 84/384) of the 427 native species of amphibians and reptiles that inhabit the TVB are included in a category of conservation concern in the IUCN Red List (i.e., Vulnerable, Endangered, or Critically Endangered), 60 (14.1% = 60/427) are placed in some category of protection by SEMARNAT (excluding not listed [NL] and subject to special protection [Pr], this last category is equivalent to the least concern [LC] category of IUCN), and 173 (43.1% = 173/401) are considered high risk by the EVS (Fig. 7, Table 5). Sixty-nine amphibians (46.9%) are included in the IUCN Red List, 16 of them listed as Vulnerable, 26 as Endangered, and 27 as Critically Endangered, most of them have a decreasing population trend, and all of them endemic to Mexico, and 31 are endemic to the TVB (IUCN 2022). Three of the four species endemic to the TVB that are not included in the IUCN Red List with a protected status were recently described: *Craugastor cueyatl* Jameson et al. 2022; *Eleutherodactylus franzi* Grünwald et al. 2023, and *Eleutherodactylus humboldti* Devitt et al. 2023. Another is considered as Data Deficient (DD): *Ambystoma bombypellum*. All of these species are facing increasing rates of habitat loss due to urbanization and transformation for agricultural use (IUCN 2022). Some are also threatened by the amphibian chytrid fungus, *Batrachochytrium dendrobatidis*, and others face the presence of predators and competitors that have been introduced to their habitat, such as bullfrogs, rainbow trout, sport fish, and crayfish (IUCN 2022). Eighteen amphibian species (11.6% = 18/155) are protected by SEMARNAT; and 69 species are considered of high risk by EVS (50.0% = 69/138) (Table 5).

For reptiles, 6.3% (15/237) are included in some category of conservation concern in the IUCN Red List, 15.4% (42/273) are listed by SEMARNAT, and 39.5% (104/263) are considered to be at high risk by EVS (Fig. 7). Six of the 15 species included in the IUCN Red List are Endangered (EN) and nine are Vulnerable (VU) (Table 5). All of the listed species are endemic to Mexico, and three are endemic to the TVB. All these species are threatened by habitat loss which is exacerbated by limited distributions. For example, lizards in the genera *Abronia* and *Barisia* have highly restricted distributions and are threatened by increasing habitat loss (IUCN 2022). In addition, species in the genus *Abronia* are targeted by the illegal pet trade, significantly impacting their populations (IUCN 2022). The convergence of these factors poses a significant threat to the survival of these species. Other species such as *T. melanogaster*, although it has a relatively wide distribution in north-central Mexico, is rapidly losing its habitat due to the contamination of the streams it occupies by pollution from agricultural and industrial waste (IUCN 2022). Eleven of the 15 species listed by the IUCN are categorized as high risk by the EVS, but only six are listed with a threatened category by SEMARNAT (Table 5).

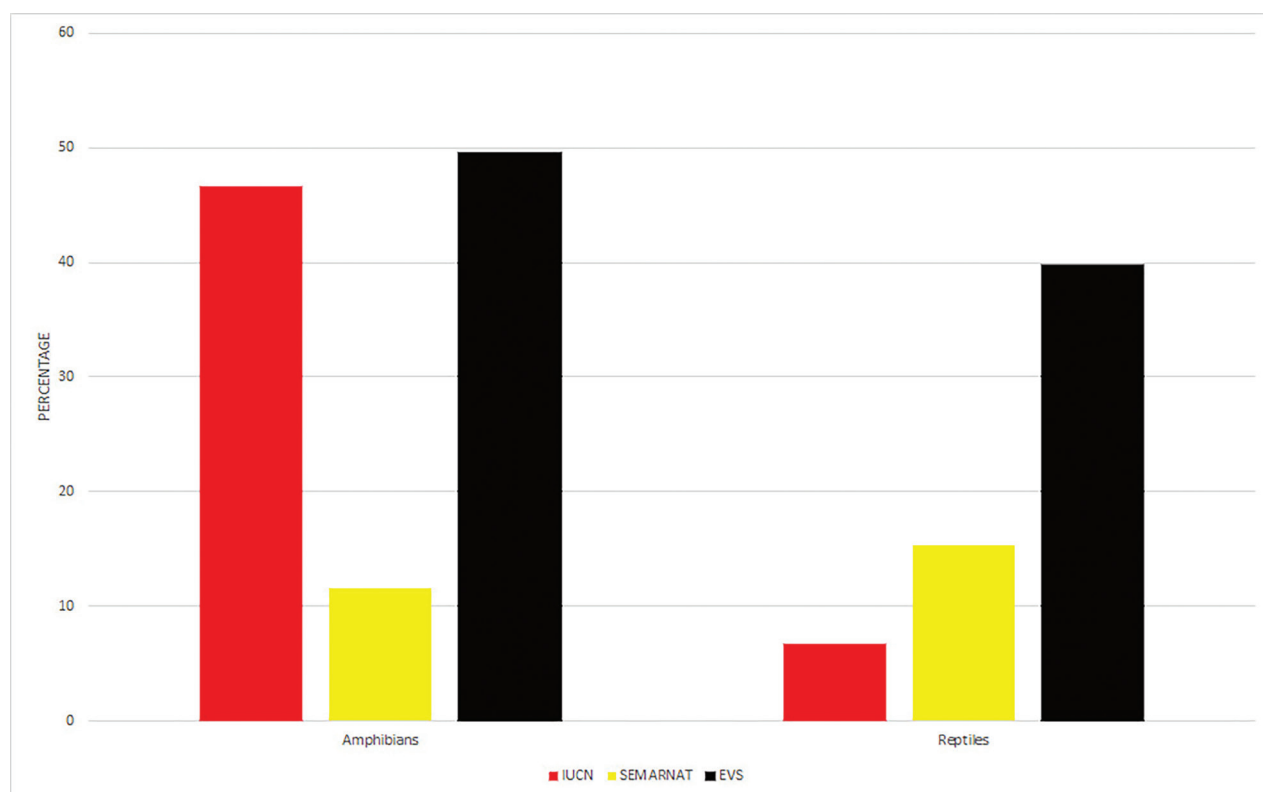
As mentioned above, the main threat to the amphibians and reptiles of the TVB is the loss of their habitats, which are being rapidly urbanized, transformed into agricultural fields, cleared, or polluted (IUCN 2022). In addition, this habitat loss is enhanced by the acid rain that occurs throughout and near the large cities found in the TVB (IUCN 2022). These threats, along with other threats including emerging diseases, such as *B. dendrobatidis*, the introduction of non-native species, and climate change demand rapid conservation and management actions to prevent the decline and ultimately the disappearance of the amphibians and reptiles of the TVB.

**Table 5.** Summary of native species present in TVB biogeographic province of Mexico by family, order or suborder, and class. Status summary indicates the number of species found in each IUCN conservation status in the order DD, LC, VU, NT, EN, CR (see Table 1 for abbreviations; in some cases, species have not been assigned a status by the IUCN and therefore these may not add up to the total number of species in a taxon). Mean EVS is the mean Environmental Vulnerability Score; scores  $\geq 14$  are considered high vulnerability (Wilson et al. 2013a, b) and conservation status in Mexico according to SEMARNAT (2019) in the order NL, Pr, A, P (see Table 1 for abbreviations).

Scientific Name	Genera	Species	IUCN	x̄ EVS	SEMARNAT
			DD, LC, NT, VU, EN, CR		NL, Pr, A, P
Class Amphibia					
Order Anura	26	95	1,64,3,10,7,4	11.3	65,21,7,2
Bufonidae	3	9	0,7,0,0,1,0	9.1	8,1,0,0
Centrolenidae	1	1	0,0,0,0,0,0	10	1,0,0,0
Craugastoridae	1	13	0,12,0,0,1,0	12.8	11,2,0,0
Eleutherodactylidae	1	19	1,9,0,4,2,0	15.3	12,7,0,0
Hylidae	14	30	0,18,2,5,2,2	10.5	20,4,6,0
Leptodactylidae	1	2	0,2,0,0,0,0	5.5	2,0,0,0
Microhylidae	1	2	0,2,0,0,0,0	5.5	1,1,0,0
Phyllomedusidae	1	2	0,2,0,0,0,0	11.7	2,0,0,0
Ranidae	1	15	0,10,1,1,1,2	11.7	6,6,1,2,
Scaphiopodidae	2	2	0,2,0,0,0,0	4.5	2,0,0,0
Order Caudata	8	57	1,7,0,6,19,23	15.5	18,30,8,1
Ambystomatidae	1	13	1,1,0,0,5,6	13.9	0,9,3,1
Plethodontidae	7	44	0,6,0,6,14,17	16	18,21,5,0
Order Gymnophiona	1	2	0,2,0,0,0,0	11.5	0,2,0,0
Dermophiidae	1	2	0,2,0,0,0,0	11.5	0,2,0,0
Subtotal	35	154	2,73,3,16,26,27	12.8	83,53,15,3
Class Reptilia					
Order Squamata	84	265	27,187,3,9,6,0	12.1	151,73,37,4
Suborder Lacertilia	28	105	5,75,1,5,4,0	12.5	62,29,11,3
Anguidae	5	12	0,5,0,1,4,0	13.5	3,5,2,2
Anolidae	1	9	1,6,1,0,0,0	11.6	7,1,1,0
Corytophanidae	3	3	0,3,0,0,0,0	9.7	2,1,0,0
Dibamidae	1	1	0,1,0,0,0,0	10	0,0,1,0
Diploglossidae	1	2	0,2,0,0,0,0	14	1,1,0,0
Eublepharidae	1	1	0,1,0,0,0,0	9	0,0,1,0
Helodermatidae	1	1	0,1,0,0,0,0	11	0,0,1,0
Iguanidae	2	3	0,3,0,0,0,0	13	0,2,1,0
Phrynosomatidae	3	42	1,33,0,1,0,0	12.8	34,6,2,0
Phyllodactylidae	1	3	0,3,0,0,0,0	13	1,1,1,0
Scincidae	3	14	2,8,0,1,0,0	12.4	8,5,1,0
Sphaerodactylidae	1	1	0,1,0,0,0,0	12	0,1,0,0
Teiidae	2	8	0,6,0,0,0,0	11.5	5,3,0,0
Xantusidae	2	4	1,2,0,1,0,0	14	1,2,0,1
Xenosauridae	1	1	0,0,0,1,0,0	9	0,1,0,0
Suborder Serpentes	56	160	22,112,2,4,2,0	11.9	89,44,26,1
Boidae	1	2	0,1,0,0,0,0	10	2,0,0,0
Colubridae	21	49	3,41,1,0,0,0	10.9	34,5,10,0
Dipsadidae	18	52	14,32,0,2,0,0	11.4	30,22,0,0
Elapidae	2	8	0,8,0,0,0,0	12.6	1,6,1,0
Leptotyphlopidae	2	5	1,3,0,0,0,0	11.4	4,1,0,0
Loxocemidae	1	1	0,1,0,0,0,0	10	0,1,0,0



Scientific Name	Genera	Species	IUCN	$\bar{x}$ EVS	SEMARNAT
			DD, LC, NT, VU, EN, CR		NL, Pr, A, P
Natricidae	4	17	1,13,0,2,1,0	12.2	6,1,10,0
Typhlopidae	1	1	0,1,0,0,0,0	11	1,0,0,0
Viperidae	6	25	3,12,1,0,1,0	14.9	11,8,5,1
<b>Order Testudines</b>	<b>4</b>	<b>8</b>	<b>1,2,2,0,0,0</b>	<b>12.3</b>	<b>1,6,1,0</b>
Emydidae	2	2	1,0,0,0,0,0	15.5	1,1,0,0
Geoemydidae	1	2	0,0,1,0,0,0	11	0,1,1,0
Kinosternidae	1	4	0,2,1,0,0,0	11.3	0,4,0,0
<b>Subtotal</b>	<b>88</b>	<b>273</b>	<b>28,189,5,9,6,0</b>	<b>12.1</b>	<b>152,79,38,4</b>
<b>Total</b>	<b>123</b>	<b>427</b>	<b>30,262,8,25,32,27</b>	<b>12.4</b>	<b>235,132,53,7</b>



**Figure 7.** Percentage of amphibian and reptile species with conservation concern status (IUCN 2022), protected by the Mexican government (SEMARNAT 2019), or deemed to have a high environmental vulnerability score (EVS), for the TVB biogeographic provinces of Mexico.

The growth of the cities of Mexico, Cuernavaca and Toluca in the center of the TVB, is especially worrying. Species such as *Eleutherodactylus grandis*, *Rana tlaloci*, *Ambystoma altamirani*, *A. mexicanum*, *Pseudoeurycea tlilicxitl*, among others, are threatened by the excessive and accelerated growth of these cities, which seems to be occurring unplanned and unchecked (J. Lemos-Espinal personal observation). This problem is largely the result of the centralization of major governmental service to urban areas by the Mexican government that began in the early 20<sup>th</sup> century. Due to the resulting lack of opportunities in rural areas and the resulting widespread poverty, people abandon the unprofitable crop fields and migrated to cities that already are expanding due to high birth rates. Of particular concern is that species whose distributions are limited to Mexico City, such as *Rana tlaloci*, often lack recent records, and the distributions

of other species have been reduced to small areas within Mexico City, such as *Eleutherodactylus grandis*, which is currently limited to the Pedregal Reserve, or *Ambystoma mexicanum* which is limited to Lake Xochimilco (Contreras et al. 2009; IUCN 2022). Other species such as *Ambystoma altamirani* that inhabit streams in the Sierra de las Cruces, are seeing their habitat reduced due to the growth of these cities which are increasingly threatening to form one continuous urban complex between them that will fragment the mountain ranges, such as of the Sierra Las Cruces, and eventually making their habitats disappear (Deutsche Gesellschaft für Internationale Zusammenarbeit 2023). The species that inhabit the ranges that surround the Valley of Mexico where Mexico City is located also face the excessive and poorly planned introduction of non-native species such as the Rainbow Trout (*Oncorhynchus mykiss*), which have negative effects on *Dryophytes plicatus* and *Ambystoma altamirani* populations in the Sierra de las Cruces (e.g., Estrella Zamora et al. 2018; Guerrero de la Paz et al. 2020). However, the Mexican government continues to promote expansion programs for Rainbow Trout farms in these areas (García-Mondragón et al. 2013), affecting species endemic to the TVB, mainly those of the genus *Ambystoma* (*A. altamirani*, *A. leorae*, and *A. rivulare* among others) (Estrella-Zamora et al. 2018; Guerrero de la Paz et al. 2020).

Another important threat in this province is the practice at the end of the dry season (March-April) of burning dry vegetation in grasslands and lower parts of the forest layers to produce green shoots to feed livestock, which produces smoke from fires that contribute to contaminating one of the most polluted regions in the country and in the world (i.e., the Mexico City metropolitan area; Rodríguez-Trejo and Cruz-Reyes 2012). In addition, the loss of forests and the pollution that this practice causes significantly damages amphibian and reptile populations. It is common to observe individuals of *Barisia imbricata*, *Phrynosoma orbiculare*, *Sceloporus mucronatus*, etc., without hands or limbs, and snakes such as *Crotalus polystictus*, *C. triseriatus*, or tree frogs such as *Dryophytes eximius*, are frequently found completely burned after these intentional fires (J. Lemos-Espinal personal observation). Unfortunately, all of these threats and an apparent failure to regulate human development in this transition province means that the TVB is in dire need of a comprehensive and effective recovery plan to protect its diverse herpetofauna.

## Conclusion

The Transvolcanic Belt (TVB) has an incredible diversity of native amphibians and reptiles, hosting 427 species, including 154 amphibians and 273 reptiles. This represents a significant portion of Mexico's herpetofauna, with the TVB housing 72.7% of the families, 79.4% of the genera, and 30.5% of the species found in the entire country. Among these species, 50 are endemic to the TVB, highlighting the importance of its unique biodiversity. It shares a considerable number of species with neighboring provinces, particularly the Sierra Madre Oriental and the Sierra Madre del Sur, indicating close biogeographic relationships. These provinces serve as sources of species that enter the TVB from various directions, increasing the richness of its fauna. While the TVB exhibits some affinity with eastern Mexico in terms of amphibian species, its reptile fauna reflects a mixture of species from different regions, including western and south-

ern Mexico. However, the conservation status of the herpetofauna of the TVB is of great concern, with a notable percentage of species classified as vulnerable, endangered, or critically endangered. The main threats to these species include habitat loss due to urbanization, agriculture, and pollution, as well as the introduction of non-native species and climate change. Additionally, seven non-native species have been introduced to the TVB, highlighting the need for careful management and conservation efforts. Overall, the TVB stands out as a critical region for the conservation of the unique herpetofauna of Mexico, emphasizing the importance of preserving its rich biodiversity for future generations. Urgent conservation actions are needed to protect the rich and unique herpetofauna of the TVB and ensure their survival in the face of these challenges.

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## Additional information

### Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Ethical statement

No ethical statement was reported.

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### Author contributions

Conceptualization: GRS, JLE. Data curation: JLE, GRS. Formal analysis: JLE, GRS. Investigation: GRS, JLE. Writing – original draft: GRS, JLE. Writing – review and editing: JLE, GRS.

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### Data availability

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

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## Appendix 1

**List of the literature sources used to create the species lists of amphibians and reptiles of the Transvolcanic Belt used in the cluster analyses. Source refers to the references from which the checklist for the Transvolcanic Belt biogeographic province was obtained**

Ahumada-Carrillo et al. (2020); Alvarado-Díaz et al. (2013); Badillo-Saldaña et al. (2018); Blancas-Hernández et al. (2019); Bryson et al. (2021); Campbell et al. (2018); Campillo-García et al. (2021); Carbajal-Márquez et al. (2020); Cavazos-Camacho and Ahumada-Carrillo (2020); Caviedes-Solis and Nieto-Montes de Oca (2018); Cox et al. (2018); Cruz-Elizalde et al. (2019); Cruz-Sáenz et al. (2017); De la Torre-Loranca et al. (2020); Devitt et al. (2023); Dixon and Lemos-Espinal (2010); Everson et al. (2021); Fernández et al. (2006); Fernández-Badillo et al. (2020); Flores-Villela et al. (2022); García-Alvarado (2016); García-

Vázquez et al. (2018, 2021); Grünwald et al. (2018, 2019, 2021a, b); Hansen et al. (2016); Hernandez et al. (2022); Hillis et al. (1983); Horowitz (1955); Jameson et al. (2022); Kaplan et al. (2020); Köhler et al. (2019); Lavin et al. (2014); Lemos-Espinal and Dixon (2016); Lemos-Espinal and Smith (2015, 2020a, b, c); Lemos-Espinal et al. (2020); Leyte-Manrique et al. (2022); Loc-Barragán and Woolrich-Piña (2020); Loc-Barragán et al. (2018, 2024); McCranie and Köhler (2004); McCranie et al. (2020); Mendoza-Hernández and Roth-Monzón (2017); Montanucci (1979); Montaña-Ruvalcaba et al. (2020); O'Connell and Smith (2018); Palacios-Aguilar and Flores-Villela (2018, 2020); Palacios-Aguilar and Santos-Bibiano (2020); Palacios-Aguilar et al. (2018); Pazos-Nava et al. (2019); Pérez-Ramos and Saldaña-de la Riva (2008); Platz (1991); Ramírez-Bautista et al. (2020); Ramírez-Reyes and Flores-Villela (2018); Ramírez-Reyes et al. (2017, 2021a, b); Reyes-Velasco et al. (2020a, b); Schätti et al. (2020); Streicher et al. (2014); Tepos-Ramírez et al. (2021); Torres-Hernández et al. (2021); Valencia-Herverth et al. (2020); Webb (2001); Woolrich-Piña et al. (2016, 2017a); Zaldivar-Riverón et al. (2004)

# Eight years after the Fundão tailings dam collapse: chaos on the muddy banks

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Eight years have passed since Brazil's worst environmental disaster, the collapse of the Samarco company's Fundão tailings dam in Mariana, Minas Gerais, Brazil. The mud spill buried the village of Bento Rodrigues and affected, with mud full of heavy metals and metalloids, more than 600 km of the river channel, and marginal habitats. It extended for hundreds of kilometers along the coast, disturbing the sea, coral reefs, mangroves, and beaches (Fernandes et al. 2016; Costa et al. 2022). At the time of the disaster, more than one million people across 35 cities were affected due to the spill of around 50 million m<sup>3</sup> of mud waste, resulting in 19 deaths, diseases, and hundreds of people displaced (Fernandes et al. 2016; Omachi et al. 2018).

The dam breach unleashed a cascade of health and social woes for people living in the area. High levels of heavy metals were found in the blood and urine of riverside populations (Paulelli et al. 2022). There was an increase in diseases arising from habitat degradation, when people are exposed to mosquitoes, such as dengue's transmitters, as well as enteric pathogens in the water along the Doce River (Nishijima and Rocha 2020). Finally, the livelihoods, health, and culture of indigenous peoples, such as the Krenak, Tupiniquim, Guaranis, and Quilombola populations (afro-descendant communities, predominantly composed of the rural and urban black population) were profoundly affected by the disaster (Oliveira et al. 2020; Zhouri and Pascoal 2022).

The ecological devastation mirrored the human tragedy (Fig. 1). Approximately 346 endangered species on the mainland were negatively impacted by the disaster (Knopff et al. 2020), and increased the risk of extinction of 13 aquatic species, becoming a major threat to three of them (Drummond et al. 2021). Furthermore, there were unexpected impacts such as the bioaccumulation of pesticides in endangered Franciscana dolphin [*Pontoporia blainvillei* (Gervais & d'Orbigny, 1844)], as the mud tsunami stirred up historic deposits of these pollutants from soil and river sediments (Nascimento et al. 2022).



**Figure 1.** Dead fish in Marliéria, Minas Gerais, Brazil, about 200 km downstream from the Fundão tailings dam. Photo credit: Elvira Nascimento.

To make it worse, heavy summer rains re-suspend contaminants in the continent, while winter cold fronts suspend pollutants from the bottom of the sea every year (Fernandes et al. 2022).

Another major concern is invasive alien species. Studies have shown that changes in the substrate and water quality altered the composition of the assemblage of fish, copepods, and rotifers, favoring non-native species from these groups (Programa de Monitoramento da Biodiversidade Aquática 2021). Furthermore, another indirect impact of contamination on lake communities was the invasion of water hyacinth (*Eichhornia* sp.), which increased in lakes and ponds, with a high correlation with increases in the concentration of vanadium, iron, and zinc found in the water (Programa de Monitoramento da Biodiversidade Aquática 2021).

Finally, it is worth highlighting the challenge of restoring ecosystems. Removing all the waste that has spread throughout the basin is practically impossible, but the longer the recovery actions take, the greater the risk that the river will be contaminated again by the mud that is still on the banks, especially during periods of rain. To prevent this from happening, the recovery of the riparian forest must be prioritized. For now, emergency actions are being undertaken to try to prevent the mud from flowing into the river, with the planting of grasses and legume trees that would have the function of keeping the land on the bank firmer. However, many of these species are exotic (personal observation) and this brings future environmental problems, unbalancing the entire ecosystem. Even the use of native species can be problematic because by introducing a



limited number of species into a given region, we can inadvertently reduce the ecological functionality of the environment, making it more homogeneous and less diverse (Pereira et al. 2024; Toma et al. 2024). Therefore, the restoration of vegetation in the Rio Doce basin needs to be carried out with a diverse range of native species so that environmental connectivity and restoration of the important ecosystem services on which we depend are more quickly promoted (Pereira et al., 2024; Ramos et al., 2024; Toma et al. 2024).

Specific efforts to compensate for socio-environmental impacts have been made by Renova since 2016, a foundation created to repair and compensate for the impacts of Fundão. The foundation has been building housing, compensating residents, and trying to help the affected human populations. However, the process has been very slow and controversial (Losekann and Milanez 2023). Conveniently, the company creates its foundation to repair its own damages. The government should be imposing several measures to solve the problems, demanding reports, assessments and results on what should be done. In other words, it is not right that the company chooses its own professionals to assess these damages. This oversight should be impartial and carried out by the government. Local representatives, research institutions, and NGOs should also have a say in what Renova is doing. It is of utmost importance to develop and implement a comprehensive program to provide the necessary assistance to affected families, in addition to implementing a comprehensive restoration and recovery program that would accelerate efforts and go beyond punctual efforts.

All mitigation measures may be wasted if the causes of such catastrophes are not addressed. The Mariana accident was repeated in Brumadinho, claiming 272 lives (Vidal et al. 2024). This brought to the fore the discussion about the need to find alternatives for mining activities in the country, which has 839 tailings dams (Agência Nacional de Mineração 2024). A law was created in Minas Gerais (Law No. 23,291, of 25/02/2019, Sistema Integrado de Informação Ambiental 2019) that prevents the construction, installation, expansion, or raising of dams where there is a community in the so-called self-rescue zone: the portion of the valley downstream of the dam where there is no time to intervene in an emergency situation. However, these laws do not appear to be obeyed and dams may still be built if there is no alternative method.

The best way to replace the problematic tailings dams is dry mining (Davies and Rice 2001). Dry ore beneficiation is ideal because it allows the ore to be processed with very little or no water. In other words, the leftovers from the ore beneficiation are filtered and placed in large piles, replacing the use of traditional dams. Dry mining also makes it possible to generate a substrate that can be used in the production of new products, including sand for asphalt paving and bricks for civil construction. In addition, this substrate can be vegetated and, in this way, contribute to the recovery of nature (Davies and Rice 2001). However, this method of beneficiating the ore is much more expensive and, unfortunately, there are still several active tailings dams in Brazil, many of them upstream and in critical condition. It is important to emphasize that it is not enough to simply deactivate these dams, as the mud remains stored and the dam could break at any time. Heavy rains can generate floods that could rupture the reservoir. Unfortunately, a common practice among mining companies is to hire technicians to assess the condition of their dams and the risk of rupture. This is as incoherent as “putting a fox to guard a henhouse”. It is up to the government



to inspect all of this and do an impartial and thorough assessment. Therefore, in addition to replacing the dams with a more modern and safer tailings processing system, it is essential that these dams be monitored and emptied to prevent overflows.

The mud spill is still affecting lives, health, economy, culture, and biodiversity. Over the years, the impact has increased in intensity, severity, and area, ranking as one of the major environmental disasters worldwide. In association with other threats such as climate change that induce extreme events such as cyclones and heavy rains, which foster pollutant resuspension, Fundão spills are even more worrisome to terrestrial and aquatic species in a wide range of globally important sites for conservation, as well as for human population that depend on these ecosystems.

The path to healing the Rio Doce requires a multi-pronged approach. Effective public policies are essential, encompassing compensation for affected communities, restoration initiatives, and long-term conservation programs. These policies, rooted in current scientific research, should prioritize rebuilding resilient ecosystems and river protections. Collaboration is paramount. Local and indigenous communities hold invaluable knowledge of the Rio Doce and fostering their inclusion provides a sense of ownership over its revival. Partnering with scientific experts ensures evidence-based restoration strategies and tracks their effectiveness. By implementing these solutions and supporting a collaborative conservation strategy, the long journey to heal the Rio Doce ecosystem and the lives it sustains can truly begin.

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## **Additional information**

### **Conflict of interest**

The authors have declared that no competing interests exist.

### **Ethical statement**

No ethical statement was reported.

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## **Author contributions**

Cássio Cardoso Pereira, Geraldo Wilson Fernandes, and Fernando Figueiredo Goulart conceived the ideas; Cássio Cardoso Pereira and Fernando Figueiredo Goulart led the writing of the manuscript. Stephannie Fernandes made edits and also contributed to the main text. All authors contributed critically to the drafts and gave final approval for publication.

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## Data availability

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

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## Research Article

# *Cymbidium kanran* can deceptively attract *Apis cerana* for free pollination by releasing specialized volatile compounds

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## Abstract

*Cymbidium kanran* is classified as a second-level protected plant in China and is also listed in the World Genetic Conservation Plant Registry. Pollen flow is an important factor influencing the genetic structure of plant populations, holding significant relevance in the conservation of endangered plants. In this study, we present a comprehensive exploration of the pollination biology of *Cymbidium kanran*, encompassing investigations into its flowering phenology, breeding system, floral volatile components, and interactions with pollinating insects. The results showed that: 1) *C. kanran* exclusively relies on external pollination mechanisms, as automatic self-pollination or apomixis mechanisms are conspicuously absent. Consequently, the natural fruit set rate is significantly lower compared to artificial pollination, highlighting a pronounced pollination limitation. 2) *Apis cerana* emerges as the primary effective pollinating insect for *C. kanran*, adeptly carrying both pollinia and anther caps during the pollination process. Notably, *C. kanran* does not provide any rewards, such as nectar or edible pollen, to entice the pollinators. 3) Contrary to expectations, our glass cylinder experiment demonstrates that the flower color of *C. kanran* lacks significant attractiveness to pollinators ( $p=0.1341>0.05$ ). However, the scent emitted by the flowers exhibits considerable allure ( $p=0.0004<0.05$ ), despite *C. kanran* boasting one of the most diverse color variations within the *Cymbidium* genus. 4) Based on dynamic fluctuations in floral volatile components during different flowering stages, we hypothesize that hexanal, heptanal, octanal, 2-pentyl furan, 4-methyl-2-pentanone, and 1,4-cyclooctadiene may serve as pivotal volatile compounds responsible for attracting pollinators. This study establishes a robust scientific foundation for the conservation efforts concerning *C. kanran*, thereby facilitating the sustainable management and protection of its wild resources.

**Key words:** *Apis cerana*, breeding system, *Cymbidium kanran*, flowering phenology, pollination mechanism

\* These authors contributed equally to this work.

## Introduction

Human activities have brought about a crisis in current biodiversity, reflected not only in a high rate of species extinction but also in the loss of interactions among species, leading to the emergence of many “zombie species” (Tylianakis et al. 2008; Traveset and Richardson 2014). Typically, researchers focus on species, proposing conservation strategies for rare or endangered species. However, such protection methods are inefficient, costly, and may not be sufficient to maintain the integrity of ecosystems (Laycock et al. 2009). Protecting species based on richness rather than functional importance may allocate substantial resources to specific species or even individual species, yielding low returns in terms of ecosystem integrity and functionality (Gotelli et al. 2012). Due to limited conservation management resources, researchers need to consider the cost-benefit ratio of conservation efforts. However, prioritizing species or habitats for protection is a complex task that requires considering the interspecific dependencies generated by interactions among species.

Ecological interactions among species provide services and functions for populations, communities, and ecosystems, constituting a crucial component of biodiversity (Kremen et al. 2007; Rader et al. 2016). Animal pollination is a common ecological interaction in terrestrial ecosystems, promoting the evolution and diversity of plants and pollinators (Wei et al. 2021). An estimated 85% of flowering plants and most crops directly consumed by humans rely on pollinators for reproduction (Ollerton et al. 2011). However, with habitat changes, climate change, and invasive species, the abundance and diversity of pollinators have sharply declined in recent years, potentially leading to more severe pollen limitation and reduced fruiting rates, affecting plant growth and community structure. Gomez et al. (2014) found that the decrease in pollinator diversity affects the growth of the generalist species *Erysimum mediohispanicum*. Biesmeijer et al. (2006) observed a correlated decline in the abundance of pollinators and their pollinated plants in the UK and the Netherlands, suggesting that the decrease in pollinator diversity may lead to a reduction in seed production and, consequently, impact the growth of plant populations for an extended period. Understanding how interactions between plants and pollinators affect species stability is crucial for predicting the consequences of species extinction (Bascompte 2009).

The Orchidaceae family stands out as one of the most highly evolved angiosperm groups, boasting approximately 800 genera and an astonishing 30,000 species distributed globally (Lu et al. 2019). Among these, *Cymbidium kanran*, a member of the Orchidaceae family, thrives as a perennial, terrestrial herbaceous plant within the moist soils of forested regions, typically at altitudes ranging from 400 to 2,400 meters. Its natural habitat spans across China, Japan, Korea, and neighboring regions. The allure of *C. kanran* is derived from its graceful foliage, diverse flower morphology, distinct fragrance, and substantial economic and ornamental value. However, these very attributes have led to the severe exploitation of its wild resources, exacerbated by the relentless encroachment upon its habitat, further jeopardizing the already precarious status of this orchid.



The floral structures of orchids exhibit remarkable specialization, featuring distinctive elements such as the specialized labellum, pollinia, and the gynandrium, formed through the fusion of stamens and pistils. The evolution of these specialized structures is thought to be the result of intricate interactions between orchids and their pollinators over their evolutionary history (Pramanik et al. 2020). To attract a broader array of pollinators, most orchids, mirroring other angiosperms, provide incentives such as nectar. However, approximately one-third of orchid species employ deceptive strategies, including sexual deception, food deception, and more, to accomplish pollination without offering any rewards to pollinating insects (Molnár et al. 2015).

Research conducted in Japan has shown that *C. kanran* can attract workers of *Apis cerana japonica* for pollination by emitting specific volatile compounds, despite these honeybees not receiving any food rewards during the pollination process (Tsuji and Kato 2010). Nevertheless, comprehensive data on the flowering phenology, reproductive biology, flower visitation behavior of pollinators, and the attractiveness of floral colors to pollinators—crucial elements in *C. kanran*'s pollination ecology—are still lacking. Additionally, the pollination strategy of the same plant species may adapt differently under diverse ecological conditions. Given that *A. cerana japonica* is less prevalent in China, it raises the intriguing question of how the pollination strategy of Chinese *C. kanran* may adjust. These intriguing inquiries have kindled our research interest. Consequently, this study delves into the flowering phenology, reproductive system, pollinators, and volatile compounds of *C. kanran*. Furthermore, it employs the Glass cylinder experiment to unravel the mechanisms behind pollinator attraction. Ultimately, this research aims to provide a scientific foundation for the conservation efforts directed towards Chinese *C. kanran*.

## Materials and methods

### Plant materials

The plant materials employed in this study included the wild population of *C. kanran* situated at Wuzhi Peak, Ganzhou City, Jiangxi Province (25°42'N, 114°40'E, elevation 632–648 m) for observations related to plant flowering phenology, breeding system experiments, insect pollination behavior, and detection of volatile components in flowers. Additionally, artificially cultivated *C. kanran* of five distinct varieties were selected for the glass cylinder experiment, namely “Lvobao” (green flowers with a pale green lip and purple spots), “Ziban” (purple-yellow flowers with a pale yellow lip), “Hongyu” (reddish-purple flowers with a pale green lip and purple-red spots), “Ehuang” (pale yellow-green flowers with a pale yellow lip and purple-red spots), and “Yincui” (silver-white sepals and petals with a pale green lip and purple spots).

### Observation of flowering phenology

From October 2021 to January 2022, we meticulously observed 30 randomly labeled *C. kanran* plants at Wuzhi Peak, adhering to the guidelines established by Dafni (1992) for recording the flowering process. The flowering period of each individual

inflorescence, single flower, and the entire population was meticulously documented. Between December 1<sup>st</sup> and 8<sup>th</sup>, we continuously recorded the number of pollinia transferred in/out and the daily temperature over the course of a week. Fifteen fully bloomed flowers were chosen randomly, and the dimensions of the flower openings were measured using a vernier caliper with a precision of 0.001 mm.

### Breeding system experiment

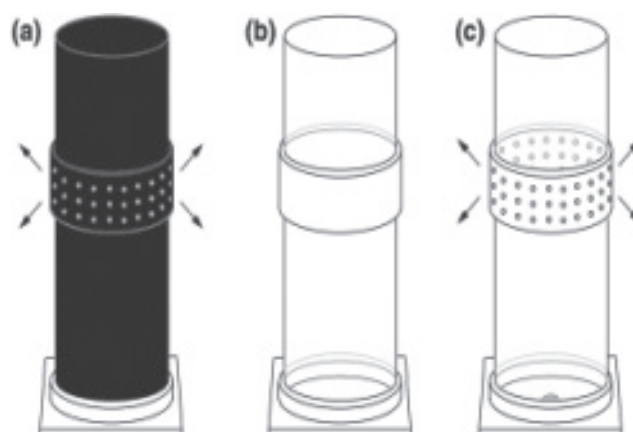
Thirty individuals of *C. kanran* were randomly selected from the wild population at Wuzhi Peak. To prevent the ingress of insects and foreign pollinia, breathable nylon bags were placed over the flower buds prior to flowering. On the first day of flowering, we removed the nylon bags and divided the individuals into six groups, each undergoing distinct treatments: (a) bagged (bag retained until flowers faded); (b) emasculation + bagged (removal of pollinia followed by bagging); (c) artificial self-pollination; (d) artificial geitonogamy; (e) artificial xenogamy; (f) Control (no bagging). Subsequently, we calculated the fruit set rate post-flowering and employed SPSS software for the analysis of differences among pollination methods.

### Observation of pollinating insects and their behavior

During the peak flowering period, spanning from 8:00 to 17:00 daily, continuous recording of *C. kanran*'s pollinating insects and their behaviors was conducted at Wuzhi Peak. A camera (LUMIX, D1000) and a video camera (JVC, GZ-R10SAC) were employed for this purpose. This encompassed documenting their behavior before approaching the flower, the process of flower visitation, their landing and removal of pollinia, the duration of their stay on the flower, and the frequency of flower visitations. Additionally, we meticulously recorded the types and quantities of pollinating insects and preserved them as voucher specimens. From this group, 15 specimens were randomly selected for morphological characterization.

### Glass cylinder experiment

To explore whether plants attract pollinators through olfactory or visual cues, an experiment was conducted employing three types of glass cylinders following the methodology outlined by Milet-Pinheiro et al. (2015): (a) a black cylinder with perforations, emitting odor without any visual cues (O cylinder); (b) a sealed, transparent cylinder, devoid of odor emission but with visual cues (V cylinder); and (c) a transparent cylinder with holes, facilitating both odor emission and visual cues (O/V cylinder) (Fig. 1). To facilitate odor emission from the cylinders with perforations, a coupled membrane pump (G12/01 EB; Rietschle Thomas, Germany) was used to circulate air through the cylinders at a rate of 1 L/min. Inflorescences of *C. kanran* were placed within O, V, and O/V cylinders to investigate the mode of attraction for pollinating insects, namely, whether the plant relied on olfactory or visual cues. Controls consisted of empty O, V, and O/V cylinders. Each type of glass cylinder was replicated three times, resulting in a total of 54 glass cylinders distributed within the wild population of *C. kanran*. Observations of *A. cerana* visitations were carried out between 8:00 AM and 4:00 PM on sunny days. The observations were conducted continuously over a span of three days, and the visitation frequency (average visits per hour) was calculated.



**Figure 1.** Glass cylinder that explores the behavior of insects **A** a black cylinder with holes (O cylinder) **B** a sealed and transparent cylinder (V cylinder) **C** a transparent cylinder with holes (O/V cylinder).

### Detection of volatile components in flowers

The volatile components of *C. kanran* at different developmental stages (including the bud stage, blooming stage, and withering stage) and at various times of the day (8:00, 10:00, 12:00, 14:00, and 16:00) were analyzed using gas chromatography-mass spectrometry (Agilent, 6890 GC). Chemical components with a high degree of matching were selected through computer spectral library retrieval, and the samples were subjected to qualitative analysis following the protocol established by Wang et al. (2020).

### Data analysis

Routine statistical analyses were carried out using IBM SPSS (version 19), while assessments of statistical significance were performed using GraphPad Prism 8.

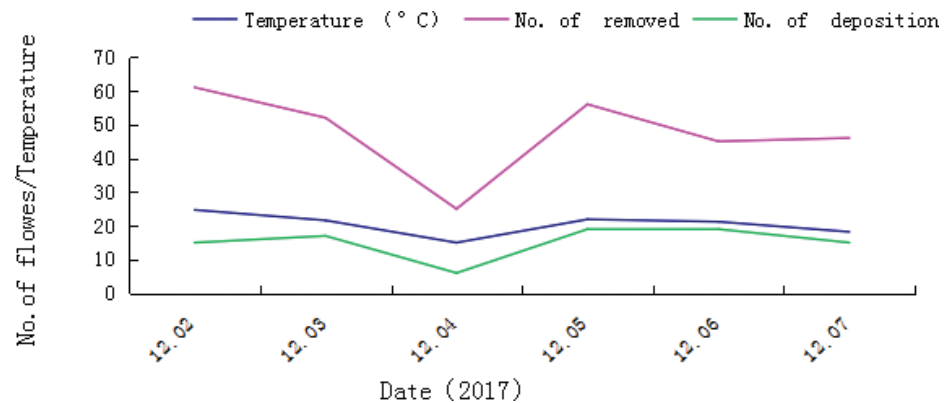
## Results

### Temperature-dependent pollinia transfer efficiency

We observed a positive correlation between temperature and the efficiency of pollinia transfer in *C. kanran*. The flowering period of the *C. kanran* population spans approximately 120 days, with individual flowers remaining in bloom for an average of 33.6 days, and single inflorescences flowering for approximately 49.2 days. Continuous temperature monitoring, coupled with the quantification of pollinia movement over a week, revealed a pronounced correlation. Specifically, *C. kanran* exhibited extensive blooming on sunny days when daily maximum air temperatures exceeded 20 °C. Consequently, there was a significant increase in the quantity of pollinia movement, as illustrated in Fig. 2.

### Insufficient media for the seed formation of *C. kanran*

Our investigation into the breeding system of *C. kanran* revealed a notable limitation in its natural seed formation potential. Both the “Bagged” and “Emasculation+Bagged” groups displayed a fruit set rate of 0, indicating the absence of



**Figure 2.** The relationship between temperature changes and the migration of pollinia.

automatic self-pollination and apomixis mechanisms in *C. kanran*. Therefore, successful seed formation in this species relies entirely on external pollination agents. In contrast, artificial self-pollination, artificial geitonogamy, and artificial xenogamy yielded fruit set rates exceeding 90%, as detailed in Table 1. Conversely, the control group exhibited a fruit set rate of less than 14%, underscoring the presence of substantial pollination limitations within the *C. kanran* population (Table 1).

### Pollinating insects and their behavior

*C. kanran* attracts a diverse array of flower-visiting insects, including *A. cerana* (Apidae), *Chalcididae spp.*, *Syrphidae spp.*, and *Scutelleridae spp.* (Fig. 3A–D). Among these, only *A. cerana* possesses the ability to effectively carry pollinia. *A. cerana* typically visits between 1 to 9 flowers within a population and displays peak activity between 10:00–14:00 on sunny days. Our observations, conducted continuously for 7 consecutive days during the peak flowering period (8:00–17:00 each day), revealed that *A. cerana* visited *C. kanran* flowers a total of 151 times, with 53 of these visits involving the transportation of pollinia. When *A. cerana* carried pollinia, its average duration of stay on the flower was 51.28 seconds ( $\pm 6.29$ ,  $n = 10$ ). In contrast, when it visited a flower without carrying pollinia, its stay was significantly shorter, lasting 15.50 seconds on average ( $\pm 9.42$ ,  $n = 10$ ). The visiting behavior of *A. cerana* was observed to follow a specific sequence: it initially landed on the labellum and then entered the passage connecting the gynandrium and the labellum (Fig. 3E, F). As it approached the base of the gynandrium with its head, it became immobilized but continued to move its abdomen up and down until it exited (Fig. 3G). During the process of exiting, owing to the slightly larger thorax height of *A. cerana* compared to the passage's height, the pollinia on the gynandrium came into contact with the insect's back and adhered to it (Fig. 3H, Table 2). Subsequently, when *A. cerana* visited the next flower, it repeated similar movements, potentially transferring the pollinia into the gynandrium and thereby completing the pollination process.

### Attraction of *A. cerana* by olfactory signals in *C. kanran*

Our findings suggest that *C. kanran* primarily attracts *A. cerana* through olfactory cues. Tukey's post-hoc test enabled us to classify the visitation frequency to the 18 distinct glass cylinders into two distinct levels. The first level displayed

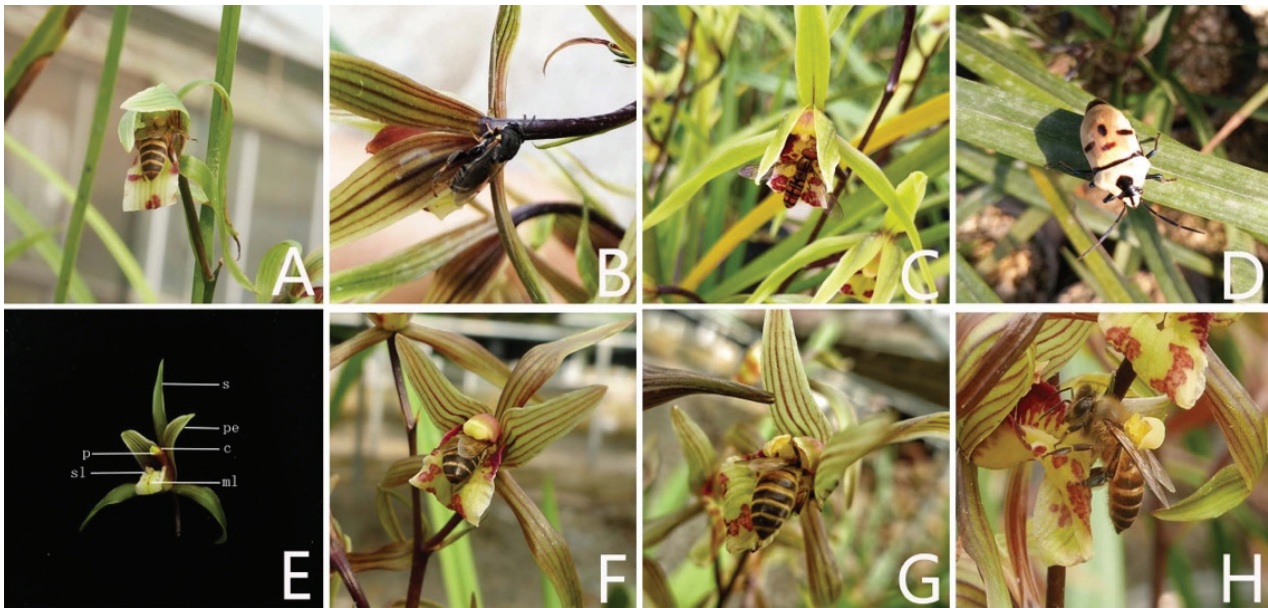
**Table 1.** The fruit set rate in different pollination types.

Treatment	No.of flowers	Fruit set in orchid garden C (%)	Fruit set in orchid garden C (%)
bagged	30	0 <sup>c</sup>	0 <sup>c</sup>
Emasculation + bagged	30	0 <sup>c</sup>	0 <sup>c</sup>
Artificial self-pollination	30	93.33 <sup>a</sup>	96.67 <sup>a</sup>
Artificial geitonogamy	30	96.67 <sup>a</sup>	93.33 <sup>a</sup>
Artificial xenogamy	30	96.67 <sup>a</sup>	100 <sup>a</sup>
Control (unbagged)	30	11.18 <sup>b</sup>	13.33 <sup>b</sup>

Multiple comparisons were conducted by one-way ANOVA followed by Tukey's post-hoc test. Different lowercase letters indicate significant differences between groups ( $P < 0.05$ ).

**Table 2.** Morphological characteristics of *C. kanran* and its pollinators.

	Sample size	Width of the passage/body width (mm)	Height of the passage/height of the thorax (mm)
<i>C. kanran</i>	15	8.982±0.812	3.474±0.176
<i>A. cerana</i>	15	6.010±0.290	3.592±0.108



**Figure 3.** Visiting insects and pollinators of *C. kanran* **A** *A. cerana* visits flower **B** *Chalcididae* spp. visits flower **C** *Syrphidae* spp. visits flower **D** *Liscutelleridae* spp. visits flower **E** the flowers of *C. kanran*. c, column; ml, middle lobe; p, pollinium; pe, petals **F** *A. cerana* enters the passage connecting the gynandrium and the labellum **G** the *A. cerana* exits the passage **H** the back of *A. cerana* is already stuck with pollinium.

low visitation frequency and encompassed O-CK (olfactory-signal glass cylinders without inflorescence), V-CK (visual-signal glass cylinders without inflorescence), O/V-CK (combined-signal glass cylinders without inflorescence), and V-inflorescence (visual-signal glass cylinders with inflorescence of different varieties). In contrast, the second level demonstrated high visitation frequency and included O-inflorescence (olfactory-signal glass cylinders with inflorescence of different varieties) and O/V-inflorescence. Statistical analysis revealed that the visitation



frequency in the first four groups was significantly lower compared to the last two groups. Importantly, no significant differences were detected among the visitation frequencies within the first four groups or the last two groups. Furthermore, our observations indicated no significant disparity in bee attraction between inflorescences of different colors placed within the same glass cylinder (Table 3).

Candidate volatile components for pollinator attraction

The composition of volatile aroma compounds in *C. kanran* exhibited significant variations across different flowering stages. During the bud stage, the predominant volatile components included cyclobutanol, pentanol, 4-methyl-2-pentanone, and hexanal, constituting 20.86%, 14.38%, 5.14%, and 59.62% of the total, respectively. In the peak flowering stage, the primary components were pentanal, hexanal, 2-pentyl furan, l-Alanine, N-(1-oxopentyl)-, methyl ester, 4-methyl-2-pentanone, cyclopropane, 1,1-dimethyl-, and 1,4-cyclooctadiene, accounting for 19.09%, 66.47%, 0.85%, 7.67%, 3.00%, 1.49%, and 1.43%, respectively. During the withering stage, the main components comprised pentanal, hexanal, heptanal, 2-pentyl furan, octanal, 2-methylbutanal, isovaleraldehyde, nonanal, and 3-methyl-2-butenal, constituting 12.96%, 69.35%, 1.76%, 6.32%, 0.77%, 1.90%, 2.83%, 0.19%, and 0.81% of the total, respectively (Fig. 4).

Table 3. Frequency of pollinating insects visiting *C. kanran* based on a glass cylinder experiment.

Treatment	Visiting frequency	Treatment	Visiting frequency	Treatment	Visiting frequency
O-CK	0.00 <sup>b</sup>	V-CK	0.04 <sup>b</sup>	O/V-CK	0.00 <sup>b</sup>
O-"Lvbao"	1.75 <sup>a</sup>	V-"Lvbao"	0.00 <sup>b</sup>	O/V-"Lvbao"	1.97 <sup>a</sup>
O-"Ziban"	1.56 <sup>a</sup>	V-"Ziban"	0.00 <sup>b</sup>	O/V-"Ziban"	1.36 <sup>a</sup>
O-"Hongyu"	1.48 <sup>a</sup>	V-"Hongyu"	0.00 <sup>b</sup>	O/V-"Hongyu"	1.28 <sup>a</sup>
O-"Ehuang"	1.25 <sup>a</sup>	V-"Ehuang"	0.00 <sup>b</sup>	O/V-"Ehuang"	1.50 <sup>a</sup>
O-"Yincui"	1.44 <sup>a</sup>	V-"Yincui"	0.00 <sup>b</sup>	O/V-"Yincui"	1.67 <sup>a</sup>

O: olfactory-signal glass cylinder; V: visual-signal glass cylinder; O/V: combined-signal glass cylinder; CK: no inflorescence placed in the glass cylinder; Green: *C. kanran* "Green Treasure" (green flowers with labellum in light green and purple spots); Purple: *C. kanran* "Purple Spot" (purple and yellow flowers with labellum in light green and purple-red spots); Red: *C. kanran* "Red Jade" (red-purple flowers with labellum in light green and purple-red spots); Yellow: *C. kanran* "Goose Yellow" (pale yellow-green flowers with labellum in light yellow and purple-red spots); Silvery white: *C. kanran* "Silver Jade" (the outer sepals and petals are silver-white, and the labellum is light green with purple spots). Multiple comparisons were conducted by one-way ANOVA followed by Tukey's post-hoc test. Different lowercase letters indicate significant differences between groups ( $P < 0.05$ ).

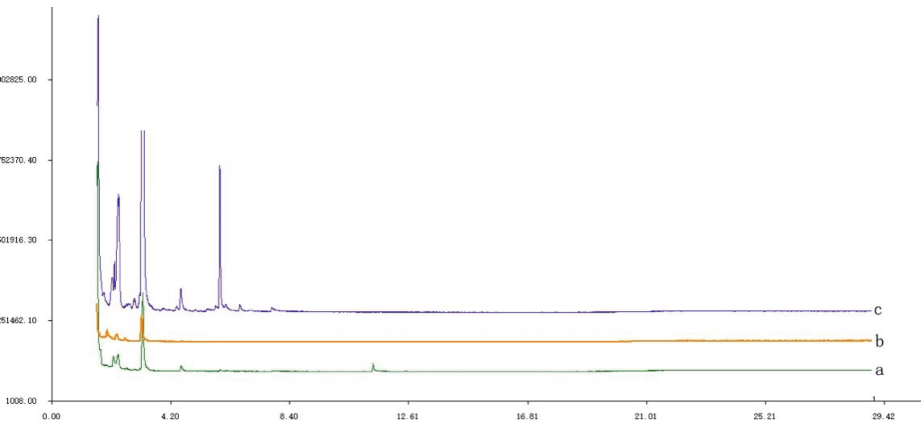


Figure 4. Total ion chromatograms of volatile components from flowers at different stages a bud stage b full bloom stage c withering stage.

Moreover, our analysis of volatile components at different times of the day (8:00, 10:00, 12:00, 14:00, and 16:00) during the full bloom stage revealed distinct patterns. At 8:00, the volatile components primarily comprised cyclobutanols, pentanols, hexanals, heptanals, 2-pentyl furan, and 3-octyne, representing 9.1%, 15.6%, 66.14%, 4.27%, 0.81%, and 4.09% of the total, respectively. At 10:00, the main volatile components were pentanals, hexanals, heptanals, 2-pentyl furan, and ( $\pm$ )-3-hydroxy-r-citronellal, accounting for 17%, 72.25%, 2.69%, 1.43%, and 4.35% of the total, respectively. At 12:00, the primary volatile components included pentanals, hexanals, 2-pentyl furan, l-Alanine, N-(1-oxopentyl)-, methyl ester, 4-methyl-2-pentanone, cyclopropane, 1,1-dimethyl-, and 1,4-cyclooctadiene, constituting 19.09%, 66.47%, 0.85%, 7.67%, 3%, 1.49%, and 1.43% of the total, respectively. At 14:00, the volatile components primarily consisted of pentanals, hexanals, heptanals, 2-pentyl furan, 4-methyl-2-pentanone, 1,4-cyclooctadiene, hexamethylcyclotrisiloxane, and octanals, making up 18.96%, 62.22%, 4.6%, 1.19%, 1.52%, 7.29%, 2.37%, and 1.84% of the total, respectively. Finally, at 16:00, the dominant volatile components were pentanals, hexanals, heptanals, and 2-pentyl furan, accounting for 17.19%, 73.74%, 4.87%, and 1.45% of the total, respectively (Fig. 5).

The combined analysis of Figs 5, 6 highlights several notable trends. Pentanals and hexanals consistently displayed relatively high contents throughout the entire flowering period. In contrast, heptanals and 2-pentyl furan were detected predominantly during the peak flowering period and were absent during the budding stage. Notably, 4-methyl-2-pentanone and 1,4-cyclooctadiene exhibited their highest abundance during the period when flower visitations by insects were most frequent (between 12:00 and 14:00), as depicted in Fig. 6.

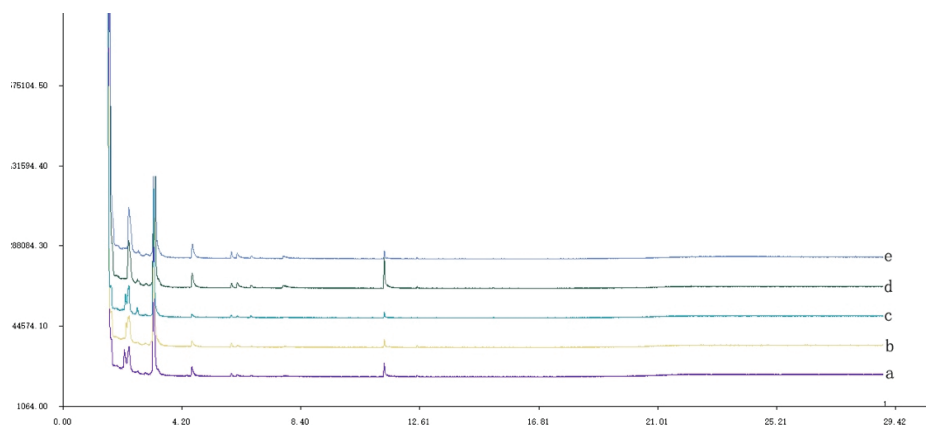
## Discussion

### Deceptive pollination strategy in *C. kanran*

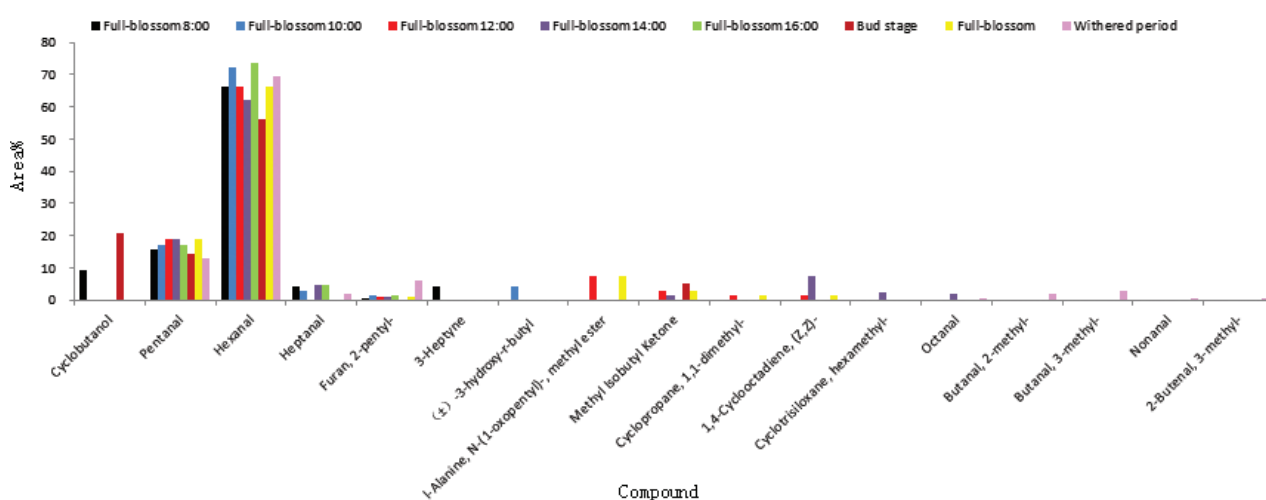
Our study reveals that *C. kanran* employs a deceptive pollination strategy to attract *A. cerana* for pollination. The comprehensive characteristics of the flower, the pollination mechanism, and the behavior of the pollinators are intricately linked to the fitness and reproductive success of the plant (Zhang et al. 2021).

*C. kanran* exhibits a unique set of traits that contribute to its deceptive pollination strategy. It blooms from October to January of the following year, producing numerous large, strongly scented flowers with an extended flowering period. Remarkably, each individual flower remains in bloom for up to 33 days. This prolonged flowering duration serves a crucial function by mitigating the adverse effects of low temperatures and potentially limited insect populations, thereby increasing the likelihood of successful pollination.

While mutualistic relationships between plants and pollinating insects are common in nature, characterized by the provision of various rewards such as nectar, oils, or lipids to attract pollinators (Burger et al. 2010; Kaiser-Bunbury et al. 2017; Wong et al. 2017), *C. kanran* stands apart. Unlike many other orchids and flowering plants, we did not observe any form of food reward for pollinators within the *C. kanran* flower. Furthermore, the flower's morphology and the behavior of the pollinators do not suggest the provision of functions such as breeding grounds or shelters.



**Figure 5.** Total ion chromatogram of volatile components in flower at full-blossom **a** 8:00 **b** 10:00 **c** 12:00 **d** 14:00 **e** 16:00.



**Figure 6.** Variation of volatile components in different time periods.

In light of these observations, we conclude that *C. kanran* primarily relies on deceptive means to entice *A. cerana* for pollination, representing a fascinating example of an orchid species that has evolved unique strategies to ensure its reproductive success. Further research is warranted to elucidate the specific mechanisms underlying this deceptive pollination strategy in *C. kanran*.

### Impact of deceptive pollination on *C. kanran*'s fruit set rate

The presence of deceptive pollination in *C. kanran* has a substantial impact on its fruit set rate. Our artificial pollination experiments provided valuable insights into the reproductive mechanisms of this orchid species. Specifically, the fruit set rate of bagged flowers that were not subjected to stamen removal was recorded at 0%, indicating the absence of apomixis in *C. kanran*. Fully bagged flowers also exhibited a fruit set rate of 0%, effectively ruling out automatic self-pollination. These findings underscore the critical reliance of *C. kanran* on pollinators for seed formation.

In natural conditions, non-rewarding orchids typically exhibit an average fruit set rate of approximately 27.7% (Jersakova et al. 2006). Intriguingly, the natural fruit set rate of *C. kanran* appears to be even lower. However, when subjected to artificial pollination, *C. kanran* demonstrated a remarkably high fruit set rate,

approaching 100%. This stark contrast between natural and artificial pollination outcomes strongly suggests that the reproductive success of *C. kanran* is significantly constrained by the availability and effectiveness of pollinators (Table 1).

It is well-documented that the fruit set rate of non-rewarding plants tends to be lower than that of rewarding plants. This phenomenon can be attributed, in part, to the learning abilities of pollinating insects, particularly in social species like *A. cerana*. Insects receiving deceptive signals from a plant, landing on floral organs devoid of rewards, are less likely to revisit similar flowers, reducing the frequency of visits to deceptive flowers. Furthermore, the success of pollination hinges on whether pollen-carrying pollinators can be deceived twice and return to the plant's flowers. Our observations indicate that after visiting non-rewarding flowers, most insects tend to avoid the population, diminishing the likelihood of a second deceptive encounter. Consequently, in many deceptive plants, the number of pollen outflows does not match the number of inflows (Johnson et al. 2003). As exemplified in this study, the number of pollinia outflows significantly exceeded the number of inflows (Fig. 2), highlighting the intricate dynamics of deceptive pollination in *C. kanran*.

### Enhanced opportunities for outcrossing via deceptive pollination

In the context of deceptive pollination, it is worth noting that this strategy may have a positive influence on promoting outcrossing in plant populations, as exemplified in species like *C. kanran*. These plants often exhibit rhizomatous growth patterns and tend to form patches in their natural habitats. Within a given population, these patches typically consist of several clones of rhizomes.

In general, for clone-forming plants, as the size of the clone base expands, individual flowers become increasingly surrounded by other flowers originating from the same clone. This spatial proximity can facilitate the transfer of pollinia within the clone while impeding the dispersal of pollinia between different clones. This scenario inherently elevates the risk of self-pollination (Handel 1985). However, our observations of *C. kanran* introduce an intriguing twist to this dynamic. Since pollinators visiting *C. kanran* do not receive any rewards, they tend to depart from the population after attempting to visit only 1–5 flowers. This behavior significantly limits the extent of self-pollination within the clone, effectively reducing the proportion of self-pollination and concurrently increasing the likelihood of outcrossing events.

In this way, the deceptive pollination strategy employed by *C. kanran* may serve as a mechanism to counteract the potential negative consequences of spatial clustering in clone-based populations, ultimately enhancing genetic diversity and contributing to the plant's evolutionary success. Further research is warranted to explore the genetic consequences of this deceptive pollination strategy and its implications for the long-term viability of *C. kanran* populations.

### Specialized pollination systems enhance reproduction in scattered plant populations

The reproductive success of plants with dispersed distribution can be significantly bolstered by the presence of specialized pollination systems. These systems are intricately linked to floral characteristics, pollinator behavior,

pollination mechanisms, and overall plant fitness (Huang and Guo 2000). Through field observations, we have established that *C. kanran*'s primary pollinator is *A. cerana*, with pollinia situated on the back of these bees. The dimensions of the entrance channel formed by the petals, labellum, and gynandrium of *C. kanran* precisely align with the average thorax height and body width of *A. cerana*, ensuring that only these bees can come into contact with the pollinia for effective pollination. This exclusive relationship indicates that *C. kanran* employs a classic specialized pollination system with a distinct pollinator.

Remarkably, *C. kanran* is not an exception among *Cymbidium* species when it comes to specialization. With the exception of *C. madidum* and *C. suave* (Davies et al. 2006), all other *Cymbidium* species, including *C. goeringii* and *C. floribundum*, also exhibit a preference for a single pollinator species (Yu et al. 2008). In the natural environment, *C. kanran* populations are dispersed over a wide area, presenting challenges for effective gene flow. Nevertheless, the specialized pollination system adapted by *C. kanran*, closely aligned with the behavior of *A. cerana*, enhances its reproductive success.

This strategic adaptation effectively overcomes the limitations imposed by scattered habitats. The synergy between the unique flower traits of *C. kanran* and the foraging habits of *A. cerana* facilitates efficient pollen transfer across considerable distances, mitigating the genetic isolation that could result from the species' fragmented habitat. Ultimately, this adaptation contributes to the maintenance of genetic diversity, enhances adaptive potential, and secures reproductive success. In essence, *C. kanran*'s specialized pollination strategy serves as a mechanism for overcoming the spatial challenges posed by its ecosystem, ensuring effective reproduction and the preservation of its genetic diversity pool (Jacome-Flores et al. 2019).

### **Some aldehyde compounds may be effective volatile compounds for attracting pollinators**

Plants employ various deceptive strategies to attract pollinators without providing any tangible rewards. These strategies encompass generalized food deception, Batesian mimicry, mimicry of oviposition sites, sexual deception, and even the release of insect pheromones. In the first two strategies, plants typically rely on visual signals to lure pollinators. However, our Glass Cylinder experiment unequivocally demonstrated that *C. kanran* primarily employs olfactory signals rather than visual cues to entice pollinators, effectively excluding generalized food deception as a viable strategy (Handel 1985; Johnson et al. 2003; Ravigne et al. 2006).

Among the volatile compounds detected in our study, pentanal and hexanal consistently exhibited relatively high levels throughout the flowering period. Additionally, heptanal and 2-pentylfuran were absent during the bud stage but became present during the full bloom stage, with their levels displaying no discernible regular patterns. Furthermore, 4-methyl-2-pentanone and 1,4-cyclooctadiene emitted volatile odors during the period when insect visitation was most frequent, particularly from 12:00 to 14:00. Hence, our speculation centers on pentanal, hexanal, heptanal, 2-pentylfuran, 4-methyl-2-pentanone, and 1,4-cyclooctadiene as potential effective volatile compounds for attracting pollinators.



It is worth noting that hexanal has previously been demonstrated to play a pivotal role in attracting *A. cerana* to *Jatropha curcas* (Luo et al. 2013). Additionally, heptanal has displayed strong attraction to *A. cerana* and is frequently employed in proboscis extension reflex tests to assess the learning ability of these bees (Luo et al. 2022). These findings suggest that these aldehyde compounds may indeed serve as effective volatile compounds for alluring pollinators to *C. kanran* blossoms.

### **The role of flower colors in *C. kanran*'s pollination strategy**

*C. kanran* exhibits a remarkable diversity of flower colors within the *Cymbidium* genus. However, behavioral experiments involving *A. cerana* have revealed that these flower colors do not hold significant attraction for these Chinese honeybees; instead, the primary allure lies in the plant's scent. This observation challenges the conventional belief that flower colors predominantly evolve to captivate pollinators, prompting inquiries into the evolutionary significance of *C. kanran*'s diverse flower palette.

Firstly, while behavioral experiments suggest that scent is paramount in attracting *A. cerana*, it is conceivable that the varied flower colors of *C. kanran* may still entice other pollinators. The species inhabits diverse ecological niches, each harboring its own spectrum of insect species. Consequently, the profusion of flower colors in *C. kanran* might cater to different pollinator preferences across distinct habitats, thereby increasing its chances of successful pollination (Kremen and Ricketts 2000; Olesen and Jordano 2002).

Secondly, the abundance of flower colors in *C. kanran* could be a testament to its evolutionary history. Throughout the evolutionary timeline of plants, flower colors might have served diverse functions in attracting pollinators, potentially at different times or under varying environmental conditions. Although current experimental evidence indicates that flower colors may not presently captivate pollinators, it does not necessarily negate their historical or future significance. The myriad flower colors of *C. kanran* may represent the outcome of genetic inheritance and intricate evolutionary processes in plants, with the possibility of these colors assuming distinct roles under varying environmental circumstances (Liu et al. 2014; Rudall 2020).

These insights into the interplay between flower colors and pollination strategies expand our understanding of the multifaceted mechanisms plants employ to ensure their reproductive success and adapt to dynamic ecological contexts.

### **Implications for conservation of *C. kanran***

Presently, all wild orchid species fall within the protective ambit of the Convention on International Trade in Endangered Species of Wild Fauna and Flora, constituting a majority of the plant species safeguarded by this international convention.

Studying the pollination characteristics of plants not only reveals their survival strategies and ecological adaptability but also provides valuable insights for the conservation of rare and endangered species. Orchids, in particular, have evolved mutualistic relationships with their pollinating insects, with some forming specialized one-to-one pollination associations. The vulnerability of

these pollinating insects directly affects the outcomes and reproductive capabilities of the corresponding orchids, thereby influencing their overall survival (Aizen 2021).

This study found that *A. cerana* is the sole pollinator of *C. kanran*, and a decrease in its population may impact the reproductive capacity of *C. kanran*. The study also revealed that the natural fruit set rate is much lower than that achieved through artificial pollination, primarily due to the insufficient presence of *Chuanpollia* bees. Increasing the population of pollinators is advantageous for enhancing the fruit set rate. Firstly, the protection of wild *A. cerana* populations is crucial, and capturing wild bee colonies should be prohibited, especially given the current popularity of wild bee honey in the Chinese market, driving locals to capture wild bee colonies for profit. Secondly, planned releases of *A. cerana* should be conducted, allowing the new bee colonies to thrive in the natural environment without human interference. Thirdly, in areas where *C. kanran* is distributed, select a location with convenient flight paths for bees, close to water sources, away from agricultural orchards, with a mix of shade and sunlight. Construct simple beehives to attract bee colonies to settle in these areas. Fourthly, during the flowering period of *C. kanran*, encourage beekeepers to relocate their bee colonies to the areas where *C. kanran* is distributed (Theisen-Jones and Bienefeld 2016; Liu et al. 2022). Fifthly, plant some nectar-producing plants that bloom simultaneously with *C. kanran* to increase the frequency of *C. kanran* visits (Dellinger 2020). Additionally, the study found that *C. kanran* communities are dispersed, with some populations located far beyond the flight range of *A. cerana*. For populations distant by 4–6 km, consider artificially planting *C. kanran* in intermediate locations to connect the dispersed *C. kanran* communities, thereby increasing the probability of pollinators carrying pollen to the stigma (Finger et al. 2014; Dellinger 2020).

Furthermore, as *C. kanran* is extensively cultivated for its ornamental value, this research underscores its potential applications in artificial cultivation. Intervarietal hybridization typically involves manual pollination. Building upon the insights gleaned from this study, the introduction of *A. cerana* to *C. kanran* plantations or the cultivation of honey plants to draw in *A. cerana* for pollination could markedly elevate the fruit set rate. Moreover, the pollination mechanism orchestrated by *A. cerana* in *C. kanran* conduces to a heightened rate of cross-breeding, facilitating an increase in intervarietal hybridization. Consequently, this augments the prospect of breeding variants displaying a broader array of phenotypes. Such an approach also has the potential to mitigate illicit harvesting of *C. kanran* by unscrupulous flower farmers.

## Conclusion

In sum, this study reveals that in its natural state, *C. kanran* must rely on pollinators for successful fruit setting, with *A. cerana* being the sole pollinator; however, the insufficient population of *A. cerana* leads to a lower fruit-setting rate in *C. kanran*. *C. kanran* does not offer rewards to pollinators but attracts them for pollination by releasing volatile compounds such as hexanal, heptanal, octanal, 2-pentyl furan, 4-methyl-2-pentanone. These research findings provide scientific guidance for the conservation of *C. kanran*, and corresponding conservation strategies have been proposed.

## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

Luo and Xiao performed most of the experiments and data analysis. Liu, Chen, and Xiong participated in the experiments. Wu and Huang participated date analysis. Yang designed the experiments. Luo, Xiao and Wu prepared the manuscript. All authors read and approved the final version of the manuscript.

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### Data availability

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

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## Short Communication

# Establishing permanent monitoring plots of narrow endemic and threatened plants of Indonesia: a case study on *Dipterocarpus cinereus* Slooten (Dipterocarpaceae)

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## Abstract

Permanent plots are commonly employed to record and monitor temporal changes in plant communities. In this study, we established a permanent plot to monitor the future population dynamics and regeneration of *Dipterocarpus cinereus* Slooten (Dipterocarpaceae), or Lagan bras, a critically endangered and narrowly endemic tree confined to the Mursala Island in Sumatera Utara Province, Indonesia. Using a quarter-hectare (50 × 50 m<sup>2</sup>) plot, we collected initial data on the population structure of *D. cinereus* and the floristic composition of the tree community within the plot. Tree inventories were conducted by measuring all individual trees with a Diameter at Breast Height (DBH) of more than 5 cm. In the 0.25-hectare plot established, we recorded 261 trees, representing 76 species, 49 genera, and 32 families of flowering plants. There were seven individuals of *D. cinereus* in the plot, of which six were in mature stages with a DBH range of 79.5–38.8 cm. The results of our study can serve as baseline data for future monitoring programs of *D. cinereus* and the accompanying tree community. Furthermore, these results can support the implementation of existing long-term conservation strategies and action plans for the species.

**Key words:** Extinct species, IUCN Red List, *Keruing*, Lagan bras, Mursala Island, Sumatera

## Introduction

Permanent plots, defined as sampling plans where each tree is permanently marked, are commonly employed to monitor vegetation change (Poso 2006; Priyadi et al. 2006). The long-term data produced by regularly sampling permanent plots is the most precise measure to record and detect temporal changes in plant communities because the plots' fixed geographical location of the plots can prevent relocation bias, which is inherent in plant resurveys (Verheyen et

al. 2018; Damgaard 2019; de Bello et al. 2020). Due to this reason, permanent plots have been listed as one of the six most essential developments in vegetation sciences over the last three decades (Chytrý et al. 2019). Data collected from permanent plots offers many benefits, including understanding vegetation dynamics, assessing the stability of a plant community, tracking the successional trajectories, monitoring species' interactions over time, and providing detailed data on the population dynamic of high-conservation-importance species (Earle-Mundil 2010; Sperandii et al. 2022).

Plant species with restricted range are more sensitive to environmental changes and threats due to their restricted distribution and often low numbers of individuals, populations, or both (Işık 2011). Therefore, they represent an important group for conservation (Fenu et al. 2015; Huang et al. 2016; Orsenigo et al. 2018). To be effective, conservation actions require reliable information on the population dynamic and demographic status of the target species. Demographic studies will provide information on the critical phases of plant life history (Caswell 2000; de Kroon et al. 2000; Menges et al. 2016) and factors that drive fluctuations and population trends (Menges 2000; Sulis et al. 2018). These are important to determine best management practices and establish effective conservation programs (Schemske et al. 1994; Menges et al. 2016; Sulis et al. 2018). The use of permanent plots to study the long-term demographic status of narrow endemic and threatened plant species is still low, yet required for effective conservation (Menges 2000; Fréville et al. 2004; Jacquemyn et al. 2007).

Indonesia is a habitat for 1,070 threatened plant species, of which 600 (56%) are endemic (IUCN 2023). Currently, none of these endemic and threatened plant species have population dynamic and demographic information derived from long-term studies (e.g. Priyadi et al. 2006; Brearley et al. 2019). This condition will prevent the authority from determining the best management practices and establishing effective conservation programs.

*Dipterocarpus cinereus* Slooten, also known as Lagan bras by the locals, is a tree species of high-conservation-importance from the family Dipterocarpaceae. The species is endemic to Mursala Island in Sumatera Utara Province, Sumatera, Indonesia (Robiansyah et al. 2020a). In 1998, *D. cinereus* was listed as extinct on the IUCN Red List (Ashton 1998) before being rediscovered in 2013 (Kusuma et al. 2013). The species is currently threatened by habitat loss due to massive illegal logging and forest conversion and is assessed as Critically Endangered by the IUCN Red List (Robiansyah et al. 2020b). Despite its critical status recognised by the IUCN Red List, *D. cinereus* is not a protected plant species at the national level. However, the tree is included as one of Indonesia's 12 priority plant species for conservation (Hamidi et al. 2019). While ex-situ conservation of *D. cinereus* has been conducted (Susilowati et al. 2021), in-situ research and conservation actions that can support the species' survival are still lacking. In addition, information on population dynamics and demography of the species is absent (Hamidi et al. 2019).

In the present study, we established a permanent plot to monitor the future population dynamic, regeneration, and demography of *D. cinereus*. Initial data on the population structure of *D. cinereus* and the floristic tree community composition within the plot were presented. The results of our study can be used as baseline data for future monitoring programs of the species and accompanying tree community. The results can also support the implementation of the existing long-term

conservation strategies and action plans for *D. cinereus* that have been launched since 2019 (Hamidi et al. 2019). Furthermore, the study can become a conservation model for other Indonesian narrow endemic and threatened plant species.

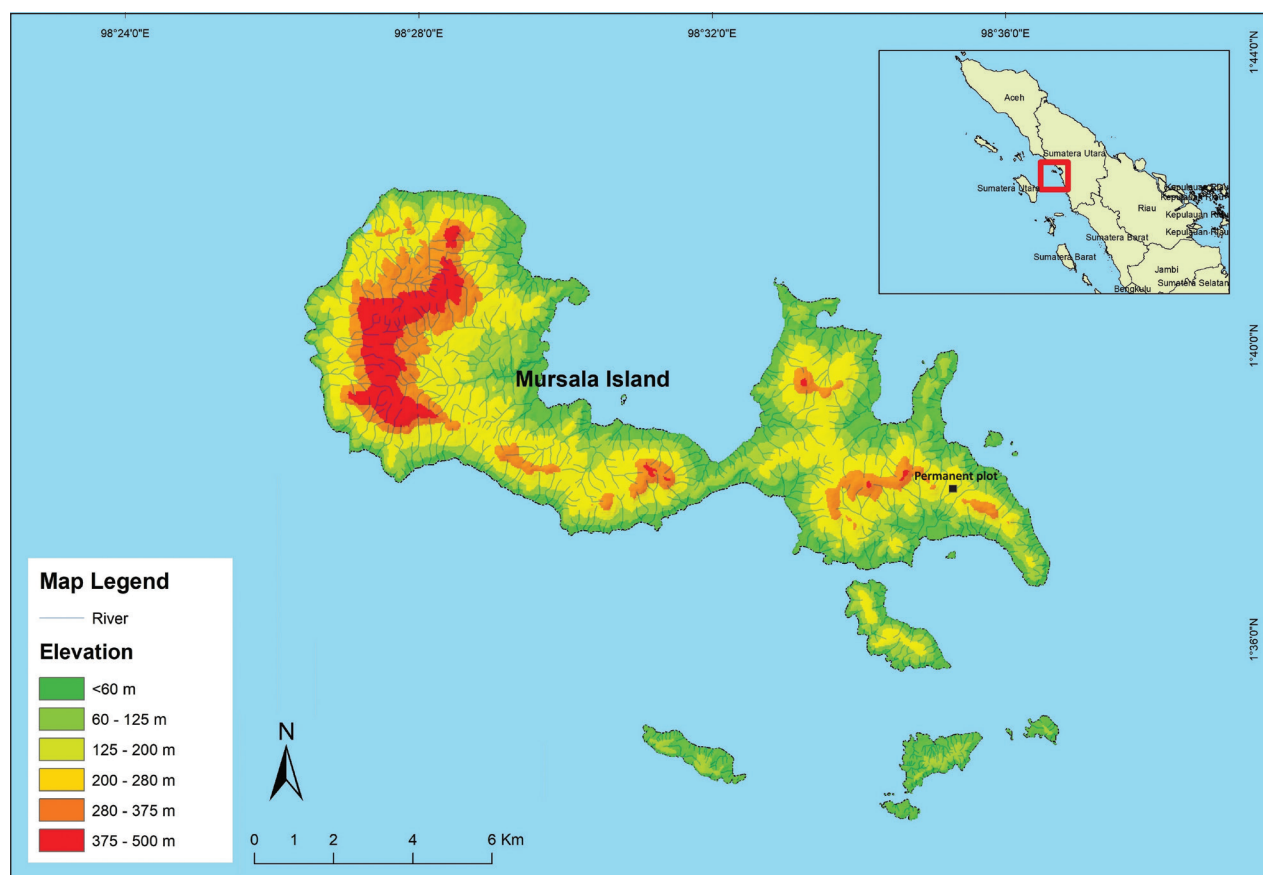
## Materials and methods

### Study site

The permanent monitoring plot for *D. cinereus* was established on Mursala, a small island located on the west coast of the Sumatera Utara Province (Fig. 1). The island has an area of about 8,000 ha with hilly topography with the highest elevation of 500 m above sea level. The island's average air temperature and humidity are 26.7 °C and 75% (Rachmat et al. 2018), whereas the average precipitation is 12.96 mm/year (BPS Kabupaten Tapanuli Tengah 2017). Mursala Island is a habitat for at least 26 dipterocarp species, of which 76.9% are threatened with extinction (Robiansyah et al. 2020a). Therefore, Mursala Island is considered one of the dipterocarp biodiversity hotspots in Indonesia.

### Tree inventories

In the present study, a quarter-hectare (50 × 50 m<sup>2</sup>) permanent plot was established in the year 2022. The plot was located on a Northeast-facing sloped area (c. 17°) with an elevation of 250 m above sea level. It was intentionally



**Figure 1.** Location of the permanent monitoring plot for *Dipterocarpus cinereus* on Mursala Island.

established at a location with a high density of mature individuals and the wild regeneration of *D. cinereus*. One of the corners of the plot was georeferenced using a high-precision GPS receiver (Garmin GPSMAP 65s). To ensure the tree measurement process and plot orientation easier, we divided the plot into workable units of 25 subplots ( $10 \times 10 \text{ m}^2$ ).

All trees with a Diameter at Breast Height (DBH) of more than 5 cm in the plot were measured at 1.3 m above ground. Each tree was given a unique identification number, and its position in the grid (x- and y-coordinates) was recorded. Herbarium vouchers were made for all measured trees for species identification purposes. Identification of tree species was based on relevant botanical and taxonomic literature pertinent to the Malesian flora and comparisons with digital herbarium images stored in the Global Biodiversity Information Facility (GBIF.org 2023) and Naturalis Biodiversity Center (<https://bioportal.naturalis.nl/>). Family and species names used in the study follow the Plants of the World Online (<https://powo.science.kew.org/>). We also checked the wood density of each species using the Tree Functional Attributes and Ecological Database developed by the World Agroforestry Center (<http://db.worldagroforestry.org/wd>). The wood density was then classified into the following groups (Melo et al. 1990): light ( $<0.5 \text{ g/cm}^3$ ), medium ( $0.5\text{--}0.72 \text{ g/cm}^3$ ), and heavy ( $>0.72 \text{ g/cm}^3$ ).

## Data analysis

We constructed a species-area curve based on the cumulative value of each subplot. The diversity level of the plot was calculated using Shannon diversity and Simpson's evenness index (Magurran 2021). Both indexes were also calculated for trees with  $\text{DBH} \geq 10 \text{ cm}$  to make this study comparable to other permanent plot data in mainland Sumatera, which mainly used trees with at least 10 cm DBH. To identify important taxa in the plot, we calculated the Importance value index (IVI) for all families and species based on the sum of their relative frequency, density, and dominance (Mueller-Dombois and Ellenberg 1974). The stand structure of the plot was identified visually using DBH classes.

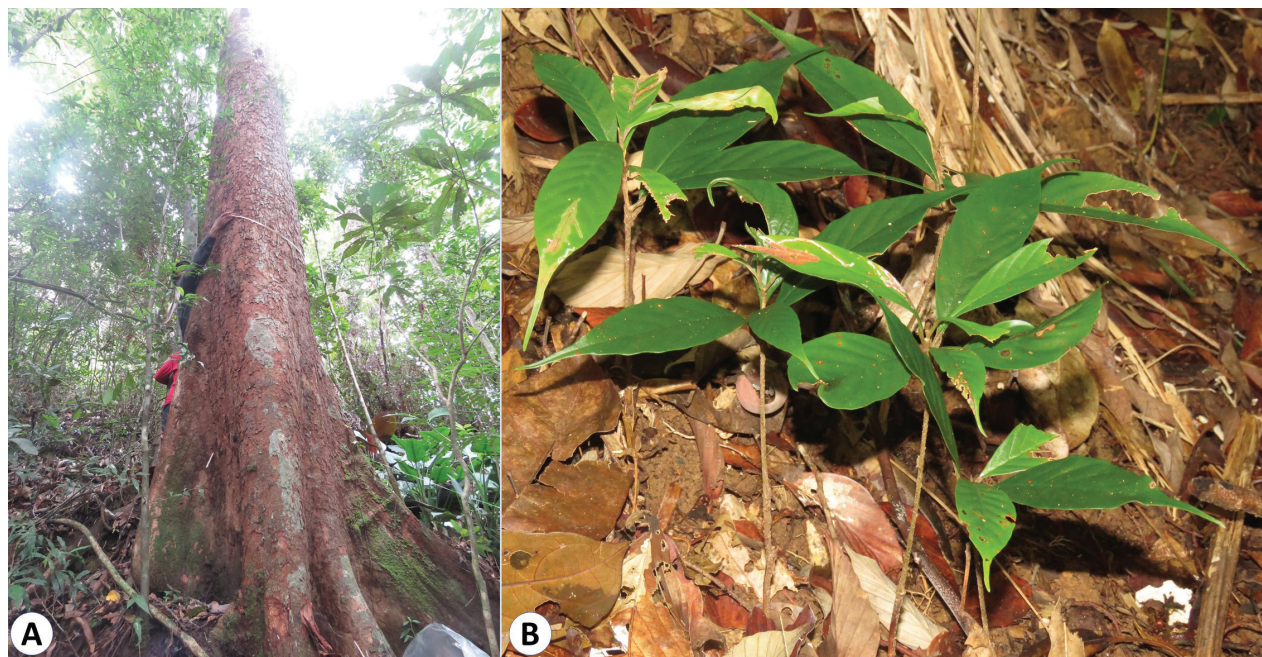
## Results and discussion

### Species diversity

According to previous studies (Robiansyah et al. 2020b, 2020a), 163 individuals of *D. cinereus* were recorded on Mursala Island. In the present study, we enumerated seven individuals of *D. cinereus* in the plot, of which six were mature (79.5–38.8 cm DBH), and one was immature with a trunk DBH of 8.9 cm. We also observed hundreds of seedlings (0.1–0.5 m height) of the species around the mature individuals (Fig. 2). Therefore, our plot represented one of the sites on the island with a high density of *D. cinereus*.

In the 0.25 ha plot, we recorded 261 trees, representing 76 species, 49 genera, and 32 families of flowering plants (Suppl. material 1). Of these 76 species, 63 were identified down to the specific level, nine taxa to the generic level, three taxa to the family level, and one was unidentified. The Shannon diversity index of the plot was 3.87, which indicated that the plot has a high plant species diversity. In general, most ecological data shows the range of the Shannon diversity





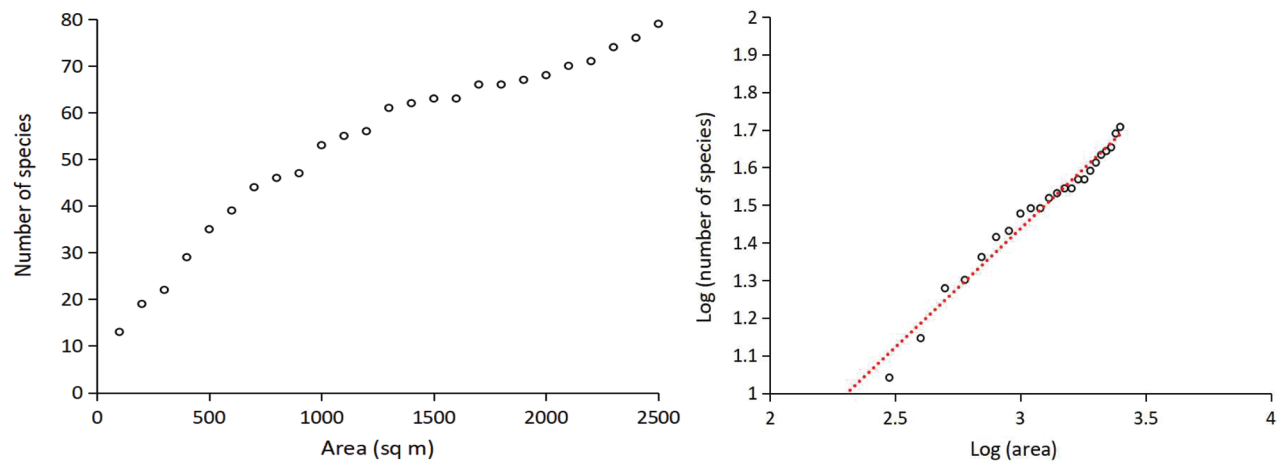
**Figure 2.** A mature individual (A) and seedlings (B) of *Dipterocarpus cinereus* Slooten found in the permanent plot.

index of 1.5–3.5 and only rarely surpasses 4.5 (Gaines et al. 1999). The Simpson's evenness index of the plot is 0.97, which indicates that the species within the plot are present in relatively equal abundance (Beisel et al. 2003).

The species-area curve is constructed to depict species diversity in relation to the increasing size of the area (Mueller-Dombois and Ellenberg 1974), and it can be used to evaluate species diversity patterns in different types of forests (Ashton 1965). Following Scheiner (2003), the species-area curve in the present study could be classified as a type II curve since it was constructed based on quadrats arrayed in a contiguous grid. This type of curve usually forms a smoothly rising pattern. Indeed, the species-area curve in the present study (Fig. 3) showed a steady increment, and at a quarter-hectare, there is no sign of levelling off. It indicated that the forest has a relatively high species diversity, as shown by the species number 51 and Shannon diversity index of 3.87.

Most permanent plots in Sumatera use trees with at least 10 cm DBH. Therefore, to make this study comparable, we presented the subset data using trees with DBH of  $\geq 10$  cm (Table 1). The permanent plot in this study had a lower tree density (516 individuals/ha) than the studies in Gunung Leuser National Park (582 individuals/ha; Basyuni et al. 2019), Bukit Duabelas National Park (540 individuals/ha; Anas et al. 2021), and Batang Gadis National Park (583 individuals/ha; Kartawinata et al. 2004). The density, however, was higher compared to another study in Gunung Leuser National Park (470 individuals/ha; Basyuni et al. 2019) and Bukit Duabelas National Park (414 individuals/ha; Rahmah et al. 2016). According to Kartawinata (1990), the range of tree density (DBH  $\geq 10$  cm) in Malesia's lowland rainforest is 350–800 trees/ha.

In terms of species number, our plot had the lowest species diversity (51 species) compared to other studies in mainland Sumatera. However, this was caused by the small area used in the present study. Using the trendline equation of the log-log species-area curve (Fig. 3), we estimated that the species number in the present study would reach up to 75 and 116 if the plot size is



**Figure 3.** A species-area curve in a 0.25 ha forest plot on Mursala Island, Indonesia. The left curve uses arithmetic axes for trees with a Diameter at Breast Height (DBH)  $\geq 5$  cm (left), whereas the right curve uses log-log axes for DBH  $\geq 10$  cm. The red line is a linear trendline with  $y=0.6313x-0.4571$  ( $R^2=0.99$ ).

**Table 1.** The tree (diameter at breast height  $\geq 10$  cm) structure and composition of the present study and comparison with other studies on mainland Sumatera.

Variable	This study (2022)	Basyuni et al. (2019)		Rahmah et al. (2016)	Anas et al. (2021)	Kartawinata et al. (2004)
Location	Mursala Island	Gunung Leuser National Park A	Gunung Leuser National Park B	Bukit Duabelas National Park A	Bukit Duabelas National Park B	Batang Gadis National Park
Area (ha)	0.25	0.5	0.5	1	1	1
Altitude (m)	250	75–100	75–100	200–300	265–327	650
Tree number	129	291	235	414	540	583
Species number	51	100	88	113	89	184

increased to 0.5 and 1 ha, respectively. Following this extrapolation, the diversity is now higher than that of Bukit Duabelas National Park (Anas et al. 2021; Rahmah et al. 2016) but still lower than Gunung Leuser National Park (Basyuni et al. 2019) and Batang Gadis National Park (Kartawinata et al. 2004). Whitmore (1986) pointed out that the differences in species diversity in lowland forests are strongly related to the variations of topography, terrain, and degree of natural disturbance. In addition, according to the island biogeography theory, the plant diversity on a small island is affected by its size and distance to the main island (Guo 2015).

### Important taxa

As the target species, *D. cinereus* had the highest IVI value (26.74) in the plot (Suppl. material 1). It was closely followed by *Shorea multiflora* (Burck) Symington (22.06), *Teijsmanniodendron holophyllum* (Baker) Kosterm. (15.87), *Shorea ovata* Dyer ex Brandis (13.77), *Pentace sumatrana* Kosterm. (12.75), and *Vatica venulosa* subsp. *simalurensis* (Slooten) P.S.Ashton (12.31). A previous study by Robiansyah et al. (2020a, b) showed that *S. multiflora* was widely distributed on Mursala Island and had an estimated density of  $21.72 \pm 10.96$  individuals/ha. This species is also commonly found in lowland forests of Peninsular Malaysia, Sumatera, and Borneo (Ashton 1982; POWO

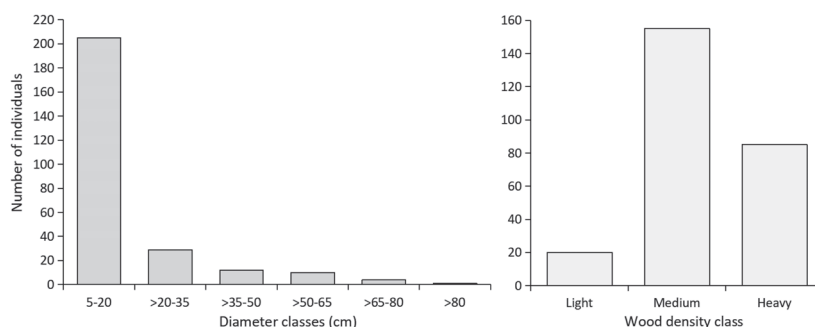
2023) and is assessed as Least Concern according to the IUCN Red List (Barstow 2018).

At the family level, the highest number of species found was in Dipterocarpaceae (seven species), followed by Myristicaceae (six species), Myrtaceae (six species), and Ebenaceae (six species). Furthermore, Dipterocarpaceae had the highest IVI value (94.38), followed by Lamiaceae (19.84), Myrtaceae (17.81), Clusiaceae (17.66), and Anacardiaceae (15.14). There were 16 families (50%) represented by a single species. Of the 26 Dipterocarpaceae species recorded for the island, only seven were recorded from the plot (Robiansyah et al. 2020a). In the lowland forests of the mainland of Sumatera, Dipterocarpaceae is also reported to be one of the most dominant families in terms of the number of species (e.g. Rahmah et al. 2016; Basyuni et al. 2019).

### Stand structure and wood density

The stand structure of a forest can be represented horizontally by the diameter class distribution (Purwaningsih and Yusuf 2005). In the present study, the diameter class distribution curve showed an inverted J-shaped (Fig. 4). This type of curve is commonly found in tropical forests and is characterized by the dominance of trees with DBH less than 20 cm, reflecting the general characteristics of dynamic tropical forests (Richards 1973). A total of 205 trees with DBH  $\leq 20$  cm were located in the present plot, which amounted to more than 78% of the total trees. All trees with DBH  $>65$  cm were from the family Dipterocarpaceae and consisted of three species, i.e. *D. cinereus*, *S. multiflora*, and *S. ovata*. The dominance of Dipterocarpaceae at the upper end of the diameter class is also observed in Bukit Duabelas National Park (Anas et al. 2021) and Batang Gadis National Park (Kartawinata et al. 2004).

Wood density is one of the reliable predictors of the successional stage of a forest (Slik 2005). The domination of trees with medium to heavy wood density indicates that a forest is in a later successional stage. In contrast, a higher proportion of trees with low wood density means they are in an early successional stage (Slik et al. 2008). In our study site, the tree community showed a characteristic of a mature forest where there were more trees with medium (59.6%) and heavy wood density (32.7%) compared to those with light wood density (7.7%) (Fig. 4). To cope with understory conditions, trees in old-growth, mature forests develop conservative strategies: low growth rate and high wood density (Chao et al. 2008; Chave et al. 2009; Poorter et al. 2021).



**Figure 4.** The stand structure of a 0.25 ha forest plot on Mursala Island, Indonesia. The structure is based on the diameter at breast height (dbh) class (left) and wood density classification (Melo et al. 1990; right).

## Comparison with other similar studies

Our study was the first to use permanent plots to examine narrow endemic and threatened plant species in Indonesia. Previous studies utilizing permanent plots in Indonesia primarily focused on monitoring above-ground carbon stock, forest recovery, and tree species richness (Brearley et al. 2019). Studies similar to ours have been conducted in Vietnam (Chien et al. 2008), Brazil (Sant'Anna et al. 2013), the Netherlands (Jacquemyn et al. 2007), and France (Fréville et al. 2004), where permanent plots were used to monitor the population demographics of threatened plant species. However, those studies concentrated on the target species and did not assess the accompanying tree species community. We believe that our method, by including measurements of the accompanying tree community, will provide more comprehensive data and can be used to monitor various factors related to the target species and the forest ecosystem.

## Conclusion

Our permanent monitoring plot represented one of the sites on Mursala Island with a high density of *D. cinereus*. The tree vegetation composition and structure in the plot showed a typical mature lowland forest with a high density of trees from medium to high wood density and an inverted J-shaped diameter class distribution. The results of our study can be used as baseline data for future monitoring programs of *D. cinereus* and the accompanying tree community. The results can also support the implementation of the existing long-term conservation strategies and action plans for the species. These include supporting the designation of Mursala Island as a protected area, listing *D. cinereus* as a protected plant species, and attracting relevant stakeholders to actively engage in the conservation of *D. cinereus* and its habitat. Finally, our study can become a model for conserving other Indonesian narrow endemic and threatened plant species.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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## Author contributions

Conceptualization: AS, EMDR, EP, AA, IR, HHR, AH. Data curation: IS, IR, MNZ, EP, AH. Formal analysis: IS, IR, EP. Funding acquisition: IR. Investigation: IR, IS, EP, AH, MNZ. Methodology: HHR, IR, EP, AH, AS, AA, EMDR. Project administration: MNZ, EMDR, IS, AA, HHR, AS, AH, IR, EP. Supervision: IR. Validation: IR, AH. Visualization: MNZ, IR, EP, IS. Writing - original draft: IR. Writing - review and editing: AH, HHR, EP, AA, EMDR, IS, MNZ, IR, AS.

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## Data availability

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

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## Supplementary material 1

### List of plant species found in the study plot

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Data type: xlsx

Explanation note: Importance value index (IVI) and wood density of tree species in a 0.25 ha plot of forest on Mursala Island, Indonesia. IVI is calculated based on the relative density (RD), frequency (RF) and dominance (RDm) of each family and species.

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# Old trees: Precious natural heritage on isolated islands

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## Abstract

Old trees on the South China Sea islands represent a unique and irreplaceable element of the region's ecological heritage. Their resilience and critical role in maintaining local biodiversity highlight the need for immediate conservation efforts to protect these natural treasures from environmental and human threats.

**Key words:** Biodiversity conservation, environmental adaptation, Old trees, South China Sea islands



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Old trees, revered for their age and resilience, are found in diverse environments ranging from cities and rural areas to dense forests and wilderness (Jim 2018). However, these old guardians of nature are exceedingly rare on isolated islands (Ibanez et al. 2021), particularly those scattered across the South China Sea. The few old trees that have managed to survive on these remote islands are not only rare but also incredibly precious, having endured and adapted to the harsh climates and poor soil conditions unique to these small land masses (Wang et al. 2011). These old trees are living testaments to the remarkable adaptability of species and play an indispensable role in the fragile ecosystems of these islands.

The existence of old trees on these isolated islands vividly demonstrates extreme evolutionary adaptation. The trying environment of the South China Sea, characterised by high winds, salty air and infertile soils (Gong et al. 2013), poses significant challenges to plant life. Nevertheless, against all odds, these trees have survived and flourished, reaching a venerable age that speaks for their hardiness and ecological importance (Lindenmayer 2017). Their continued existence offers invaluable insights into the mechanisms of plant adaptation and resilience (Cannon et al. 2022), providing a living laboratory for studying evolution in extreme conditions.

Old trees on these small islands are critical components of the local ecosystems. They offer a crucial assortment of micro-habitats for various species, particularly those that rely on the unique and often stressful environmental conditions provided by the islands. Seabirds and epiphytes are amongst the many organisms that depend on these trees for shelter, nesting and sustenance (Li et al. 2021). The trees' canopies also support a diverse array of epiphytes with companion organisms, contributing to the islands' overall biodiversity (Díaz et al. 2012). Essentially, these old trees are keystone species (Lindenmayer et al.

2014) and their presence is collaterally or synergistically vital to the survival and reproduction of numerous other species on and around these faraway islands.

Given the critical role of old trees in maintaining the ecological balance of the South China Sea islands, we advocate that steps must be taken promptly to ensure their protection. Unfortunately, the current lack of focused conservation efforts leaves these old trees vulnerable to the threats posed by relentless natural disasters and human perturbations. Without immediate and effective protection measures, we risk losing these irreplaceable natural treasures and the unique ecosystems they support.

We, therefore, call on the relevant Chinese authorities to prioritise the conservation of old trees on the small islands of the South China Sea. This action should include establishing protected areas reinforced by statutory instruments and management measures, whereupon these trees can be safeguarded from artificial interference and environmental degradation (Chen 2011). Enhanced understanding of the ecology and diversity of old trees on the South China Sea islands constitutes a crucial scientific challenge that demands attention. Additionally, efforts should be made to raise public awareness about the eclectic importance of these trees, not only for their ecological value, but also as symbols of resilience and adaptation in the face of adversity. By protecting these old trees, we are preserving biodiversity and safeguarding the ecological heritage of the South China Sea islands for future generations. We suggest that the Chinese government and relevant agencies take immediate steps to ensure the survival of these natural treasures, thereby preserving a vital part of our planet's ecological diversity.

## **Additional information**

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The authors have declared that no competing interests exist.

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No ethical statement was reported.

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### **Author contributions**

XC and CYJ conceived and designed the research; XC performed research; XC Wrote the original draft; CYJ reviewed and edited. All authors had read and agreed to the published version of the manuscript.

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### **Data availability**

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

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# Lost species, neglected taxonomy, and the role of natural history collections and synonymization in the identification of the World's forgotten biodiversity

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## Abstract

A recent paper highlighted the need for increased attention towards species that have vanished from scientific literature and proposed a new nomenclature in view of needed conservation actions, based on the time interval since their disappearance. Here, we propose to implement this concept by showcasing a taxonomic perspective that is often overlooked in conservation studies. In recent decades, research on vertebrates showed that, in several cases, species considered as undescribed were, in fact, first discovered and already named a long time ago. Quite often they fell into oblivion while being considered synonyms of other taxa until they were re-evaluated in subsequent taxonomic revisions. In this context, we are proposing the concept of “long-lost synonymized” species, asking for greater attention to the discipline of taxonomy, the relevance of specimen-based taxonomy and the value of natural history collections within conservation and academic communities.

**Key words:** Conservation, long-lost synonymized species, natural history museums, taxonomy, vertebrates

In a recent paper, Long and Rodriguez (2023) highlighted the cases of species that have dropped off science's radar and proposed a new nomenclature to guide conservation actions. They stated that species absent for at least 50 years should be termed “long-lost” species, those missing for more than 10 years as “lost species”, and those not found for 5–10 years as “missing species”. However, the authors omitted to address taxonomy in their contribution, a fundamental scientific discipline that itself can also be considered missing, threatened or lost. It is a well-known fact that the ranks of taxonomists are shrinking all around the world, and natural history museums (NHMs), where many of them usually work, are suffering a parallel decline in support (Pearson et al. 2011; Engel et al. 2021; Löbl et al. 2023).

In our opinion, unambiguously identified taxa are crucial to many biological disciplines, especially conservation biology, as they represent the first compulsory steps to enable the confirmation or rejection of any scientific study that



refers to them (Gippoliti and Amori 2002; Dubois 2003). More properly, Long and Rodriguez (2023) focused on distinct and poorly known vertebrate taxa. Obviously, no one can highlight and conserve species that are not yet known to science and/or that are not formally described, and for this taxonomy, once more, plays a crucial role. As a well-known fact, species must be formally described prior to being subjected to red list assessments, as undescribed and/or candidate species simply do not exist in terms of conservation priorities. This is why taxonomic actions are often, albeit informally, encouraged (i.e., Zachos et al. 2014).

We here report some cases regarding vertebrates, as they are better known and easier to deal with in terms of nomenclature and taxonomy. As a remarkable example we may cite amphibians, recently confirmed as the most threatened terrestrial vertebrates, with more than 41% of described species reported on IUCN Red Lists (8761 according to AmphibiaWeb 2024), but, at the same time, with many new species discovered and constantly being described (Luedtke et al. 2023). In fact, we believe that there is another category of “long-lost” species that are often overlooked by conservation literature: the synonymized (and often forgotten) taxa. Indeed, the 20<sup>th</sup> century was dominated by an overly synthetic and “lumping” approach to the study of biological diversity that resulted in a significant decrease in the number of recognized species, especially in vertebrates. Several nominal species were forgotten because they were synonymized or because they were moved to the sometimes-neglected sub-specific rank (Burgin et al. 2018). Such an approach concerns mainly the relatively well-known vertebrates, for which red lists are already available. For invertebrates the situation is quite different, with species often known from just a single specimen, the so-called “singletons” (Wells et al. 2019), and with red lists, with just a few exceptions (Neubert et al. 2019), missing.

As a consequence, the “taxonomic inertia”, a term used to describe the persistence of incomplete knowledge of biodiversity due to a lack of taxonomic interest during most of the 20<sup>th</sup> Century (Gippoliti et al. 2017), propagated the remarkable prevalence and persistence of taxonomic errors. So far, the subspecies category has lost much of its scientific credibility and therefore many subspecies were *de facto* forgotten by conservation biology (Gippoliti et al. 2018). Subspecies are often not even considered worthy of conservation attention except in a few cases, mostly involving charismatic animals such as the mountain gorilla *Gorilla beringei beringei* Matschie 1903 (Gippoliti and Amori 2007). Among mammals, other than the well-known example of primates (Rylands and Mittermeier 2014), even among supposedly well-known groups, such as felids, there are overlooked species and resurrection of old synonyms, such as in the case of the Neotropical genus *Leopardus* (Carnivora, Felidae) with the recent re-validation of *L. guttulus* Hensel 1872 (do Nascimento and Feijo 2017). African canids are also little known from a taxonomic perspective. For example, the newly identified lineage of the African wolf *Canis anthus* F. Cuvier 1820 (Rueness et al. 2011) probably includes several distinct species (Allen 1939) that were later considered subspecies of a phylogenetically distinct species, the golden jackal *C. aureus* Linnaeus 1758 (Ellerman and Morrison-Scott 1951) and that consequently disappeared from science’s radar for more than half a century. This seems to be the case for the recently observed wolf in Danakil that has been preliminarily assigned to *C. mensesi* Noack 1897 (Gippoliti and

Lupi 2020), a long-lost synonymized taxon. New World deer of the tribe Odocoileini offer a clear example of the effect of taxonomic inertia. At the end of the 20<sup>th</sup> century most authors recognized a total of 12 species in six genera (Corbet and Hill 1986). Nowadays, after a revival of interest in their taxonomy and the contribution of molecular techniques, the number of accepted genera is 10 with around 20 species (Gutiérrez et al. 2017; Morales-Donoso et al. 2023; Barrio et al. 2024). Interestingly, the 40% increase in recognized species in about forty years is almost completely due re-evaluation of synonyms (the only exception being the newly discovered *Pudella carlae* Barrio et al. 2024). *Bisbalus citus* Osgood 1912 (Artiodactyla; Cervidae) is another good example of a “long-lost synonymized” species. In fact, the genus *Bisbalus* is based on *Mazama americana cita*, a taxon that has been overlooked, as most other subspecies, for almost a century (Sandoval et al. 2024).

The importance of a revisionary and taxonomic working activity is even more evident in other speciose vertebrate groups, like amphibians and reptiles. In these cases, the intensification of field surveys and the use of integrative tools (i.e., biomolecular, acoustics, etc.) often leads to the reevaluation of the fauna of understudied geographic areas. Of course, the list of these works would become almost endless and is one reason for the continued description of new herpetological species (i.e., Köhler et al. 2005). As an example, in a recent work that described 24 new species in the genus *Mantidactylus* (Anura; Mantellidae) from Madagascar, a detailed comparison of historical museum types allowed the re-validation of four old taxonomic names that were formerly synonymized (and forgotten) for more than one century (Scherz et al. 2022). The availability of new techniques to extract genetic and genomic data from historical museum vouchers also led to the development of the exciting field of “museomics” that offers a further hope for filling a gap between natural history museums and biodiversity researchers (Fong et al. 2023). Furthermore, incorporating available names by generating molecular data for types of synonymized species is definitely feasible meanwhile and should be a standard protocol when describing new species using proper taxonomy. This would help in bringing these names back into scientific process and helping to avoid nomenclatorial chaos.

It is also well-known that the historical vouchers may be the only evidence for distinct and probably already extinct species, as is the case with the reptile *Lygodactylus neglectus* Ceríaco and Passos 2023 found in the herpetological collections of the Museu Nacional, Rio de Janeiro (Brazil) (Ceríaco and Passos 2023). First evidence for new species may originate directly from museum-based revisions, as in the case of the Somalian mole-rat *Fukomys ilariae* Gippoliti and Amori 2011 in the collection of the Museo Civico di Zoologia in Rome (Gippoliti and Amori 2011) or, more recently, the two new species of Neotropical small-eared *Cryptotis* shrews discovered at the Smithsonian National Museum of Natural History (Woodman 2023). In other cases, museum specimens offer definitive evidence regarding the former distributions of species and open new taxonomic questions, as is the case of the recently verified Mediterranean occurrence in historical times of the small-eye hammerhead *Sphyrna tudes* (Valenciennes 1822) (Carcharhiniformes; Sphyrnidae) confirmed by the (re)description of the specimen preserved in the NHM of Pisa University (Collareta and Farina 2023). This species is today reported only from the Atlantic coast of South America, from Venezuela to Uruguay (Pollom et al. 2020).

Hammerhead sharks have declined in the Mediterranean Sea by more than 99.99% since the late 19<sup>th</sup> century (Ferretti et al. 2008) and the reappraisal of historical sphyrid specimens from natural history collections could help to clarify the taxonomic identity and the true extent of species diversity, and its decline, in the Mediterranean Basin.

Finally, as reported by Wheeler (2024), we underline here the central role of natural history collections in systematics, and the pivotal role of NHMs as centers for taxonomic research and preservation of voucher specimens, thus reflecting species diversity as fully as possible, even of the most obscure taxonomic groups on Earth (Suarez and Tsutsui 2004; Fisher 2019). It is therefore crucial that taxonomists and museum communities persist, and are fully integrated, into worldwide efforts to map the biodiversity of the planet, including the specific mission to rediscover and conserve lost and long-forgotten species. Only with this in mind is it possible to re-align historical collections with the new role of biodiversity assessment. And, obviously, taxonomy plays an important role in resurrecting lost species, most of which are lost in museums. It is time that international bodies, such as IUCN, recognize that biodiversity conservation needs to be fully embedded in a wider vision that is fully aware of the crucial role of botanical and zoological collections and of the people that care for them and study them. Success relies on a future with a stronger collaboration between the conservation and taxonomy communities. This will certainly help in developing conservation actions that are urgently needed.

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## Data availability

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

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## Research Article

# Valuing ecosystem services of community gardens in developing countries: a case study of Dezful City in Iran

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## Abstract

Allotment (AGs) and community gardens (CGs) are types of urban gardens that provide diverse ecosystem services (ESs) such as climate regulation, beautiful landscapes, biodiversity and food security. Despite this, there are no AGs or CGs in many developing countries such as Iran. One of the main reasons for the lack of development of CGs in developing countries is the lack of proper understanding by policy-makers and citizens regarding the economic value of the ESs provided and the lack of disclosure of the benefits of its construction in urban areas. The purpose of this study is to analyse the preferences of citizens, to examine the existence of a potential market, to estimate the willingness to pay (WTP) for the creation and operation of CG services and to investigate the factors affecting residents' WTP in Dezful, Iran. For this purpose, a choice experiment, based on the stated preferences valuation approach, was applied through interviews with 170 respondents living in Dezful urban area. The data were collected during the autumn of 2022 and analysed using conditional logit model. The results indicated that the responding citizens are willing to pay 4.57 USD per month to create CG and operate its ESs in Dezful. Amongst the attributes and ESs of the community garden, citizens valued cultural services the most, followed by provisioning services. In addition, the variables "age", "being native", "education", "household expenditure level" and "awareness and recognition of CG ecosystem services" had a significant effect on WTP. According to the results, it is suggested to follow the policy of creating CGs with the participation of citizens instead of focusing only on the creation of urban green space with a government budget and heavy financial burden. CGs, while providing diverse ESs, can generate sustainable incomes for municipalities and accelerate the movement toward sustainable urban development.

**Key words:** choice experiment, community garden, economic valuation, ecosystem services, urban green space



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## Introduction

The world population will reach 10 billion people by 2050, two-thirds of whom will live in cities (Alivand 2022). The expansion of urbanisation brings environmental externalities such as a reduction in green spaces, an increase in ecological fragility, growing air pollution, destruction and fragmentation of agricultural lands on the outskirts of cities and disconnection between urban dwellers and

the natural environment (Meng 2019; Albaladejo-García et al. 2021). A reduction in urban green space as well as recreation and natural tourism centres and the disconnection of urban residents from the natural environment can lead to a decline in the well-being (Cao and Li 2024), mental health reduction (Elrafie et al. 2023; Werder et al. 2024) and mental fatigue (Li et al. 2024; Osa et al. 2024) of citizens. One of the solutions to address these problems is urban agriculture, which can be considered a new solution to address the environmental, economic and social challenges of cities. Additionally, “urban agriculture” can be promoted as an effective way to ensure food security (Steenkamp et al. 2021; Slater and Birchall 2022; Desalegn et al. 2024).

Urban agriculture plays a significant role in preserving natural and semi-natural landscapes and areas around cities (Contesse et al. 2018; Zabala et al. 2021). Urban agriculture and horticulture create green spaces in and around cities (Azunre et al. 2019) and improve social well-being (Rao et al. 2022). In many cities worldwide, the development of urban agriculture has become one of the fundamental and important features of urban planning and, in addition to its complementary role in providing food, it also plays other effective and beneficial roles in the framework of urban life (Alivand 2022). Urban gardening is one of the dimensions of urban agriculture in which a group of city residents or around the city are engaged in gardening in a plot of land and there is an element of collective action in it. In general, due to the contributions of these gardens to food security, economic and environmental sustainability and the protection and creation of green spaces (Azunre et al. 2019), the multipurpose role of these gardens has been accepted (Orsini et al. 2013).

According to their location and management model, there are different types of urban gardens, such as allotment gardens, home gardens, community gardens (CGs), school gardens, as well as balcony and rooftop gardens (Ferreira et al. 2018; Kwartnik-Pruc and Droj 2023). One type of urban garden is community gardens (CGs). This type of urban garden, as an asset of a city, provides various ecosystem services (ESs), such as preserving biodiversity (Cabral et al. 2017) and creating habitats for various species (Speak et al. 2015), food production (Song et al. 2022), adjusting the local climate (Tsilini et al. 2015), regulating water and runoff (Breuste and Artmann 2015), cooling through evaporation and transpiration (Knight et al. 2021), providing agricultural education and understanding of agronomic knowledge (Ding et al. 2022), improving health (Li et al. 2024) and recreation (Bretzel et al. 2018) and creating social cohesion in cities (Barthel et al. 2013). CGs improve the quality of a city's social environment by enhancing participation, creating social cohesion, providing educational activities and strengthening social structures (Chalmin-Pui et al. 2021; Rao et al. 2022). Note that CGs were formed in the early 19<sup>th</sup> century to deal with food insecurity and reduce the effects of poverty in Europe (Keshavarz and Bell 2016). The wide range of services of CGs has increased the interest of policy-makers, social organisations and researchers in this issue (Kingsley et al. 2019; Doyle 2022) and CGs have been accepted as a significant tool in sustainable urban development (Alivand 2022). Investigating the uses and services of CGs can be useful in designing local policies and urban management (Egerer et al. 2024).

Neglecting the importance and not understanding the real value of the environmental as well as socioeconomic services of CGs in many developing countries, including Iran, has led to a lack of planning for the creation and

development of this type of urban garden. Many city managers, with a pessimistic view, believe that this innovation does not have feasibility in the urban green space. Additionally, many decision-makers consider the creation of CGs, AGs and other types of urban gardening and farming in cities to be political gestures or luxury entertainment and do not accept them as practical plans for sustainable urban development (Alivand 2022). One of the reasons for such a view is citizens' livelihood and economic hardships together with the existence of other political priorities for city management in developing countries, which cause the social, economic, environmental and cultural services and benefits of CGs to be neglected.

One of the most important elements of the development of urban CGs, especially in developing countries, is the understanding of their ESs by consumers and the formation of demand, based on consumer preferences. Indeed, without potential demand, planning to create a CG will fail. Analysing consumer preferences and investigating the existence of a potential market, especially in developing countries such as Iran, which have not yet had the experience of creating a CG, can help in policy-making and planning for city managers to create and operate CGs. Additionally, studies in this area, while informing decision-makers, allowing them to be more efficient in urban planning and making decisions, are accepted by the majority of citizens (Albaladejo-García et al. 2021).

Since there is no CG in many developing countries (Alivand 2022; Zheng et al. 2022), one way to investigate the existence of potential demand is to use economic valuation methods and estimate people's WTP. Economic evaluation provides the possibility of estimating the demand for CG to benefit from the expected ESs of these agricultural systems. It also estimates the social, economic and demographic factors affecting the demand and WTP of citizens (Ye and Yoshida 2019). Estimating the social demand for this type of urban garden leads to creating a specific legal framework that citizens will widely accept (Ribeiro et al. 2020).

A review of literature shows that economic valuation studies on the social-environmental benefits and services of allotment and community gardens is very limited (Scott et al. 2018; Albaladejo-García et al. 2021). There is extensive literature on the WTP, economic value and welfare effects of other types of natural urban ecosystems, such as parks (Latinopoulos et al. 2016; Halkos et al. 2022; Silva et al. 2022; Yousofpour et al. 2024), forests and forest parks (Chen and Qi 2018; Kim et al. 2021; Zegeye et al. 2023), as well as other types of urban green spaces (Xu et al. 2020; Gelo and Turpie 2021; Salm et al. 2023). Numerous studies have also been conducted regarding socio-economic factors (such as age, household expenses, gender, income, education, environmental attitudes etc.) and spatial factors (residence location, distance to recreational places and green spaces, distance to alternative places, air pollution status) affecting the willingness to pay and demand for urban green spaces (Tiraeyari et al. 2019; Ye and Yoshida 2019). Additionally, there are many studies regarding different fields of urban agriculture (Ayoni et al. 2022; Yuan et al. 2022; Pradhan et al. 2023), urban gardens (Čepić et al. 2024), ecosystem services of allotment and community gardens (Cabral et al. 2017; Haase and Gaeva 2023), barriers to the creation and development of social gardens (Asl and Azadgar 2022; Ding et al. 2022), socio-psychological effects of allotment and community gardens (Young and Bauer 2022) and other issues related to community gardens



(Raneng et al. 2023). However, in few studies in developing countries, the potential demand and people's WTP to create CGs and the economic valuation of CG ecosystem services have been investigated (Albaladejo-García et al. 2021).

The purpose of the present study is to explore the potential demand and estimate the WTP for creating and exploiting the ecosystem services of CGs, identify the factors affecting the demand and determine the total economic value of the ESs provided through the creation of CGs in barren and abandoned urban lands of Dezful City, Khuzestan Province, Iran. For the following reasons, this study can contribute to the development of the limited literature on estimating the demand and preferences of citizens for community gardens and valuing the resulting ecosystem services:

1. In the Middle East countries, no study has been done regarding the estimation of demand, willingness to pay for the creation of CGs and the valuation of its resulting ecosystem services. Thus, the results of the present study can answer this gap and serve as a guide for other developing countries.
2. The valuation method used in this study is the Choice Experiment method, based on the stated preferences approach, which, unlike methods such as contingent valuation (CV), can value multiple ecosystem services and analyse citizens' preferences regarding each of them. In addition, this method can identify which of the CG ecosystem services for citizens has more importance and higher value (by estimating the WTP for each of them). The understanding of this issue by policy-makers and decision-makers of urban management leads to more efficient urban planning and is accepted by the majority of citizens.

Due to the lack of CGs in Iran and, accordingly, the lack of knowledge about the issue amongst urban decision-makers, as well as awareness about the acceptance or non-acceptance of CGs by citizens, together with the novelty of the investigated issue and the lack of similar domestic research on the WTP of people for the use of CGs, the present study tries to provide an appropriate response to this information and research gap.

## Methods

The choice experiment (CE) method was used for investigating the objectives of this study, which, in addition to valuing ecosystem services (ES) and estimating citizens' willingness to pay (WTP), also estimates the factors affecting the WTP for valued attributes and ESs. The following subsection provides more details on the study area, theoretical foundations of the CE, experimental design, data collection and model estimation.

### Study area

Dezful County is one of the counties of Khuzestan Province and is located in south-western Iran. The area of this county is 4762 km<sup>2</sup>, which is located at 48°20' to 48°31' longitude and 32°22' to 32°57' latitude. As a major part of the Khuzestan Plain, Dezful is one of the most populated cities in Khuzestan Province due to its fertile lands and the presence of the Dez River, which runs

through the centre of this city and it has significant potential for creating and exploiting the capacity of CG. This city has a Mediterranean winter and autumn and its green ecosystem is very pleasant from early autumn to early spring. In addition to fertile plains in the central and southern regions, it has foothills and spectacular mountains in the north. The minimum and maximum temperatures in this area are between 2 and 50 degrees Celsius (Eidipour 2023).

However, the development of urbanisation and suburbanisation has reduced urban green space and the presence of abandoned and unused land in some old parts of the city has distorted the beauty of the city. The emergence of CGs in potential cities such as Dezful can play a significant role in reviving waste and unused lands. The presence of a river with rich water resources and suitable weather conditions can accelerate the creation of CGs and their ecosystem services (Eidipour 2023). Fig. 1 shows the geographical location of Dezful city.

### Choice experiment method

The choice experiment is a valuation method based on stated preferences, in which people are asked to state their preferences for different attributes of goods in a hypothetical market by creating alternative hypothetical scenarios, as well as offering non-market goods and services at different levels and attributes (Petcharat et al. 2020). Each “good” alternative has several attributes with different levels. One of the reasons for using this method for valuation in the current study is that CG provides multiple ecosystem services, each of which has value and the CE method (unlike the conditional valuation method) provides the possibility of estimating the value of several attributes at different levels.

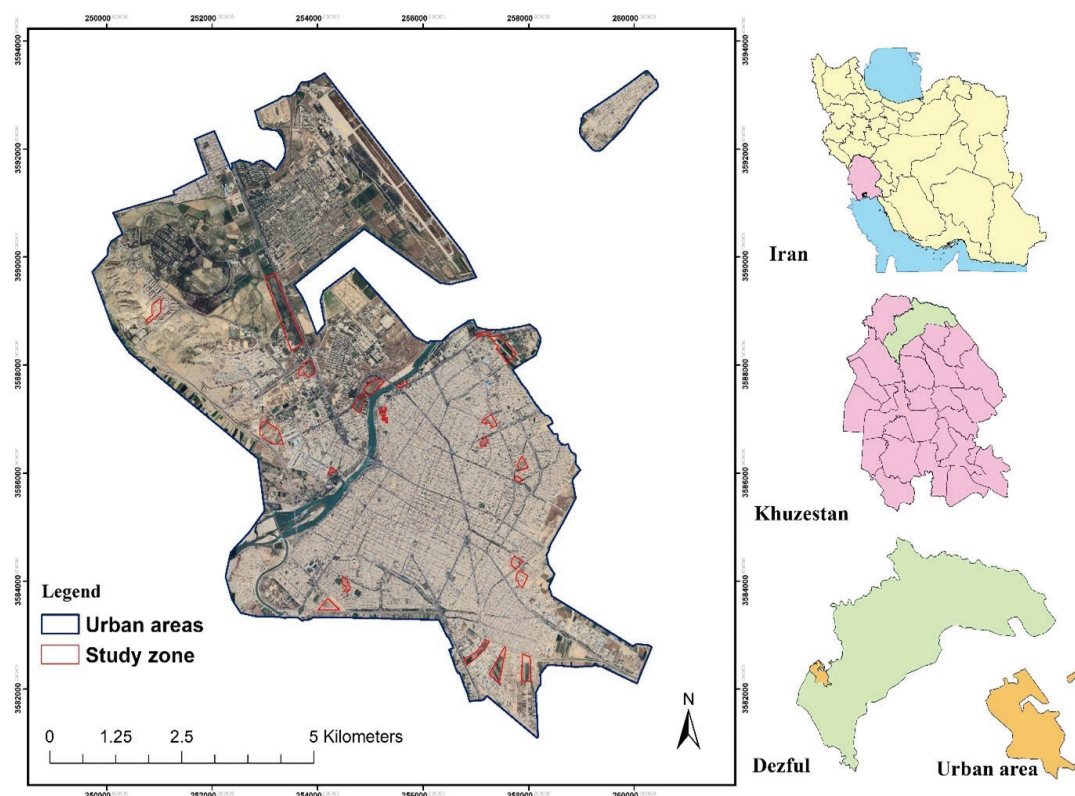


Figure 1. Geographical location of the study area.

## Theoretical foundations of the choice experiment

The theoretical basis of the choice experiment method is Lancaster's characteristics theory of value, random utility theory and experimental design (Hanley et al. 1998). According to Lancaster's theory of demand, the total utility of a product or service is equal to the sum of the utilities of each of the characteristics of that product (Lancaster 1966). The random utility model assumes that people gain "utility" from choosing a product or environmental attribute (choosing an option) (Aguiar et al. 2023). In this framework, the choice of each option is shown by the differences between a set of alternatives (Petcharat et al. 2020). In the random utility model, each alternative (options A and B or alternatives to the status quo) is described by a utility function that has a deterministic component ( $V_i$ ) and a random error component, being expressed as Equation (1) (Salm et al. 2023):

$$U_i = V_i + \varepsilon_i \quad (1)$$

It is assumed that a person chooses an option or an alternative that has a higher utility. For example, a person chooses alternative  $i$  if  $U_i > U_j$  for all  $i \neq j$ . Since utility has a random component, the probability of choosing alternative  $i$  by an individual can be expressed as follows (Caporale et al. 2024):

$$Pr_i = Prob\{V_i + \varepsilon_i > V_j + \varepsilon_j; \forall j \in C\} \quad (2)$$

where  $C$  is the choice set amongst the possible options and the variable  $V_i$  includes the attributes of the situation. In this study, there are three options (current situation, option A and option B). It can be shown that, if the error terms ( $\varepsilon_i, \varepsilon_j$ ) have standard type I extreme value distributions which are independently and identically distributed, the probability of choosing option  $i$  by the respondent is expressed as follows (Maddala 1983):

$$Pr_i = \frac{\exp(\lambda V_i)}{\sum_j \exp(\lambda V_j)} \quad (3)$$

This distribution is specified by the scale parameter  $\lambda$ , which is inversely dependent on the variance of the error term and the location parameter  $\delta$  (Petcharat et al. 2020). It is assumed that the chosen distribution is the standard Gumbel distribution with  $\lambda = 1$  and  $\delta = 0$  (Ben-Akiva and Lerman 1985). Assuming that the systematic component of utility is linear, the utility function for alternative  $i$  can be defined as Equation (4):

$$U_i = \alpha_i + \sum_{j=1}^n \beta_j X_j + \sum_{k=1}^m \theta_{ki} Z_k + \varepsilon_i \quad (4)$$

In the above relationship, coefficient  $\alpha_i$  indicates the "opt out" or "status quo" option,  $X_j$  represents the attributes or ecosystem services related to each option,  $Z_k$  is a vector of individual socioeconomic characteristics,  $\varepsilon_i$  shows a component of random error and  $\beta_j$  and  $\theta_{ki}$  are estimated parameters of the model (Liu et al. 2019). Finally, citizens' WTP for benefitting from the attributes or ecosystem services of CGs can be calculated through dividing the coefficients of the ecosystem attributes by the price attribute, which is expressed as follows (Salm et al. 2023):

$$WTP_j = \frac{\beta_j}{\beta_{price}} \quad (5)$$

where  $\beta_j$  is the coefficient of the selected attributes and  $\beta_{price}$  is the coefficient of price, rent and input rate or municipal tax for each household per month.

## Experimental design

The purpose of this study is to examine the preferences of citizens and the existence of a potential market to determine the economic value of ESs and to estimate citizens' WTP and the factors affecting it in the City of Dezful, Iran. For these purposes, the CE method was used to determine the value of the attributes and ESs of the CG. Since no CG currently exists, the "status quo" is the absence of a CG, where two levels of creating CGs (options A and B) were designed and presented to the respondents. In the questionnaire, along with the complete introduction of the CG and its extensive environmental and socio-economic services, the ESs of options A and B were explained. It was stated that these two options can improve the urban environment. Each of the three available options is described by four ecosystem attributes, which can be assigned to the existing status (status quo), good and excellent level and citizens can state their WTP for each of the ecosystem services with defined levels using the price attribute (with different levels). According to literature and studies conducted with the CE method, the evaluation with this method has four main stages (Petcharat et al. 2020): selecting attributes and attribute levels, creating choice sets, drafting questionnaires and pretests and conducting surveys.

In the first stage, attributes and ecosystem services were selected by referring to literature and other studies, subject to geographical, local and climatic features, as well as services of other gardens in the study area. The first attribute is recreation and nature or urban tourism, which is a kind of cultural service offered to citizens through the natural environment of these gardens. The second attribute is the presence of natural landscapes and beautiful perspectives in the city, which are provided by the green space of these gardens. The third attribute is protecting water and soil resources (preventing soil erosion and helping water infiltration through vegetation cover in unplanted and abandoned urban lands), which is a regulating service. The fourth attribute is access to healthy, organic, nutritious and fresh food, which is realised through the allocation of CGs to citizens for urban agriculture and is considered a type of provisioning service. Each of these four attributes has three levels: no change, good and excellent. Additionally, the price attribute was designed with payment options of 750,000 Rials (2.19 USD), 1,000,000 Rials (2.92 USD), 1,200,000 Rials (3.51 USD) and 1,500,000 Rials (4.39 USD), which actually show the WTP and the subjective value of CG ecosystem services amongst citizens. The attributes (ESs) and their levels are presented in Table 1.

The questionnaire was designed after this stage. Each questionnaire has three parts (Baniasadi et al. 2016). The first part of the questionnaire examined and measured the respondents' level of awareness of CG and its services and investigated their attitudes towards natural resources, the environment and urban green space. In the second part of the questionnaire, while defining and expressing the background of the CGs, all attributes and ecosystem services

**Table 1.** Attributes (ES resulting from the creation of a community garden) and related levels.

Attribute	Level
Recreation and tourism	Status quo: Absence of community garden and EC resulting from it, the existence of desert, abandoned and unused lands
	Good: Increasing the urban tourism environment by converting 50% of the potential lands into community gardens
	Excellent: Converting all unused urban land into a community garden and significantly increasing the tourism environment
Beauty and landscape	Status quo: Absence of community garden and EC resulting from it, the existence of desert, abandoned and unused lands
	Good: Just a little of green landscape and reducing boring and polluted landscapes
	Excellent: Green landscapes, favourable vegetation, absence of boring and polluted landscapes
Protection of natural resources (water and soil)	Status quo: Absence of community garden and EC resulting from it, the existence of desert, abandoned and unused lands
	Good: Moderate destruction of base natural resources (reduction of destruction)
	Excellent: Conservation of water and soil resources (favourable situation)
Access to healthy and organic food	Status quo: Absence of community garden and EC resulting from it, the existence of desert, abandoned and unused lands
	Good: The people of the region only have access to fresh food
	Excellent: Access of the people of the region and other regions to healthy, organic and fresh food
Price (exploitation fee or municipal tax)	0, 750,000 IRR (2.19 USD), 1,000,000 IRR (2.92 USD), 1,200,000 IRR (3.51 USD), 1,500,000 IRR (4.39 USD)

are presented and, after introducing the product (ES) and creating a hypothetical market, the respondents are asked to express their WTP to create and benefit from the ESs (attributes) of the CG in the form of choice sets (Fig. 2). That is, individuals should choose an option between the current situation without a payment option (a lack of CG) and creating and benefitting from the ecosystem services of the CG in the form of options A and B with a hypothetical payment based on their preferences. The choice experiment part includes eight choice sets, with four attributes and three levels of “status quo”, option A and option B in each choice set. Fig. 2 presents an example of a choice set in the questionnaire.

The third part of the questionnaire also included personal information and socioeconomic characteristics of the respondents. In the next step, to determine the sample size as well as to modify the original questionnaire and the bid-amounts, a pretest was conducted through interviews with 30 citizens. In the last stage, the questionnaires were completed through face-to-face interviews. The sample size was obtained from Equation (6) presented by Orme (1998) to determine the sample size in the CE method (Rose and Bliemer 2013; Baniasadi et al. 2016).

$$N = 500 \times \frac{N_{lev}}{N_{alt} \cdot N_{set}} \quad (6)$$

In the above relationship,  $N_{lev}$ ,  $N_{alt}$  and  $N_{set}$  are the maximum number of attribute levels, the number of alternatives in the choice set and the number of the choice set, respectively. Based on Equation (6), the minimum sample size was 83 respondents and 170 respondents were interviewed for statistical certainty. According to eight choice sets and three options (alternatives), a total of 4080 observations were created.

## Model estimation

For the data analysis, the conditional logit model and Maximum Likelihood estimation method were used (McFadden 1973). The data from interviews with 170 citizens were analysed using Stata 14 software. The estimated coefficients















Attribute	Status quo	option A	option B
Recreation and tourism	No change: Lack of CG 	No change: Similar to the status quo 	Excellent: Converting all unused urban land into CG 
Beauty and landscape	No change: Lack of CG 	Good: A little of green landscape 	No change: Similar to the status quo 
Protection of natural resources (water and soil)	No change: Lack of CG 	No change: Similar to the status quo 	Good: Reducing erosion 
Access to healthy and organic food	No change: Lack of CG 	No change: Similar to the status quo 	Excellent: Healthy, organic and fresh food production 
Payment (rent, exploitation fee or municipal tax)	0	750,000 IRR	1,000,000 IRR
Please choose the most appropriate	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Figure 2. An example of the choice set in the questionnaire.

related to personality and socioeconomic characteristics show the factors affecting citizens' WTP and their preferences towards accepting or not accepting the options of creating a CG. Additionally, the estimated coefficient related to the bid amounts (monetary attribute) has been used to estimate the WTP of citizens, which was explained in the subsection "Theoretical foundations of the Choice Experiment" (equation 5).

## Results

### Characteristics of the respondents

In this study, 170 household heads were interviewed. Since in the study area, the majority of household heads were men, the majority of the respondents were men accordingly (90.6%). The average age of the respondents was 44 years and young people (20–35) constituted only 15.3% of the sample. The average years of education of the respondents were 14.88 years and they mostly had a bachelor's degree. The average income of the respondents was 111.3 million Rials (269.6 USD) per month and the majority of the respondents (66.5%), after explaining the attributes and ecosystem services of CGs and changing the face of the city following the construction of CGs in the barren and abandoned urban lands, were willing to pay for the construction and protection of the CGs. Some socioeconomic characteristics of the respondents are presented in Fig. 3a–f.

Considering the novelty of the CG issue in developing countries and the absence of CG in Iran, first, the community garden, along with its attributes and ecosystem services, was fully explained to the respondents. Then, they were asked to what extent they knew this information and had knowledge about this type of urban green space.

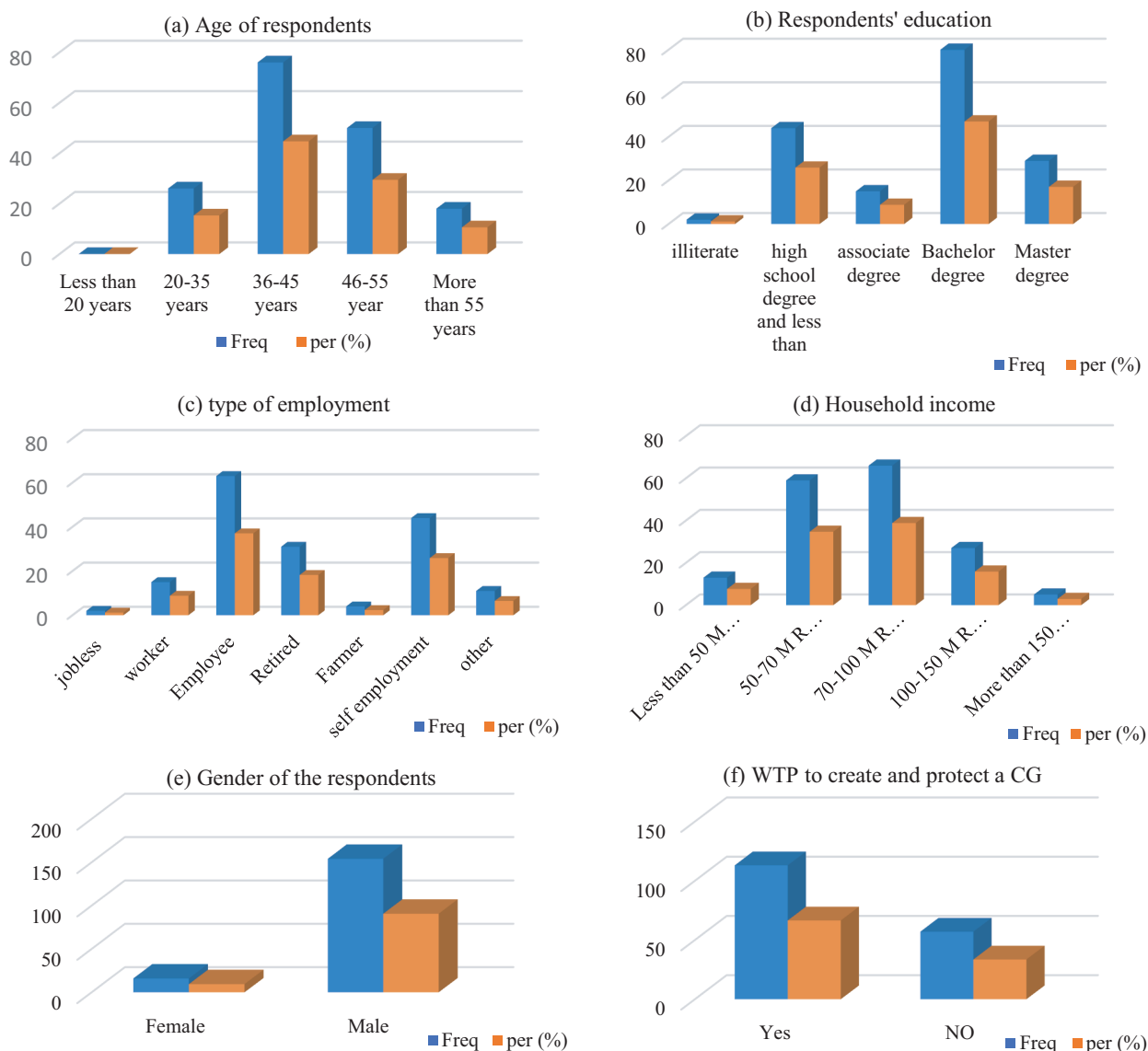


Figure 3. Socioeconomic characteristics of the respondents.

According to Table 2, the majority of respondents, that is, approximately 89.4% of individuals, stated they were unaware of the CGs or their attributes and services or, if they had partial information, most of the described details were new to them. Amongst the respondents, only one person had full knowledge about CGs and their ESs in the urban environment.

Table 2. Awareness and understanding of the community garden.

Knowledge and understanding of the subject (newness of the subject in Iran)	Number	Percent
It was completely new	61	35.89
It was new	49	28.82
Little of it was new	42	24.71
I knew a little	17	10
I knew completely	1	0.58
Total	170	100

### Measuring preferences, tendencies, attitudes about CGs and urban green space

After explaining the attributes and ES of the CG together with the experience of developed countries in creating various types of urban gardens (such as community, allotment gardens etc.), the attitude and preferences of the respondents towards the urban green space and some ecosystem services of the CGs were evaluated, with the results reported in Table 3.

First, the first item about the disadvantages of the existence of barren and unused land in the urban space was proposed. More than 99% believed that the existence of such land makes the city look ugly and can lead to social insecurity. Items 2, 3 and 4 referred to provisioning (food production, food security), regulating (air regulation) and cultural (public participation, social solidarity, recreation, urban beauty) ecosystem services. For all these items, more than 85% of the respondents agreed or fully agreed. Additionally, the majority of the respondents (29.42% agree and 70% completely agree) believed that, since the natural environment, from the habitat provision point of view, is dependent on the vegetation cover (supporting ES), the urban ecosystem should also have a share of the vegetation cover. More than 90% of respondents believe that, if natural resources and the environment are exploited, these valuable resources should be preserved for future generations.

The evaluation of respondents' opinions indicates that the majority of individuals are environmentally friendly. They believe that CGs can change the urban atmosphere of Dezful City and provide ecosystem services. This attitude shows the existence of a potential market for creating and operating

**Table 3.** Measuring tendencies, attitudes and preferences about the environment and urban green space.

Items	Percentage of respondents					Mean (0–5)	St. Dev.
	Completely Disagree (Score: 1)	Disagree (Score: 2)	Indifferent (Score: 3)	Agree (Score: 4)	Completely Agree (Score: 5)		
1) The existence of barren and unused urban lands has led to the ugliness of the city's face and can create an environment of social insecurity.	0	0	0.58	35.89	63.53	4.63	0.49
2) The increase in green space and vegetation will lead to air conditioning in the city, beautiful scenery and a healthy environment for walking and recreation.	0	0	0.58	24.12	75.30	4.75	0.45
3) The existence of a CG (according to its attributes and services) can lead to an increase in public participation and social solidarity.	0.58	0	5.9	49.41	44.11	4.36	0.65
4) Creating an environment inside the city for citizens' agriculture, in addition to helping production and food security, can cause employment and citizens' health.	1.77	4.71	7.05	51.76	34.71	4.13	0.87
5) Considering the dependence of the natural environment on vegetation, the urban ecosystem must also have a share of vegetation	0	0	0.58	29.42	70.00	4.69	0.47
6) Exploitation of natural resources and the environment to generate income, recreation and increase the well-being of the current generation should be accompanied by investment to preserve the environment for future generations.	2.35	0	4.70	40.60	52.35	4.41	0.79
7) I have to pay for the protection of natural resources even if I do not use or see them.	7.64	16.48	28.24	31.17	16.47	3.32	1.16

community gardens. For the last item, “I have to pay for the protection of natural resources even if I do not use or see them”, 47.64% of people chose the agree and completely agree option, 24.11% of people chose the disagree or completely disagree option and 28.24% of people chose the indifferent (neither agree nor disagree) option. Additionally, part (f) of Fig. 1 shows that 66.5% of the respondents tended to pay to create, operate and maintain a community garden, which indicates the existence of a potential market and consumer preferences for a community garden.

### Conditional logit model

To calculate the WTP, two models were estimated: the conditional logit model with only environmental attributes and the second model with socioeconomic variables. Table 4 presents the estimation results of both models.

According to the results of models 1 and 2, the environmental attributes “recreation and tourism”, “beauty and landscape” and “access to healthy and organic food” are significantly positive. The significance of the first two attributes reveals that the respondents believe that the creation of a CG has led to the improvement of the green space and the beauty of the city and, while adjusting the air of the city, it has led to an increase in urban tourism and the formation of an environment for recreation and sports. The significance of the attribute “Access to healthy and organic food” also shows that the respondents believe that the creation of a CG in the city leads to an increase in access to healthy and organic food. The positive sign of these attributes also indicates that improvement in these ecosystem services leads to an increase in citizens’ WTP. The attribute “protection of natural resources” is not statistically significant, demonstrating that, from the point of view of the respondents, CG does not lead to the preservation of basic water and soil resources.

**Table 4.** Estimation results of the conditional logit model.

variable	Model 1			Model 2		
	Coefficient	Z-Statistic	P-Value	Coefficient	Z-Statistic	P-Value
Recreation and tourism	0.3055	4.11	0.000	0.4005	5.21	0.000
Beauty and landscape	0.3245	4.96	0.000	0.2601	3.80	0.000
Protection of natural resources (water and soil)	0.1778	0.27	0.787	-0.2793	-0.43	0.668
Access to healthy and organic food	0.2096	3.63	0.000	0.2213	3.80	0.000
Price	-0.00011	5.45	0.000	-0.00002	-7.53	0.000
Age				-0.4287	-7.38	0.000
Being native				1.1082	4.48	0.000
Education				0.0547	2.73	0.006
Household expenditure level				0.0311	2.22	0.026
Awareness and recognition of ES of the CG				0.6018	8.92	0.000
Number of visits of urban green space				-0.0429	-8.31	0.000
<b>Good fit criteria of model</b>						
LR (likelihood ratio test)	56.38 (0.000)			41.261 (0.000)		
Log likelihood	-1465.92			-1363.41		
No. respondents	170			170		
No. observation	4080			4080		

The socioeconomic variables in the second model show that people who are younger, native, have a higher level of education, a higher level of living expenditure and are more aware of CG have greater WTP for the creation and operation of CG. These variables are significant with an expected sign. However, the variable “number of visits to urban green space” is significant with a negative sign, indicating that people who visit more urban green spaces have less WTP for the creation of CG. Additionally, the price variable is significant, with expected sign, showing that the lower the bid-amount is, the greater the probability of accepting the bid amount.

### WTP estimation

After estimating the coefficients of each of the attributes and bid-amount (price) variables, the citizens' WTP was estimated, with the results outlined in Table 5.

According to Table 5, the highest WTP of the household, equal to 2.07 USD, is related to the “recreation and tourism” attribute, followed by the “beauty and landscape” attribute, which is equivalent to 1.35 USD in second place and “access to healthy and organic food”, which is in third place, with 1.15 USD per household per month. The lack of significance of the attribute “protection of natural resources” indicates that the citizens of Dezful City believe that CG does not lead to the preservation of water and soil resources, so they do not value this attribute. The sum of the above three amounts, equivalent to 4.57 USD, indeed shows the total WTP for a household in Dezful City to benefit from the ecosystem services of the CG. According to the latest data obtained from the Iranian Statistics Center, 134,730 households live in Dezful City and, based on the data collected from the sample, 66.5% of them are willing to pay to create and operate a CG in Dezful City. Thus, it can be estimated that the people of Dezful City are willing to pay 4.91 million dollars annually for the creation and operation of CGs.

### Discussion

The current study aimed to estimate the WTP of people for the creation and operation of a CG in barren and abandoned urban lands in the City of Dezful in south-western Iran. Since plots of land have been left unused in different parts

**Table 5.** The WTP of people for the creation and operation of a community garden (Rial/Dollar).

Attributes	WTP (Person/Month)		WTP (Household/Month)		WTP for the entire area per month		WTP for the entire area per year	
	IR* Rial	US dollar	IR Rial	US dollar	Billion IRR	1000 USD	Billion IRR	Million USD
Recreation and tourism	200,250	0.59	709,250	2.07	63.545	185.8	762.543	2.23
Beauty and landscape	130,050	0.38	460,614	1.35	41.269	120.7	495.224	1.45
Protection of natural resources (water and soil)	Non-significant	–	–	–	–		–	–
Access to healthy and organic food	110,650	0.32	391,903	1.15	35.112	102.7	421.350	1.23
Total WTP for all attributes per year	440,950	1.29	1,561,767	4.57	139.926	409.1	1,679.118	4.91

\*- Rial (R) is the currency of Iran. The average US dollar (USD) in 2022 was 342028 Rials (Sources: Central Bank of I.R. Iran).



of the city and dry lands without green spaces have created unfavourable views in the city, the proposal to create a community garden, based on the experience of advanced countries, can lead to a change in the face of the city. Note that abandoned urban land can become a place for all kinds of crimes and endangers social security. Due to having sufficient water resources (the presence of Dez River) and a hot and humid climate, Dezful City has suitable conditions for creating community and allotment gardens. This innovation, for the first time in Iran, can lead to the improvement of environmental indicators and social reconstruction in dry and abandoned urban lands around the Dez River in Dezful City.

The results of this study indicate that the value of the ecosystem services of the CG for the residents of Dezful City is influenced by various socioeconomic factors. Younger people have greater WTP to create as well as operate a CG. The results of the studies of Albaladejo-García et al. (2021) and Lackey et al. (2021) also show that younger people attach more importance to cultural services such as recreation, tourism and education and, therefore, have greater WTP. Additionally, people with higher education levels were willing to pay more to create a CG in Dezful City. People with higher education, due to their greater awareness of the importance of environmental issues, are willing to pay more to protect the environment and create urban green space (Xu et al. 2020; Idris et al. 2022).

The evaluation of the ecosystem services of creating a CG indicates that people who have a higher level of living expenditure are willing to pay more for the creation of a CG. Since people in developing countries do not state their real income level, the expenditure level is used as a proxy of people's income in the model. Other studies, such as Albaladejo-García et al. (2021) and He et al. (2016), have confirmed the positive relationship between higher income (expenditure level) and WTP. He et al. (2016) believe that people with higher incomes consider urban green space to be an available option for recreation and tourism and are therefore willing to pay more.

The results indicated that native people have a greater WTP than non-native people and immigrants from other cities or provinces. The main reason for this could be their permanent residence in Dezful, greater benefit from ecosystem services and spatial dependence as well as a sense of belonging to the homeland. The results also revealed that the variable "awareness and recognition of ESs of the CG" led to an increase in WTP. Indeed, citizens who have more accurate information about CG ecosystem services have greater WTP (Meng 2019). In particular, those who have been studying more about the experiences of advanced countries in this new type of urban service have learned more about a variety of ecosystem services, such as regulating, provisioning, cultural and supporting services. Some research shows that initiatives, such as community gardens that improve ecosystem services, are significantly important to citizens (Martinez-Paz et al. 2019). Additionally, Albaladejo-García et al. (2021) found that ecosystem services are the main determining factor in valuing all kinds of urban gardens, so citizens' information about these ESs affects their WTP. Similar studies in European countries also confirm this finding (Palau-Salvador et al. 2019).

The statistical significance of the "Recreation and Tourism" and "Beauty and Landscape" attributes demonstrates that respondents believe that creating CGs in Dezful's abandoned and destroyed lands will improve cultural services such as urban beauty, green space, beautiful landscapes and a suitable environment for recreation and tourism. Numerous studies, such as Borysiak and

Mizgajski (2016) and Cabral et al. (2017), have shown that CGs provide cultural ecosystem services.

The attribute of "Access to Healthy and Organic Food", which is one of the provisioning ESs of CGs, also affects individuals' WTP. The results of Adekunle (2013), Church et al. (2015) and Albaladejo-García et al. (2021) showed that provisioning services, such as food production in CGs, lead to increased welfare for elderly people. Similarly, Partalidou and Anthopoulou (2017) argued that urban gardens, including CGs, help improve the food security of low-income households. The production of food and agricultural activities in communities and allotment gardens in urban environments provide physical mobility and vitality for all individuals, especially elderly individuals. The production of healthy and organic products can also improve the food safety of urban households.

The model results showed how much understanding of CG ecosystem services is effective in the economic evaluation of CGs. Due to the lack of CGs in Iran thus far, there may be no understanding of CG ecosystem services amongst citizens, so the results of the study are obtained according to the creation of a hypothetical market, the expression of CG ecosystem services as well as similarities to the types of urban gardens and green spaces. Indeed, after the construction of the community garden and its exploitation, citizens will develop a more accurate understanding, awareness and knowledge of the CG and its ecosystem services. Tian et al. (2020) also found that understanding and awareness of ecosystem services provided by urban green spaces, including CG, affect citizens' WTP.

The WTP of households in this study was 4.57 USD per month, which is a significant figure for citizens of developing countries under international sanctions. To better understand this number, it is worth noting that in a similar study of allotment garden valuation in Spain, the figure of 5.4 (€) euros (equivalent \$5.7) for the ecosystem services of CG was estimated to be close to the WTP in this study. In another study, Petcharat et al. (2020) estimated that the WTP for Bangkok's largest urban green space was \$42 per year, equivalent to \$3.5 months/household.

Due to the population of Dezful City and the percentage of the sample studied who would be willing to pay for the creation of a CG, Dezful citizens are expected to pay \$409.1 thousand monthly and \$4.91 million annually to create and benefit from CG ecosystem services. These amounts can generate significant income for the municipality and cover the operating plus maintenance costs of CGs. This financial source can separate the construction of a CG by the municipality from the public budget. The sustainability and continuity of CG will not depend on the public budget.

## Conclusions

This study is important as there are not all kinds of community, allocation etc. gardens in Iran and estimating the WTP as well as analysing the stated preferences of consumers can be useful for directing and starting construction projects for this type of urban garden. Socioeconomic valuation of the creation and exploitation of CGs in barren, unused and abandoned urban lands has not been carried out in Middle Eastern countries thus far and has not been emphasised in economic valuation studies in this region. Valuation studies and projects are suitable not only for estimating the value of non-market benefits of creating

CGs, but also as a tool for understanding citizens' preferences, opinions and public beliefs about environmental issues (Martínez-Paz et al. 2021). Thus, the present study can help urban planners better understand the preferences of citizens and this understanding will lead to decisions to improve social welfare.

In this study, cultural services (beauty and landscape, recreation and tourism) were valued most highly by citizens, followed by provisioning services (healthy and organic food production). Therefore, citizens pay special importance to urban green space. However, the creation of large dimensions of urban green space has a significant financial burden. The estimation of social preferences reveals that the construction of a CG, in addition to providing urban green space and providing ecosystem services, can afford its costs because of public participation and, at the same time, generate sustainable income for the municipality. Thus, the construction of such gardens in urban spaces, in addition to the aforementioned benefits, has also economic justification. Accordingly, it is recommended that municipalities take advantage of the assignment or rent of CGs or the entrance fees to them as a stable income and spend this income on the creation and development of CGs in the initial stages. Additionally, granting some facilities to encourage the private sector to convert their lands into CGs, providing the possibility of income earning from CGs and extensive advertising to buy healthy and organic food products produced in CGs, can be effective in the development of CGs.

In addition, the results of the study indicated that citizens attach less value to regulatory services and habitat services or do not care about them (no value). Indeed, citizens care about the ecosystem services that are directly used by them (use ESs) and care less about the non-use values of the environment, while these ESs are very valuable and contribute to the sustainable development of the city. Thus, by informing citizens and providing evidence, it is necessary for policy-makers to explain the non-use values of ecosystem services to citizens and influence their WTP. Studies by Livesley et al. (2016) and Petcharat et al. (2020) also confirm it.

In addition to creating urban green space and providing ecosystem services, CG leads to a decline in social insecurity. Note that abandoned and destroyed urban lands are currently a gathering place for criminal and addicted people, but when they become CG, they bring with them sustainable social security. At the same time, CG can become a place for agricultural education and sports for elderly people and provide the basis for enhancing social cohesion and the growth of social activities. As such, it is necessary to identify and evaluate the social effects and benefits of creating CGs in the region in future supplementary studies.

The field survey, municipal documents and the evaluation results of the present study show that, for the creation and exploitation of CGs, there are key elements, i.e. abandoned and apt urban lands (place) and citizens interested in the exploitation of community gardens (people). Nevertheless, it seems that the lack of a legal or political framework is one of the most important obstacles to the creation and exploitation of CGs. Additionally, institutional and legal concepts should have a clear definition (such as "property rights", "land use change", "the method of transfer of ownership" or "the method of transferring the right of exploitation", "the conditions of expropriation" or "prevention of exploitation and reclaiming of land" etc).

Countries with successful urban CGs projects usually have correct policies and appropriate legislations on CGs. For example, countries such as Germany (Act of

Small Gardens 1983), United Kingdom (Allotments Act 1950) and Denmark (Allotment Gardens Act 2001) can be mentioned (Ricci and Conrad 2018). Hence, it is recommended that, for the better implementation of the project of creating CG, legal studies for legislation, as well as proper policy-making in the field of urban land management to create CGs, should be done and policy-makers should use the experiences of leading countries in this field to implement appropriate policies.

Another point that should be noted is that there is another type of urban garden called allotment gardens (AGs), where plots of land are leased to applicants for urban agriculture or other uses. The experience of other countries indicates that the WTP of citizens for this type of urban garden is greater than the WTP for CGs (Albaladejo-García et al. 2021). Therefore, examining the strengths and weaknesses, as well as the technical feasibility of creating this type of urban garden and comparing it with the CG, is proposed as a suggestion for further studies. Another suggestion for future studies is the identification of suitable lands and technical-economic feasibility studies for creating CGs. Soil quality, road access to lands, topographical features, access to water resources and some other issues should be investigated in feasibility studies.

Finally, it should be noted that the present study, in addition to examining the preferences of citizens and assessing the social acceptance of CGs, revealed that the creation and exploitation of CGs can have financial-economic justification and generate a sustainable income for municipalities in developing countries.

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The authors have declared that no competing interests exist.

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### Author contributions

MB conceptualisation, methodology, software, data curation, validation, formal analysis, visualisation, writing - original, review & editing. ME Initial writing, data collection, data curation, analysis.

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### Data availability

Data will be made available on request.

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## Short Communication

# Invertebrate-biased diet of burrowing owls in a newly-restored coastal grassland

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## Abstract

Recovering biodiversity across trophic levels is a major challenge in restoration ecology. Specifically, predator population recovery depends on the timely re-establishment of their preferred prey species in restored habitats. Here, we evaluate potential dietary factors contributing to the loss of western burrowing owls (*Athene cunicularia hypugaea* (Bonaparte, 1825)) from a newly-restored coastal grassland. We examined owl pellets and found that burrowing owl diets were relatively low in vertebrate prey during their brief occupation of the restoration site (2.6% of prey items; found in 61.8% of sampled pellets). We suggest that preferred food limitation may have been one contributor to the loss of owls from the restoration site. These findings suggest the need to prioritise re-establishment of prey communities for effective long-term recovery of burrowing owls in restored landscapes.

**Key words:** *Athene cunicularia*, burrowing owl, food web, raptor, restoration ecology, rodent



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## Introduction

Grassland ecosystems have undergone severe degradation due to human activity and are among the most threatened ecosystems globally (Samson and Knopf 1994; Gang et al. 2014; Bardgett et al. 2021). Habitat restoration is one of the principal tools for recovering grassland biodiversity and ecosystem services (Martin et al. 2005; Déri et al. 2011; Török et al. 2021). Because restoration efforts often employ a bottom-up approach focused on re-establishing native plant communities, a major ongoing challenge is supporting higher trophic levels in restored grasslands (Serrouya et al. 2011; Fraser et al. 2015). As such, monitoring ecosystem functionality in terms of trophic interactions is crucial for holistically assessing the success of restoration efforts (Martin et al. 2005; Wortley et al. 2013; Vander Zanden et al. 2016).

In California, where grasslands have undergone large-scale degradation (Hamilton et al. 2002), restoration has been emphasised as a tool for providing foraging habitat for threatened raptor populations, with variable success (Woodbridge 1998; Watson 2018; Wolf et al. 2018). In particular, the success of these interventions depends on the concomitant re-establishment of prey



communities (Wolf et al. 2018). Western burrowing owls (*Athene cunicularia hypugaea* (Bonaparte, 1825)) are widely distributed dietary generalists that subsist on a broad range of arthropod and vertebrate prey (Martin 1973). Once considered common throughout California, burrowing owls have declined across the State, with the most severe declines occurring in coastal grasslands (Dawson 1923; Martin 1973; DeSante et al. 2003; Kidd et al. 2003; Sauer et al. 2022; Center for Biological Diversity 2024). The species is now classified as a Species of Special Concern (Shuford and Gardali 2008) and was recently petitioned to be listed as Endangered or Threatened under the California Endangered Species Act (Center for Biological Diversity 2024). Grassland restoration could help mitigate habitat loss for burrowing owls, provided that their associated prey communities can be successfully re-established.

Here, we present a case study of the diet of a small burrowing owl population occupying a newly-restored coastal California grassland. Following initial restoration efforts, wintering burrowing owls temporarily colonised the restoration site, but did not persist at the site. We evaluate the diet of burrowing owls during their occupancy of the restoration site, in particular, assessing their relative intake of vertebrate prey (e.g. rodents), which may be limited in young restoration sites (Patten 1997; Wolf et al. 2018). In doing so, we provide insights into potential challenges faced by burrowing owls foraging in newly-restored grasslands.

## Methods

### Restoration site and burrowing owl population

We surveyed the diet of burrowing owls nesting at the North Campus Open Space (NCOS) restoration site on the University of California, Santa Barbara campus in Goleta, California (34.419758, -119.875793); (Cheadle Center 2016). NCOS represents 0.5 km<sup>2</sup> of upland and wetland habitats restored from the site's previous conversion to the Ocean Meadows golf course in the 1960s. Restoration of the site began in 2017 and is ongoing. This restoration site is characterised by grasslands, coastal sage scrub and salt marsh habitat bordered by eucalyptus windrows. After initial site grading, approximately 60 refuge features were created by partially filling 1-metre-deep holes with off-set concrete slabs, with the intention of providing refugia for small mammals, birds and reptiles. In 2020, six additional artificial burrows with longer passageways and an inner chamber were installed specifically to support burrowing owls.

### Pellet collection, dissection and morphological identification of faunal remains

To assess burrowing owl diet composition, we collected 34 pellets from three artificial burrows between September 2020 and April 2021. To prevent disturbance, we collected pellets no more than once per week and only when owls were not present. After collection, we stored pellets at -20 °C until dissection.

We followed standard protocols for owl pellet dissection (Lyman et al. 2003; Moulton et al. 2006; Chandler et al. 2016) and identified faunal remains to order, genus or species where possible. Rodent identifications were performed

by Paul W. Collins (Curator Emeritus of Vertebrate Zoology, Santa Barbara Museum of Natural History) and invertebrate identifications were performed by Katja C. Seltsmann (Cheadle Center for Biodiversity and Ecological Restoration, University of California, Santa Barbara). We counted individuals in each pellet as the minimum possible number of individuals inferred from anatomical fragments (e.g. two rodent femurs found in one pellet would be scored as a single rodent; two earwig cerci would be scored as a single earwig). For each prey category, we calculated the frequency of occurrence in pellets as the percentage of pellets containing at least one individual of that prey category. To estimate biomass of each prey category, we sourced body mass values from literature, inferring at the genus level and averaging across sexes where necessary (Gettinger 1984; Thompson 1985; Velarde et al. 2007; Hodson et al. 2011; Kovac and Stabentheiner 2012; Ball et al. 2015; Lefebvre et al. 2019). For the unidentified *Rodentia*, we assumed mass was equal to that of the most common rodent sample in our study (*Reithrodontomys megalotis* Baird, 1857). Likewise, for the unidentified Coleoptera, we assumed mass was equal to that of the most common beetle genus in our study (*Calanthus* sp. Bonelli, 1810). All pellets and contents were deposited in the UCSB Vertebrate Zoology Collection (see Suppl. material 1 for accession numbers).

## Results

Between winter 2018–19 and winter 2020–21, three burrowing owls were observed overwintering in the artificial burrows at NCOS. No breeding behaviour was observed, as no owls persisted at the site into the breeding season. Across the 34 pellets sampled from this small population, we identified a total of 1533 prey individuals (Table 1). Pellets contained on average 45.1 individuals per pellet (std. error = 6.1). Every pellet contained invertebrates (all arthropods), which represented 97.4% of prey by number and 22.8% by biomass (inferred from literature estimates). Remains of orthopterans (Gryllidae and Caelifera) were identified in 22 pellets, but no body parts were sufficiently recognisable to reliably count and so are excluded from these estimates. Twenty-one pellets contained vertebrate prey (2.6% by number, 77.2% by biomass), all of which were rodents.

## Discussion

In this study, we provide evidence that an ephemeral burrowing owl population subsisted primarily on arthropods during its brief occupancy of a newly-restored grassland. In Santa Barbara County, CA, where burrowing owls have been nearly extirpated (Lehman 1994), grassland restoration is a crucial first step in efforts to conserve threatened raptors. However, small mammal populations may not immediately or reliably re-occupy restored grasslands (Wolf et al. 2018), limiting food opportunities for predatory species. Given the local precarity of burrowing owls, our focal population was small and transient, limiting our pellet sample size and the generalisability of our findings. However, samples like these that capture temporary occupancy by locally rare species can provide valuable insights into the ecological factors driving local population declines.

**Table 1.** Prey items represented as the total number of individuals found across all 34 pellets ( $n$ ), the percent of the total number of prey items across all pellets, the frequency of occurrence (percentage of pellets containing at least one of a given taxonomic group) and the total estimated biomass across all samples.

Prey Item	Common Name	$n$	% of prey items	% freq.	Estimated Biomass (g)
<i>Forficula auricularia</i> Linnaeus, 1758	European earwig	728	47.5	94.1	55.3
Coleoptera	Beetles	394	25.7	82.3	43.3
<i>Armadillidium</i> sp. Brandt, 1831	Woodlouse	331	21.5	32.3	32.4
<i>Vespula pennsylvanica</i> (de Saussure, 1857)	Western yellowjacket	40	0.2	11.8	3.0
<i>Reithrodontomys megalotis</i> Baird, 1857	Western harvest mouse	24	1.6	29.4	208.7
Unidentified Rodentia sp.	Rodent	10	0.6	20.6	87.0
<i>Mus musculus</i> Linnaeus, 1758	House mouse	5	0.3	8.8	53
<i>Thomomys bottae</i> Eydoux & Gervais, 1836	Botta's pocket gopher	1	0.1	2.9	103.8
Orthoptera	Grasshoppers, locusts, crickets	NA	NA	64.7	NA

Prey species identified in our sample were consistent with known burrowing owl diet preferences (Coulombe 1971; Barrows 1989; Littles et al. 2007; MacCracken et al. 2021; Gonzalez Rojas et al. 2022). Pellet contents were dominated by European earwigs (*Forficula auricularia* Linnaeus, 1758); (as in Coulombe 1971), which tend to associate with owl burrows and which were identified in all but two pellets. The western harvest mouse (*Reithrodontomys megalotis* Baird, 1857) was the most common vertebrate prey species, identified in nearly a third of pellets. The one notable exception to known burrowing owl prey in our sample was the western yellowjacket [*Vespula pennsylvanica* (de Saussure, 1857)], found in four pellets, with one pellet consisting entirely of 28 yellowjackets. Some birds are known to prey on stinging social wasps (Birkhead 1974; Raw 1997), though, to our knowledge, this behaviour has not been reported for burrowing owls. Repeated consumption of aggressive, venomous prey is another possible indicator that preferred prey may be limiting at the restoration site.

The relative abundance of invertebrates in burrowing owl pellets is highly variable across seasons and habitats (Table 2). In our study, invertebrates represented 97.4% of prey items by number, placing it at the upper end of known values from published burrowing owl pellet data (mean = 82.1% invertebrates, range = 55–98%); (Marti 1974; Schlatter et al. 1980; Thompson and Anderson 1988; Barrows 1989; Schmutz et al. 1991; Plumpton and Lutz 1993; Moulton et al. 2006; Littles et al. 2007; Nabte et al. 2008; Mrykalo et al. 2009; Trulio and Higgins 2012; Gonzalez Rojas et al. 2022); (Table 2). Notably, invertebrates were identified in all pellets, in contrast to previous studies that identified invertebrates in fewer than two-thirds of pellets (Thomsen 1971; Tyler 1983; Barrows 1989; Mills 2016; MacCracken et al. 2021). Importantly, our invertebrate frequency and biomass estimates are underestimates, due to our exclusion of orthopterans, which we were unable to reliably count, but which were found in two-thirds of pellets.

Because burrowing owl diet composition is thought to be strongly related to prey availability (Errington and Bennett 1935; Barrows 1989; Plumpton and Lutz 1993), the low proportion of vertebrate prey in pellets suggests low rodent abundance and/or strong competition for rodent prey at the restoration site. This possibility is supported by small mammal survey data from the site (Rickard 2023). The live-trap capture rate of rodents in in spring 2021, the time of our study, was 0.04 individuals per trap per night (4% trapping success). Survey data from an adjacent natural reserve site (Coal Oil Point Reserve, 34.408212, -119.877952)

**Table 2.** Relative proportion of invertebrates in burrowing owl diets characterised in previous pellet analyses. Only studies reporting absolute frequencies of prey items are included. Where multiple years or sampling sites were reported in a single study, we report the mean value.

Reference	Sampling time	Study location	% Invertebrates (by number)	% Invertebrates (by mass)
Present study	Fall 2020–spring 2021	Santa Barbara Co., CA, USA	97.4	22.8
Marti (1974)	Year-round, 1966–1970	Larimer Co., CO, USA	90	NA
Schlatter et al. (1980)	Summer 1973 – spring 1974	La Dehesa, Chile	78.6	NA
Thompson and Anderson (1988)	Summers only, 1982–1983	Natrona Co., Goshen Co., WY, USA	88	5
Barrows 1989	Year-round, 1986–1988	Coachella Valley, CA, USA	73.7	NA
Schmutz et al. (1991)	Summer 1990	South-eastern Alberta, Canada	64	NA
Plumpton and Lutz (1993)	Summers only, 1990–1991	Adams Co., CO, USA	55.5	NA
Moulton et al. (2006)	Spring only, 2001–2002	South-western Idaho, USA	95	NA
Littles et al. (2007)	Winter only, 1999–2004	Southern coastal Texas, USA	98	29
Nabte et al. (2008)	Winter and summer, 2001–2002	Chubut, Argentina	77.2	5.6
Mrykalo et al. (2009)	Year-round, 2003–2004	Southwest FL, USA	97.5	NA
Trulio and Higgins (2012)	Year-round, 2005–2006	Santa Clara Co., CA, USA	94	30
Chandler et al. (2016)	Autumn 2010 – spring 2011	Southeast Farallon Island, CA, USA	66	1.5
Gonzalez Rojas et al. (2022)	Winter only, 2002–2005	Llano la Soledad, Nuevo Leon, Mexico	90	16

suggest a similar rodent community, but higher capture rates (9% trapping success); (Conroy 2005). This survey was similarly conducted in the spring, but years prior to our study (2005); interannual fluctuations in rodent populations prevent us from drawing strong conclusions from the comparison. However, rodent survey data from other regions of the burrowing owls' range lend additional support to the hypothesis that rodent prey was not abundant at our site (Moulton et al. 2006: 14.5% trapping success, Mills 2016: 33.6% trapping success).

As dietary generalists, burrowing owls often subsist on diets high in invertebrates (Moulton et al. 2006; Littles et al. 2007; Trulio and Higgins 2012). However, fledgling success and productivity may increase with the availability of vertebrate prey (Poulin et al. 2001, 2001; Ronan 2002). While our case study points to the possibility of preferred prey limitation at the site, our sample size was strongly limited by the small and transient nature of our focal burrowing owl population. Larger studies are necessary to understand the extent to which diet influences site fidelity in restoration sites, though such studies are made increasingly challenging by burrowing owl population declines.

Diet quality is one of many interacting ecological factors that determine habitat suitability for burrowing owls. We observed large raptors at the site, which may present competition for rodent prey as well as predation risks to burrowing owls. During the study period, we observed two instances of burrowing owl predation (one attempted, one successful) by red-tailed hawks (*Buteo jamaicensis* (Gmelin, 1788)) and one instance of a burrowing owl being repeatedly pursued by a peregrine falcon (*Falco peregrinus* Tunstall, 1771). The risk of predation and effects of interspecific competition may have additionally reduced the attractiveness of the restoration site for burrowing owls and contributed to their eventual abandonment of the site. Together with our diet findings, these observations emphasise the need to consider predator and prey populations when providing habitat for burrowing owls in restored habitats.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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## Author contributions

Conceptualization: KCCS, MMO, KS, AR, LS. Formal analysis: MMO. Investigation: KCCS, AR, KS, LS. Writing - original draft: MMO. Writing - review and editing: LS, MMO, KCCS.

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## Data availability

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

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## Supplementary material 1

### Pellet contents raw data

Authors: Madeleine M. Ostwald, Kyra Sullivan, Lisa Stratton, Alison Rickard, Katja C. Seltmann

Data type: csv

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## Research Article

# Conservation priorities for threatened mammals of Vietnam: Implementation of the IUCN's One Plan Approach

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## Abstract

Vietnam belongs to one of the most well-known global biodiversity hotspots. However, its biodiversity is being threatened by multiple factors such as rapid growth of the human population, habitat destruction, overexploitation, and climate change. To better prioritize conservation measures for Vietnam's mammal fauna, this study attempts to identify the threat status, level of endemism, and protected area coverage for approximately 329 recognized taxa. In addition, *ex situ* conservation measures are investigated by analyzing zoo databases. Our results show that as many as 112 mammal species (~34%) in Vietnam are officially listed as threatened, 36 (~11%) are country endemics, and nine (~3%) are micro-endemic. Nearly all threatened species are currently covered by protected areas, except for two species of high conservation importance, i.e., the micro-endemic *Murina harpioloides* (Chiroptera) and the threatened *Lepus sinensis* (Lagomorpha). Our results also suggest that such areas as Hoang Lien Range, northeastern Vietnam, and the central and southern Annamite Mountains require more conservation attention, as they harbor a high number of endemic species. Analyses of the Zoological Information Management System reveal that roughly 60% (67 species) of threatened species are currently kept in zoos (*ex situ*) across the world, while none of the species listed as "Data Deficient" are currently represented in any *ex situ* program. Up to 66% of 89 species kept in zoos show a good number of breeding successes in the past 12 months. It is recommended that future conservation efforts focus on unprotected threatened mammals and on evaluating the threat status for "Data Deficient" species. Additionally, the existing protected area system's effectiveness should be improved by creating corridors to provide connectivity for highly mobile and widely distributed mammal species.

**Key words:** Conservation breeding, diversity analysis, endemic species, mammal conservation, protected area coverage, richness analysis, threat assessment

## Introduction

The consequences of anthropogenic climate change and destructive land use have led, and are leading, to the global biodiversity crisis (Habel et al. 2019; Ceballos et al. 2020). The International Union for Conservation of Nature and Natural Resources (hereafter IUCN) currently lists about 41,000 species as threatened with extinction. With regard to mammals, up to 27% are currently classified as threatened (IUCN Red List 2024).

Climate change is currently affecting 19% of species listed as threatened or near threatened by the IUCN (Maxwell et al. 2016). Besides climate change, the biggest threats to biodiversity today are overexploitation of wild animals and natural resources, as well as habitat loss due to intensification of agriculture (Maxwell et al. 2016). Wild animals are most threatened by the global unsustainable use of bush meat, while at the same time, many people around the world are dependent on this source of protein for nutrition (Mainka and Trivedi 2002). As the anthropogenic climate change is accelerating, a higher proportion of wild animals is expected to be threatened in the future (Habel et al. 2019). Hence, it is expected that greater numbers of species will require urgent conservation actions. Thus, the conservation goal today is not only to avoid extinction but also to focus on species recovery (Redford et al. 2011).

For enhancing conservation measures, the Conservation Planning Specialist Group (CPSG) of the IUCN Species Survival Commission (SSC) proposed the One Plan Approach to Conservation, which aims to combine conservation efforts, from both inside (*in situ*) and outside (*ex situ*) species natural ranges (Byers et al. 2013). On top of that, it outlines the objective to develop one integrated conservation plan for all threatened species (IUCN/SSC 2014).

In order to at least slow down or at best prevent further loss of biodiversity, species need to be safeguarded within protected areas (PAs), an effective measure to achieve long-term conservation of nature and healthy ecosystems (MacKinnon et al. 2020). As a complementary action to *in situ* conservation, whether because it is difficult or impossible to carry out at time, *ex situ* conservation has been demonstrated to be a crucial solution through building up assurance colonies of animals, viz. by keeping and breeding them in zoological institutions. In comparison to *in situ* conservation, the main difference is the ability to buy time (Byers et al. 2013). Successful breeding programs, which enable the restoration of wild populations at a later stage once problems on site are resolved, have saved many species from extinction (Price 1989; Goodwin and Langenhorst 2014; Larska and Krzysiak 2019). In addition to maintaining assurance colonies of threatened species, zoos also actively support *in situ* conservation measures with monitoring and restocking projects or expertise acquired through *ex situ* conservation programs (Che-Castaldo et al. 2018).

As one of the global biodiversity hotspots, Vietnam hosts a large variety of ecosystems, with high mountains reaching up to more than 3000 m above sea level and a 3260 km long coastline (Rambaldi et al. 2001) and inland lakes and rivers like the Red River and Mekong River with their significant deltas (Sterling et al. 2006). The country is home to more than 300 mammal species (Nguyen et al. 2020), with 20% categorized as globally threatened (IUCN 2024). Within Vietnam, there are a number of areas with exceptional species richness and often a high proportion of endemic species. One example for those endemism centers

is the Annamite Mountain Range stretching primarily along the Lao-Vietnamese border with massive mountain ridges (Sterling et al. 2006; Tilker et al. 2019; CEPF 2020). Vietnam's mammal species are currently threatened by habitat loss and degradation (McDonald et al. 2018), poaching (Mainka and Trivedi 2002), and a lack of knowledge on species distribution and threat status (Borgelt et al. 2022).

The aim of the present study is to assess priorities of *in situ* and *ex situ* conservation for threatened mammals of Vietnam. Specifically, we investigated: 1. How is the mammal fauna of Vietnam distributed when accounting for species specific habitat preferences and where are centers of local endemism that require special conservation attention? 2. Which species are not yet covered by protected areas? 3. Which proportion of the mammal fauna is sufficiently represented in zoological collections to serve as backup populations, and how do global zoological institutions invest in conserving Vietnam's mammal fauna? 4. Which actions are needed to improve both *in situ* and *ex situ* efforts?

## Materials and methods

### Species list

The initial species list was based on the "Checklist of Mammals in Vietnam" (Dang et al. 2008). Additional species were incorporated using more recent publications. While the majority of taxa were included in analyses based on the species level, six subspecies were incorporated in the study, because they are valid taxa and reported to occur in Vietnam (*Chodsigoa parca lowei*, *Callosciurus finlaysonii honnghensis*, *Mustela nivalis tonkinensis*, *Murina lorelieae ngoclinhensis*, *Panthera tigris corbetti*, and *Rhinolophus rex paradoxolophus*). *Euroscaptor parvidens ngoclinhensis* was elevated to species status, *Euroscaptor ngoclinhensis* (Bui et al. 2020), and *Muntiacus puhoatensis* was synonymized with *M. rooseveltorum* based on findings of recent molecular analysis (Le M, unpublished data).

### Species distribution

Species distribution data was obtained from the IUCN Red List in August 2022. For species whose distributions were not clearly specified in the list (Table 1), shapefiles were generated using software package QGIS 3.24. Their distribution ranges were generated based on records extracted from various research papers as well as those available at the Institute of Ecology and Biological Resources (IEBR) in Hanoi (Nguyen ST, Motokawa M, and Oshida T, unpublished data). As range estimate, radial buffers of 20 km around each record were used. *Mus cervicolor* and *Olisthomys morrisi* were not included, because these species ranges are currently unknown.

Species range estimates were refined using relevant functions of the redlist (Gearty et al. 2022) and raster package v3.6-26 (Hijmans et al. 2023) for R 4.2.2. by accounting for the availability of suitable microhabitats within the range polygons as mapped by Jung et al. (2020). The microhabitat map had a spatial resolution of 100 m and is based on habitat types as defined by the IUCN (Jung et al. 2020). Subsequently, we assessed for each protected area, in which species are likely to occur given its suitable habitats. Finally, we computed species richness maps and identified areas of high local endemism using

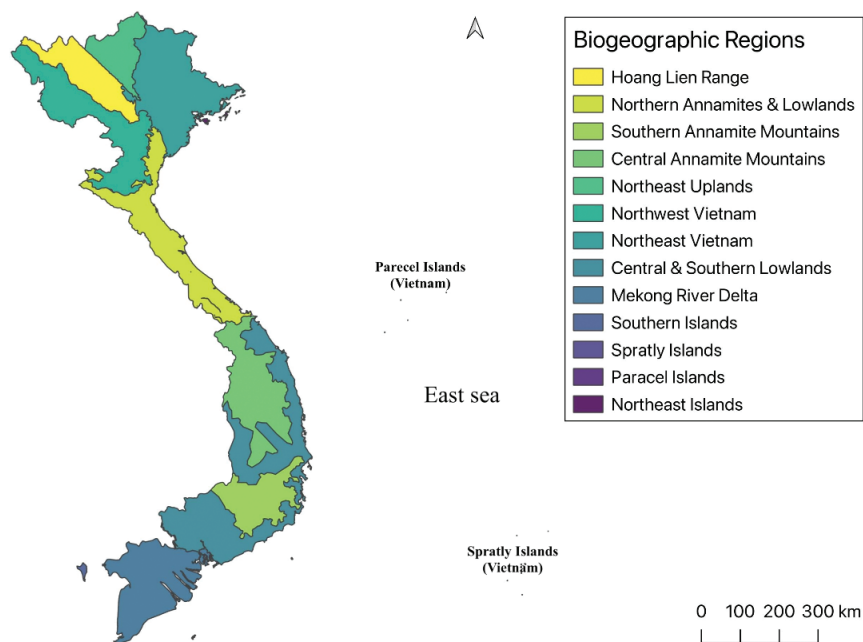


the corrected weighted endemism approach (Crisp et al. 2001). While species richness only measures the total count of species per grid cell, the weighted endemism approach specifically focuses on range-restricted species. The species distribution areas get scored by how many grid cells they obtain. The more grid cells they cover, the lower the score per species. The scores of all endemic species per cell are summed up to calculate the weighted endemism.

### Distribution categories

For classifying species distribution, QGIS was used to construct a map of Vietnam's biogeographic regions (see Fig. 1). The regions are based on those proposed by Bain and Hurley (2011) and the "Terrestrial Ecoregions of the World" (Olson et al. 2001). Two areas of endemism, Cat Ba Island and Hoang Lien Mountain Range, were added based on the number of endemic species reported from the area. The map's underlying shapefiles were drawn from the World Wildlife Fund's map (Olson et al. 2001).

Species distributions were categorized as summarized in Table 1.



**Figure 1.** Biogeographic regions of Vietnam sorted from highest elevation values to lowest.

**Table 1.** Criteria for classifying distribution areas of mammals in Vietnam.

Distribution Category	Criteria
<b>Endemic</b>	Exclusively distributed within Vietnam borders
<b>Widespread</b>	Distributed over multiple biogeographic regions in Vietnam
<b>Regionally Endemic</b>	Exclusively distributed within Vietnam borders. Distributed in only/mostly one biogeographic region in Vietnam
<b>Micro-Endemic</b>	Exclusively distributed within Vietnam borders. Distributed in only one biogeographic region and the distribution area covering at most 1% of the total area of Vietnam ( $\leq 3,317 \text{ m}^2$ )
<b>Cross-Border Species</b>	Not endemic to Vietnam but the distribution area within Vietnam covering at most 1% of the total area of Vietnam

Some species were categorized as regionally endemic and widespread. The former applies to species which are distributed in two bordering biogeographic regions, but at least 80% of the distribution is in one of the regions.

### **Threat status assessment**

For assessment of the individual threat status of each species, four different data sources were used: the IUCN Red List (IUCN 2024; version 2024.1), the Vietnam Red Data Book (VNRDB 2024) (in press), appendices of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), and the updated Decree on management of endangered, precious and rare forest plants and animals and implementation of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (hereafter Decree 84/2021). Where data sources showed distinct discrepancies in ranking a species, the most recent assessment was used. Decree 84/2021 includes threatened species of wild fauna and flora of Vietnam. It was developed according to the Vietnamese law on biodiversity enacted in 2017 (Law No. 16/2017/QH14 on Forest Law 2017) and serves as a legal document for species protection, focusing on endangered and heavily traded taxa.

Species are considered threatened if they are either classified as VU, EN or CR by the IUCN Red List and/or by the VNRDB or listed in appendices CITES as of 2022 and/or in Decree 84/2021. Species which are referred to as “Data Deficient” (DD) are those not listed in any of the databases used for assessing threat status or listed as DD in the existing databases.

### ***In situ* populations / Protected area and OECM coverage**

The World Database of Protected Areas (WDPA) and the World Database of Other Effective Area-Based Conservation Measures (WDOECM) are the most comprehensive databases on terrestrial and marine PAs and OECMs. For Vietnam, there are no OECMs listed in the database. Protected Areas in Vietnam are designated in eleven different categories. All designations are followed when referring to PAs.

### ***Ex situ* populations / Husbandry database**

The Species 360 Zoological Information Management System (ZIMS) (Species360 2022) was used to analyze the representation of Vietnamese mammals in zoos worldwide. The ZIMS data was downloaded on August 19<sup>th</sup> and 20<sup>th</sup> 2022. All institutions listed in ZIMS are in the following referred to as zoos. Although this is the most comprehensive database for animal holdings, it is important to note that not all zoological institutions worldwide contribute their data to ZIMS.

Information of species categorized as threatened in Vietnam currently kept in zoos worldwide was included in our analysis. Of particular interest was the number of threatened, micro-endemic and DD species and their breeding success in the past 12 months. To create an overview of Vietnamese mammals in zoo holdings worldwide, a map was generated. It contains the number of zoos per country which are keeping mammals documented in Vietnam and the number of individuals per zoo. The mammal species diversity of each institution was illus-

trated using the Shannon-Weaver Index ( $H = -\sum p_i \log(b)p_i$ ) (Shannon and Weaver 1994) computed with the R package *vegan* v2.6-4 (Oksanen et al. 2022), where  $p_i$  is the proportion of species  $i$ , and the sum is over the total number of species.

## Results

### Mammal diversity

The total number of mammal taxa in Vietnam was estimated at 329, including the six subspecies *Chodsigoa parca lowei*, *Callosciurus finlaysonii honnghensis*, *Mustela nivalis tonkinensis*, *Murina lorelieae ngoclinhensis*, *Panthera tigris corbetti*, and *Rhinolophus rex paradoxolophus*. A list of species with endemism levels is presented in Suppl. material 1. With 128 species, Chiroptera is the most speciose order and makes up around 39%, followed by Rodentia with 77 species, accounting for 23.4% of the total mammal diversity in Vietnam. Five orders, only represented by very few species, include Lagomorpha with three, Pholidota and Scandentia both with two, and Dermoptera and Proboscidea with only one species each. Among 36 families of mammals recorded in Vietnam, the ten most diverse families belong to six orders with greatest species richness, i.e., Chiroptera, Rodentia, Carnivora, Eulipotyphla, Primates, and Artiodactyla (Suppl. material 1).

### Species distribution and richness analysis

Vietnam is home to 36 endemic mammals, making up approximately 11% of the country's mammal diversity. The distribution analysis revealed a total of nine micro-endemic species listed in Table 2, *Tragulus versicolor* (Artiodactyla), *Trachypithecus poliocephalus* (Primates) and *Murina kontumensis* (Chiroptera) are also listed as threatened. Another five micro-endemic species are classified as DD.

**Table 2.** Micro-endemic mammal species in Vietnam (distribution area is 1% of the total area of Vietnam at most).

Order	Family/Subfamily	Scientific Name	Distribution
<b>ARTIODACTYLA</b>	<b>Tragulidae</b>	<i>Tragulus versicolor</i>	Southern Annamite Mountains (Ninh Thuan, Khanh Hoa)
<b>CHIROPTERA</b>	<b>Vespertilionidae</b>		
	Murininae	<i>Murina harpioloides</i>	Central Highland Mountains (Lam Dong)
<b>EULIPOTYPHILA</b>	<b>Soricidae</b>		
	Crociturinae	<i>Crociturus annamitensis</i>	Northern Annamite Mountains (Huong Son Camp, Ha Tinh)
		<i>C. guy</i>	Northeast Vietnam (Tuyen Quang)
		<i>C. phuquocensis</i>	Southern Island (Phu Quoc)
		<i>C. sokolovi</i>	Central Annamite Mountains (Ngoc Linh, Kon Tum)
<b>PRIMATES</b>	<b>Cercopithecidae</b>		
	Colobinae	<i>Trachypithecus poliocephalus</i>	Northeast Vietnam Islands (Cat Ba)
<b>RODENTIA</b>	<b>Sciuridae</b>		
	Callosciurinae	<i>Callosciurus honkhoaiensis</i>	Mekong River Delta (southern island, Hon Khoai Island)
	<b>Muridae</b>		
	Murininae	<i>Rattus germaini</i>	Southern Island (Con Son Island)

**Table 3.** Important cross-border species with threat status assessment.

Order	Family/Subfamily	Scientific Name	Threat Status (IUCN 2024/VNRDB 2024)
CHIROPTERA	Emballonuridae	<i>Saccolaimus saccolaimus</i>	LC (IUCN)
	Hipposideridae	<i>Hipposideros khaokhouayensis</i>	VU (IUCN) EN (VNRDB)
	Pteropodidae	<i>Pteropus hypomelanus</i>	NT (IUCN) EN (VNRDB)
	Rhinolophidae	<i>Rhinolophus osgoodi</i>	LC (IUCN)
	Vespertilionidae		
	Myotinae	<i>Myotis annamiticus</i>	DD (IUCN)
	Murinae	<i>Murina chrysochaetes</i>	DD (IUCN)
EULIPOTYPHILA	Soricidae		
	Crocidae	<i>Crocodylus wuchihensis</i>	DD (IUCN)
RODENTIA	Muridae		
	Murinae	<i>Saxatilomys paulinae</i>	DD (IUCN) VU (VNRDB)
		<i>Tonkinomys daovantieni</i>	DD (IUCN) VU (VNRDB)

According to the analysis, some species were revealed not to be endemic to Vietnam, but their distribution range within the country corresponded at most 1% of the landmass. Those nine species classified as important cross-border species are listed in Table 3. *Crocodylus wuchihensis* (Eulipotyphla), *Murina chrysochaetes*, and *Myotis annamiticus* (Chiroptera) are important cross-border species which are listed as DD. Four other species are important cross-border species and are also threatened, comprising *Hipposideros khaokhouayensis* (Chiroptera), *Pteropus hypomelanus* (Chiroptera), *Saxatilomys paulinae* (Rodentia), and *Tonkinomys daovantieni* (Rodentia).

The species richness analysis suggested that the richness hotspots are located mostly in montane areas (Fig. 2). “Northwest Vietnam”, “Hoang Lien Range”, “Northeast Uplands”, “Northeast Vietnam” and the “Annamite Mountains” harbored a high level of mammal richness with a great proportion of threatened species.

The southern part of “Northwest Vietnam”, the “Northern Annamites” and the northern part of the “Central Annamite Mountains” showed an exceptional richness density. Analyses of micro-endemic and cross-border species richness confirmed their high concentration on island habitats like Cat Ba in the North-east or Con Dao and Phu Quoc in the South. The larger area that stands out on the micro-endemic species map belongs to the distribution range of *Tragulus versicolor* (Artiodactyla). The cross-border species marked with a red area in the “Northwest Vietnam” region is *Rhinolophus osgoodi* (Chiroptera) (Fig. 2). The weighted endemism analysis revealed a slightly different picture in emphasizing the importance of northern Vietnam, central and southern Annamite Mountains, and offshore islands.

### Threat assessment

In total, 112 species are currently categorized as threatened. This makes up around 34% of all mammal species in Vietnam and leaves 217 species without threat status (see Suppl. material 1). Out of the 11 orders of mammal species in Vietnam, nine include threatened species (Fig. 3). Scandentia contains no threatened taxa. Dermoptera and Proboscidea are each only represented by one species in Vietnam, and both are regarded as threatened (*Galeopterus variegatus*,

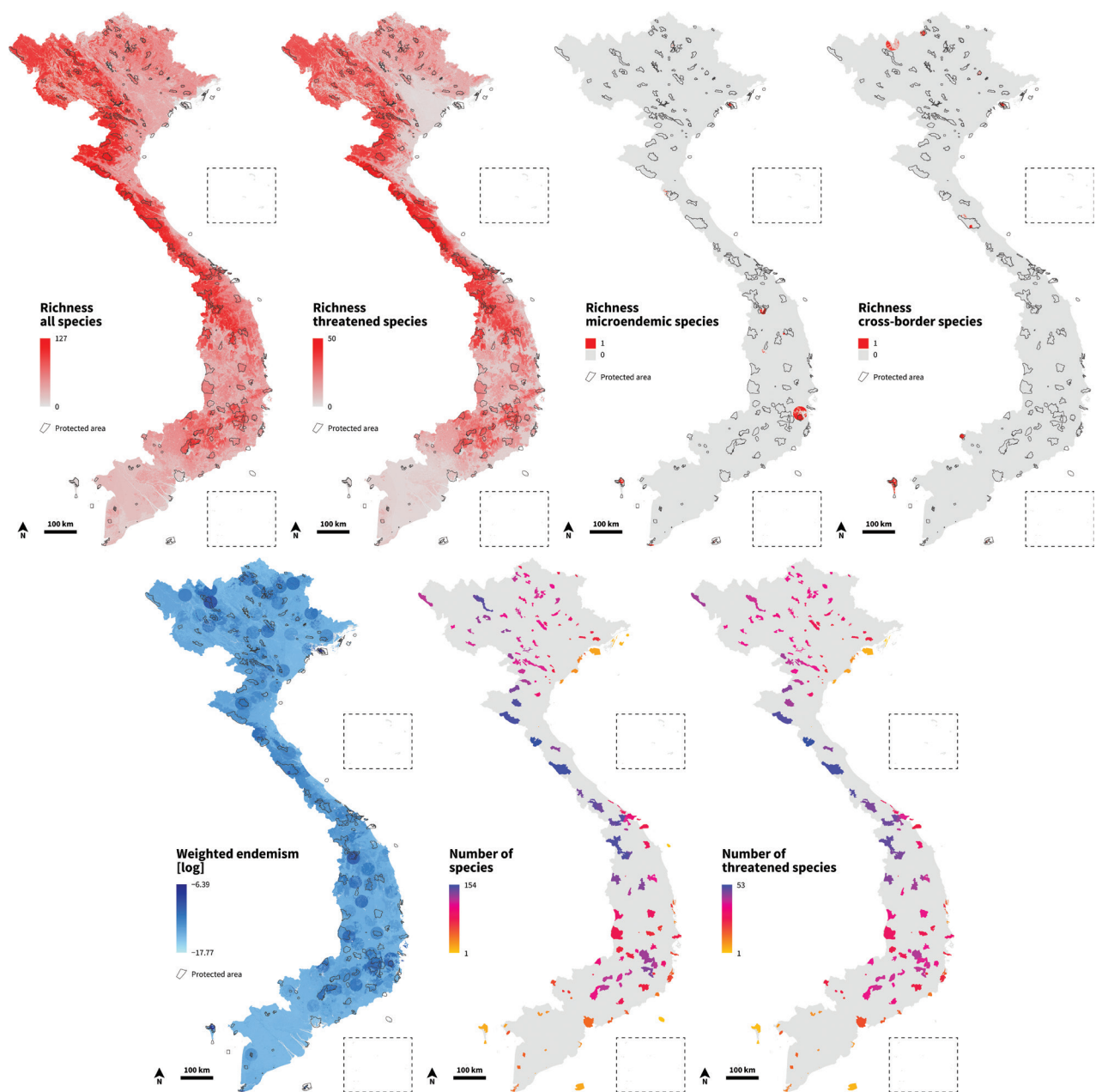


Figure 2. Richness analysis, weighted endemism, and protected area coverage.

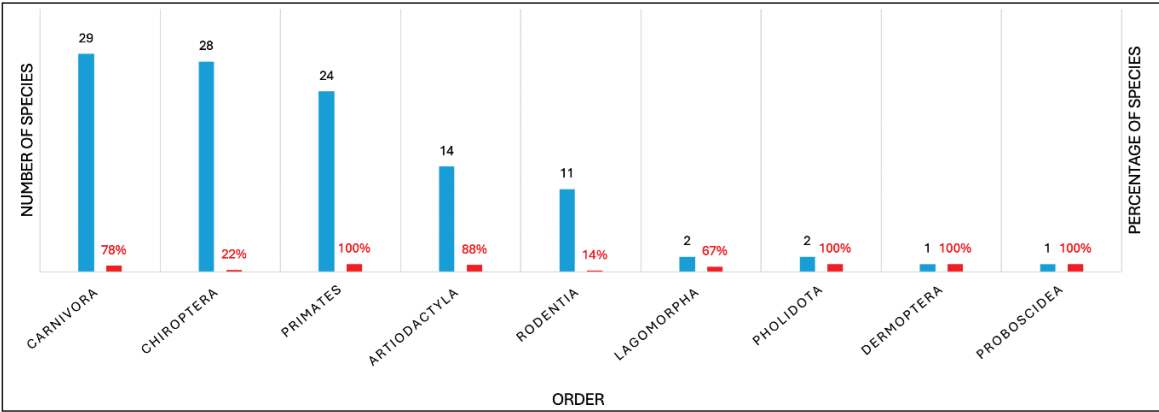


Figure 3. Number and percentage of threatened mammal species in Vietnam per order.

*Elephas maximus*). The Pholidota and Lagomorpha both consist of two threatened species each, namely *Manis javanica* and *M. pentadactyla*, *Nesolagus timminsi* and *Lepus sinensis*, respectively.

Carnivora, Primates, Chiroptera, Artiodactyla and Rodentia are the five orders with the highest numbers of threatened species, three of which comprise more threatened than non-threatened species. Specifically, approximately 78% of Carnivores and 88% of Artiodactyla in Vietnam are considered threatened but even more concerning is 100% of Primates assessed as the same status. While 95% of the 112 threatened species are listed in the IUCN Red List and 96% in the VNRDB, only 17% are listed in CITES and 68% in Decree 84/2021. One species listed as micro-endemic was also categorized as threatened, and therefore incorporated with both groups. This concerns the Cat Ba Langur (*Trachypithecus poliocephalus*) (Cercopithecidae, Primates). In Vietnam 61 species are categorized as “Data Deficient” (DD) (see Table 4). The important cross-border species *Crociodura wuchihensis* and *Murina chrysochaetes* are also classified as DD. Five of nine (56%) micro-endemic taxa fall into this category. In the following, these species were included in both datasets, i.e., DD and micro-endemic.

Most DD species belong to the order Eulipotyphla (21 species), followed by Chiroptera (20 species) and Rodentia (16 species). Those species are all quite small and elusive, making them difficult to study in the field.

**Table 4.** Data deficient (DD) species or subspecies, not listed in any of the databases used for the threat status assessment or classified as DD.

Order	Family / Subfamily	Scientific Name
CARNIVORA	Herpestidae	<i>Urva javanica</i>
		<i>U. urva</i>
	Mustelidae	
CHIROPTERA	Mustelinae	<i>Melogale cucphuongensis</i>
		<i>Mustela nivalis tonkinensis</i>
	Emballonuridae	<i>Taphozous longimamus</i>
	Miniopteridae	<i>Miniopterus fuliginosus</i>
	Rhinolophidae	<i>Rhinolophus chaseni</i>
		<i>R. perniger</i>
		<i>R. yunnanensis</i>
	Vespertilionidae	
	Murinae	<i>Murina chrysochaetes</i>
		<i>M. walstoni</i>
	Myotinae	<i>Myotis alticraniatus</i>
		<i>M. ancricola</i>
		<i>M. annamiticus</i>
		<i>M. annatessae</i>
		<i>M. indochinensis</i>
		<i>M. montivagus</i>
		<i>M. phanluongi</i>
	Vespertilioninae	<i>Cassistrellus yokdonensis</i>
		<i>Hypsugo dolichodon</i>
		<i>Mirotrellus joffrei</i>
		<i>Tylonycteris fulvida</i>
		<i>T. malayana</i>
		<i>T. tonkinensis</i>



Order	Family / Subfamily	Scientific Name
EULIPOTYPHLA	<b>Soricidae</b>	
	Crocidae	<i>Crocodylus porosus</i>
		<i>C. dracula</i>
		<i>C. guy</i>
		<i>C. kegoensis</i>
		<i>C. phuquocensis</i>
		<i>C. rapax</i>
		<i>C. sapaensis</i>
		<i>C. sokolovi</i>
		<i>C. wuchihensis</i>
	Soricinae	<i>Chimarrogale varennei</i>
		<i>Chodsigoa caovansunga</i>
		<i>C. hoffmanni</i>
		<i>Episoriculus baileyi</i>
		<i>E. umbrinus</i>
	<b>Talpidae</b>	
	Talpinae	<i>Euroscaptor kuznetsovi</i>
		<i>E. orlovi</i>
		<i>E. ngoclinensis</i>
		<i>E. parvidens</i>
		<i>E. subanura</i>
		<i>Mogera latouchi</i>
		<i>Uropsilus fansipanensis</i>
RODENTIA	<b>Muridae</b>	
	Murinae	<i>Berylmys mackenziei</i>
		<i>Chiromyscus langbianis</i>
		<i>C. thomasi</i>
		<i>Dacnomys millardi</i>
		<i>Hapalomys suntsovi</i>
		<i>Leopoldamys herberti</i>
		<i>L. revertens</i>
		<i>Micromys erythrotis</i>
		<i>Niviventer bukit</i>
		<i>N. lotipes</i>
		<i>N. mekongis</i>
		<i>Rattus germani</i>
	<b>Sciuridae</b>	
	Callosciurinae	<i>Callosciurus finlaysonii honnghensis</i>
		<i>Dremomys gularis</i>
		<i>Tamias maclellandii</i>
	Sciurinae	<i>Olisthymys morrisi</i>

### ***In situ* populations / Protected area coverage**

In our analyses, 175 PAs were reviewed. Fig. 2 shows the number of species covered by each PA. During analysis of the species' PA coverage, the focus was placed on threatened and micro-endemic species. Approximately 83% of those are already protected by at least three PAs in Vietnam with eight more species currently distributed within at least two protected areas. Four of those eight are micro-endemic and five are threatened species. One micro-endemic species is listed as threatened, while the other three micro-endemic species are DD. Table 5 lists all threatened and micro-endemic species, which occur in only one or zero PAs in Vietnam. Of a total of ten species, seven are threatened. Species currently not covered by any PA include micro-endemic *Murina harpioloides* (Chiroptera) and threatened *Lepus sinensis* (Lagomorpha).

### Ex situ records/Husbandry database

A total of 89 mammal species reported from Vietnam (27% of all species) are currently kept in zoos and about 75% (67 species) of those are classified as threatened. This makes up roughly 60% of all threatened species, leaving as many as 45 threatened species unprotected by *ex-situ* measures (Table 6). They consist of three important cross-border species, i.e., *Hipposideros khaokhouayensis* (Chiroptera), *Saxatilomys paulinae* and *Tonkinomys daovantieni* (Rodentia) as well as two micro-endemic species, namely *Callosciurus honkhoaensis* (Rodentia) and *Tragulius versicolor* (Artiodactyla), which is also listed as threatened. None of the species listed as DD are currently kept in zoos.

The Cat Ba Langur (*Trachypithecus poliocephalus*) is the only micro-endemic species currently kept in a zoological facility, the Endangered Primate Rescue Center in Cuc Phuong National Park, northern Vietnam. Of all threatened species, around 48% are kept in two institutions or fewer.

Fig. 4 presents the husbandry data of the threatened mammals included in this study. It shows the number of zoos per country, as well as specific numbers of held individuals. There is a high abundance of zoos keeping threatened mammals in the USA, Europe, the United Kingdom, and Japan. However, those in India and Southeast Asian have much greater diversity index and number of held individuals.

Regarding the number of species held in zoos, there are a few only represented by a single individual, including four following threatened species: *Pygathrix nigripes*, *Hylopetes alboniger*, *Petaurista elegans*, and *Prionodon pardicolor*. A total of 56 species, roughly 88% of threatened taxa, are kept in same-sex populations in at least one institution. Assessing the breeding success of threatened species in zoos has shown that most threatened species (66%) are being successfully bred under human care. For 22 species (34%), breeding has remained unsuccessful in the past 12 months (before August 2022) including the ones held in same-sex groups.

**Table 5.** Threatened and micro-endemic mammal species in Vietnam, which occur in one or zero PA and their threat status assessment.

Order	Family/ Subfamily	Scientific Name	Distribution Category	Threat Status (IUCN 2024/VNRDB 2024)
CHIROPTERA	<b>Vespertilionidae</b>			
	Murinae	<i>Murina harpioloides</i>	micro-endemic	EN (VNRDB) EN (IUCN)
	Myotinae	<i>Myotis formosus</i>	widespread	NT (IUCN)
EULIPOTYPHILA	<b>Soricidae</b>			
	Crocidae	<i>Crocodylus annamensis</i>	micro-endemic	DD (IUCN)
		<i>C. guy</i>	micro-endemic	DD (IUCN)
LAGOMORPHA	<b>Leporidae</b>	<i>Lepus sinensis</i>	widespread	EN (VNRDB)
RODENTIA	<b>Diatomyidae</b>	<i>Laonastes aenigmamus</i>	widespread	VU (VNRDB)
	<b>Muridae</b>			
	Murinae	<i>Saxatilomys paulinae</i>	Important Cross-Border Species	VU (VNRDB)
		<i>Tonkinomys daovantieni</i>	Important Cross-Border Species	VU (VNRDB)
	<b>Sciuridae</b>			
	Callosciurinae	<i>Callosciurus honkhoaensis</i>	micro-endemic	

**Table 6.** Threatened mammal species or subspecies, which are currently not kept in zoos.

Order	Family / Subfamily	Scientific Name	Endemic
ARTIODACTYLA	<b>Bovidae</b>		
	Bovinae	<i>Pseudoryx nghetinhensis</i>	N
	<b>Cervidae</b>		
	Cervinae	<i>Muntiacus truongsongensis</i>	N
		<i>M. vuquangensis</i>	N
		<i>M. rooseveltorum</i>	N
CARNIVORA	<b>Moschidae</b>	<i>Moschus berezovskii</i>	N
	<b>Tragulidae</b>	<i>Tragulus versicolor</i>	Y
	<b>Mustelidae</b>		
CHIROPTERA	Lutrinae	<i>Lutra sumatrana</i>	N
	Mustelinae	<i>Mustela strigidorsa</i>	N
	<b>Hipposideridae</b>	<i>Coelops frithii</i>	N
		<i>Hipposideros alongensis</i>	Y
		<i>H. griffini</i>	Y
		<i>H. khaokhouayensis</i>	N
		<i>H. lylei</i>	N
		<i>H. scutinares</i>	N
		<i>H. swinhoei</i>	N
	<b>Molossidae</b>	<i>Mops plicatus</i>	N
	<b>Pteropodidae</b>		
		<i>Macroglobosus minimus</i>	N
	<b>Rhinolophidae</b>	<i>Rhinolophus episcopus</i>	N
		<i>R. marshalli</i>	N
		<i>R. rex paradoxolophus</i>	N
	<b>Vespertilionidae</b>		
	Kerivoulinae	<i>Kerivoula picta</i>	N
	Murinae	<i>Harpiola isodon</i>	N
		<i>Murina harpioloides</i>	Y
		<i>M. kontumensis</i>	Y
		<i>M. lorelieae ngoclinhensis</i>	N
		<i>Phoniscus jagorii</i>	N
	Myotinae	<i>Myotis formosus</i>	N
		<i>M. pilosus</i>	N
	Vespertilioninae	<i>Barbastella darjelingensis</i>	N
		<i>Ia io</i>	N
		<i>Scotomanes ornatus</i>	N
		<i>Thainycteris aureocollaris</i>	N
LAGOMORPHA	<b>Leporidae</b>	<i>Lepus sinensis</i>	N
		<i>Nesolagus timminsi</i>	N
PRIMATES	<b>Cercopithecidae</b>		
	Colobinae	<i>Rhinopithecus avunculus</i>	Y
		<i>Trachypithecus crepusculus</i>	N
		<i>T. francoisi</i>	N
	<b>Hylobatidae</b>	<i>Nomascus nasutus</i>	N
RODENTIA	<b>Diatomyidae</b>	<i>Laonastes aenigmamus</i>	N
	<b>Muridae</b>		
	Murinae	<i>Saxatilomys paulinae</i>	N
		<i>Tonkinomys daovantieni</i>	N
	<b>Platacanthomyidae</b>	<i>Typhlomys chapaensis</i>	N
	<b>Sciuridae</b>		
	Sciurinae	<i>Belomys pearsonii</i>	N
		<i>Hylopetes phayrei</i>	Y
		<i>Petaurista philippensis</i>	N

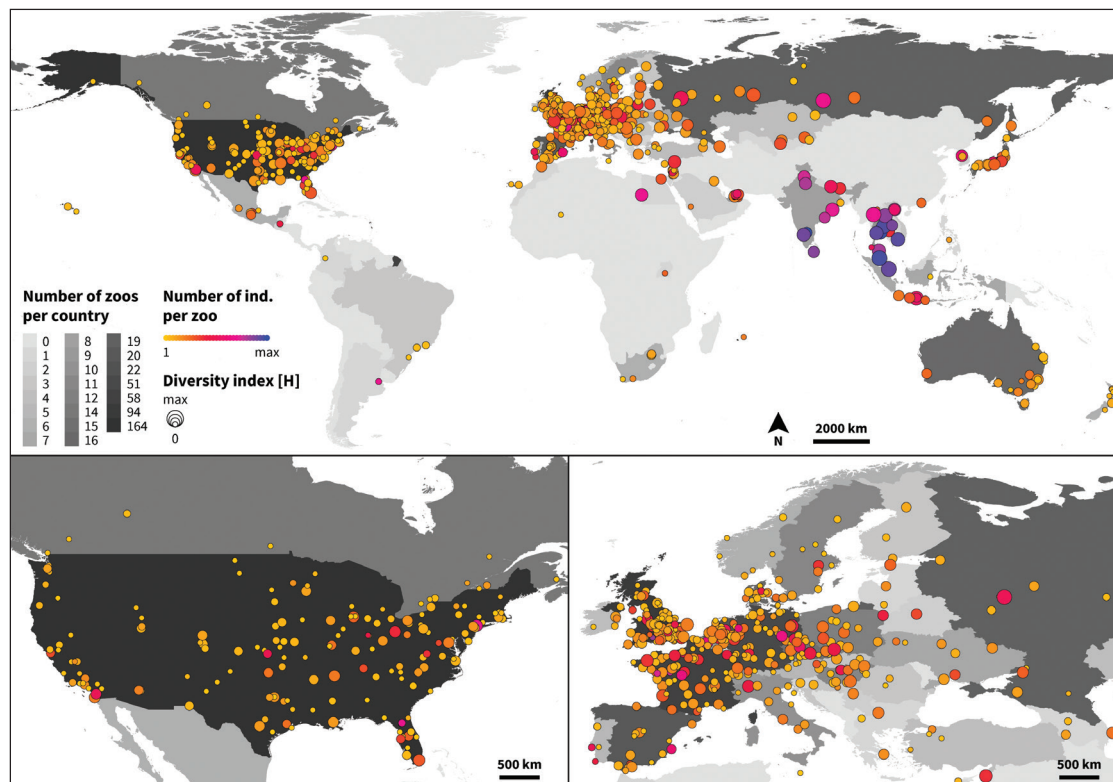


Figure 4. Ex situ preservation of threatened Vietnamese mammals worldwide.

## Discussion

### Assessment of conservation priorities

This study shows that up to 34% of the 329 mammals in Vietnam are currently listed as threatened. The weighted endemism analysis highlights mammal diversity hotspots in montane areas, such as central and southern Annamites. These areas of endemism were also previously highlighted for conservation prioritization in a study on Vietnamese amphibians (Krzikowski et al. 2022). The distribution range of the micro-endemic species *Murina harpioloides* (Chiroptera) also falls within central Annamite Mountains. Micro-endemic species will especially benefit from urgent conservation measures, as they will be particularly vulnerable to extinction risks due to their restricted range. *In-situ* conservation measures are apparently needed for the threatened *Lepus sinensis* (Lagomorpha), as it is currently not protected in its natural habitat. Additionally, five other threatened species (*Myotis formosus*, *Laonastes aenigmamus*, *Saxatilomys paulinae*, *Tonkinomys daovantieni* and *Callosciurus honk-hoiensis*) are currently covered by only one PA. This raises concern because they are not sufficiently safeguarded and therefore, other distribution areas of the species should also be a focus when it comes to designating new PAs.

Regarding *ex situ* conservation measures, a total of 45 threatened species from Vietnam are not currently represented in any zoos (Table 6). Several others are present in only one or two institutions. Nonetheless, keeping species in a network of facilities is important for stable, resilient backup populations in case a zoo population is lost unexpectedly, e.g., by a disease outbreak (Ziegler et al. 2020). In addition, well-organized studbooks help to protect the gene pool from depletion (Glatston 2001). Assurance populations in the country of origin

are optimal in terms of reduced bureaucracy and high feasibility of restocking, whereas facilities in other range states are helpful in case of disease outbreaks, natural catastrophes, or political unrest at the sites. In any case, the buildup of assurance colonies and a conservation breeding network help to buy time in case *in situ* conservation measures cannot be implemented quickly enough and prevent any impending extinction crises from happening (Byers et al. 2013).

The Cat Ba Langur (*Trachypithecus poliocephalus*) (Primates) is the only micro-endemic species currently listed in ZIMS. It is held in a primate rescue center (the Endangered Primate Rescue Center – EPRC, Cuc Phuong National Park) in Vietnam with a breeding success listed in the last 12 months. Another eight micro-endemic species are not represented in any holdings around the world. It is also important to underline the 61 species listed as DD (Table 4). More than half of them are predicted to be threatened, emphasizing the priority for assessing their conservation status in the future (Borgelt et al. 2022). Although they account for 18.3% of all mammal species in Vietnam, none of them are currently kept in zoos.

The fact that 5 of 9 micro-endemic species are DD and lack information on their threat status is particularly concerning. Species with such small distributions have a much higher risk of being extirpated (McDonald et al. 2018) and critically need immediate conservation actions. This is also the reason why important cross-border species are included in this study. While the species are on top of the list for conservation priorities in Vietnam, they might not be protected in the neighboring countries either. Evaluating the importance of conserving these species in Vietnam requires further studies covering, amongst other things, their conservation status in other countries. In addition, for the species, transboundary conservation measures should be seriously considered in developing priority actions to secure their natural populations.

### Data limitation

Assessments by the IUCN are not always up to date, as discernible from Suppl. Material 2 when the dates of the last assessments are noted. Numerous sources of error exist regarding ranges and distribution areas. This study presents an effort in reducing those by means of habitat analysis based on the shapefiles from IUCN and the newly generated ones. Despite that, distribution areas' accuracy varies widely, reflecting the details in data collected and research conducted until now (Ariño and Otegui 2015). For example, distribution areas of taxa like Eulipotyphla and Rodentia are often inaccurate (Kennerley et al. 2021). This is also a potential source of error in the species ranges areas created as part of this study. Due to a lack of information on the actual size of the distribution areas it was not possible to adjust the buffer zones for each species. We, however, used all records available for each species to estimate the distribution range in Vietnam as accurately as possible.

Furthermore, species ranges under a changing climate are shifting more rapidly than ever. This implies that accurate representations of species distributions today may change quickly in the next decades (Blair et al. 2022; Nguyen et al. 2022; Trinh-Dinh et al. 2022). This underscores the importance of updating information when it comes to conservation needs assessment. Achieving the goal to protect all threatened species of the world demands large scale exchange of information, topical analyses, and overcoming cross-border communication gaps (Bain and Hurley 2011).

Another potential source of error regarding species distribution is posed by ever-changing taxonomic knowledge, such as increasing recognition of cryptic taxa, viz. taxa previously being hidden under a name but in fact representing a species complex. Many recent studies are addressing cryptic diversity with integrative taxonomic approaches, combining morphological, ecological, and genetic evidence to discover new species. Such an example is Chiroptera, which potentially harbors a high level of cryptic diversity (Francis et al. 2010; Srinivasulu et al. 2019). In this study, we only include species recognized until the end of 2022, but since then several new taxa have been discovered (e.g., Blair et al. 2023; Bui et al. 2023).

Regarding the ZIMS database, there is the possibility of missing animal husbandry institutions because not all zoos contribute their data to ZIMS. Also, not all taxa reported to occur in Vietnam and kept in zoos derive from founders from Vietnam, and not all founders of ex situ populations were genetically screened for purity and/or geographic provenance. In addition, there are instances where a colony of species is currently kept in zoos, but the wild population no longer exists in Vietnam and future release of kept individuals is uncertain, e.g., *Cervus nippon pseudaxis*. We did not include such species in our analyses until the situation becomes clearer.

## Outlook

Designating new PAs and increasing the representations of threatened and micro-endemic species held in zoos will constitute important and much needed conservation measures in a first instance, but in the long term this approach alone might not be enough to achieve successful outcomes. For *in situ* conservation it will thus be important to improve management effectiveness of existing PAs and continuously measure the management effectiveness rather than quantity of PAs (Geldmann et al. 2019). For this, other local socio-economic issues, like poverty and the dependency on natural resources such as bush meat, must be addressed through livelihood improvement programs (Mainka and Trivedi 2002). Besides that, political corruption has been shown to be closely correlated with poor conservation outcomes, as it reduces the effectiveness of conservation investments and overlooks illegal overexploitation (Bradshaw et al. 2009). Recent evidence shows that in most cases well-managed, well-connected protected areas have more likely achieved their conservation objectives (MacKinnon et al. 2020).

These problems cannot be resolved only by designating new protected areas, but by involving local people, resource extraction companies, governments and scientists in conservation actions (Milner-Gulland et al. 2003). Future tools for conservation planning should also integrate models of urban growth (McDonald et al. 2018), as growing human populations are increasingly in conflict with designating new PAs. *In situ* conservation could further be improved by establishing wildlife corridors between PAs. This measure does not apply to conservation of micro-endemic species, as their distribution areas are very small. However, it will certainly benefit a suite of other species, especially mobile and wide-ranging mammals and in the context of the global climate change where species shift their ranges in an unprecedented rate (Thapa et al. 2017). As Northern Annamites and Lowlands harbor concentrated species richness and some of the biggest PAs in Vietnam, creating corridors to connect the PAs could substantially aid the species conservation in the region. However, it



should also be considered that with changing habitats, it might be necessary to adjust prioritization for conservation towards species with a low chance of maintaining their natural habitat in the future. Taking this into account, further research is necessary combining ecosystem modifications due to climate change and species-specific habitat requirements.

Keeping and breeding Vietnam's threatened, micro-endemic and vastly overlooked taxa will be crucial for subsequent restoration of diminished or even extinct natural populations. The rewilding approach needs coordinated conservation breeding networks in zoos and stations both inside the country of origin and also outside as assurance colonies follow the modern ark principle. This is especially true for an effective implementation of the One Plan Approach, where the concepts of *ex situ* and *in situ* conservation support each other. In the case of Vietnam, the One Plan Approach is more important than ever because many Critically Endangered species either are extirpated/extinct or their populations are so severely depleted that it is difficult for them to recover without our timely interventions.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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### Author contributions

TZ, MDL, STN conceptualized the study; HH, STN, PHD, MM, TO, DR, HTB, TQN, MDL, HTB, TZ led the data analysis and data curation; HH, TZ, MDL, DR led the writing and all authors edited and approved the manuscript.

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## Data availability

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

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## Supplementary material 1

### List of all mammal species of Vietnam and their endemism analysis

Authors: Hanna Höffner, Son Truong Nguyen, Phuong Huy Dang, Masaharu Motokawa, Tatsuo Oshida, Dennis Rödter, Truong Quang Nguyen, Minh Duc Le, Hai Tuan Bui, Thomas Ziegler

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## Supplementary material 2

### List of all threatened mammal species of Vietnam and their detailed threat assessment

Authors: Hanna Höffner, Son Truong Nguyen, Phuong Huy Dang, Masaharu Motokawa, Tatsuo Oshida, Dennis Rödter, Truong Quang Nguyen, Minh Duc Le, Hai Tuan Bui, Thomas Ziegler

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## Research Article

# Plant functional structure varies across different management regimes in submontane meadows

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## Abstract

Seminatural grasslands are among the most biodiverse habitats in Europe, and they have great conservation value. However, in recent decades, they have been threatened by either intensive fertilization or afforestation or, conversely, by abandonment due to changes in agricultural practices. The impact of management, its frequency or intensity on seminatural grassland communities is traditionally evaluated through views on the composition of communities and their diversity. A better understanding of the functioning of plants in managed grassland ecosystems could be achieved by considering plant functional traits (PFTs). In this study, we investigated whether sites with different management practices differ from each other in terms of the representation of the main PFTs. We studied a permanent plot series of 30 grassland sites in central Slovakia that had been managed or abandoned for over 10 years. Individual management consisted of low-intensity mowing (MGM), medium-intensity grazing (MGP), and abandonment (MGA). Hemicryptophytes, perennials, and semi-rosette species were dominant under all management regimes. We found significant differences in the coverage of the studied PFTs among the sites managed by phytomass removal (mowing, grazing) and abandoned sites. Compared with the MGA sites, mowed and grazed sites were characterised by high proportional coverages of species with medium plant heights (0.3–0.6 m), rosette species, and graminoids. The MGA sites presented high coverages of species with high plant heights (> 0.6 m), competitors, phanerophytes, forbs, geophytes, species with vegetative and seed reproduction types, species with long flowering periods (3 months or more), and species with summer green leaves. The MGM sites supported species with large seeds (seed mass > 2 mg), reproduction type by seed (seeds), and species with short flowering times (1–2 months), whereas the MGP sites supported species with small plant heights (plant height < 0.3 m) and species with persistent green leaves. The communities of submontane Carpathian meadows with different types of management differ in terms of the representation of selected plant functional traits, especially between managed and abandoned sites. This approach is useful not only for understanding the mechanisms involved in the application of different management methods but also for predicting changes in the responses of the functional properties of plants when abandoning grassland habitats.



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**Key words:** Abandonment, grazing, mowing, plant functional traits, seminatural grasslands



## Introduction

Seminatural grasslands are important types of biotopes with species-rich communities of plants and animals, providing several important functions and ecosystem services (Hájková et al. 2007; Rodwell et al. 2007; Wilson et al. 2012). These grasslands are elements of the landscape structure that were created by humans and used for centuries for agricultural production. Mowed meadows and pastures constitute an important part of the cultural landscape (Hejcman et al. 2013), form its typical character and reflect its history. They occur on relatively fertile soils suitable for hay production or cattle grazing (Rodríguez-Rojó et al. 2017). However, in recent decades, they have often been threatened by agricultural intensification or abandonment in Central Europe due to land-use changes (Poschlod et al. 2005; Stoate et al. 2009; Pavlů et al. 2011). One of the main tasks of nature protection, also within the framework of EU agricultural-environmental schemes, is the maintenance of seminatural meadows by appropriate management (Kahmen and Poschlod 2008).

The preservation of seminatural grassland biotopes is conditioned by continuous human economic activity in the form of mowing or grazing of varying levels of intensity. The combination of management and ecological conditions is reflected in the specific species composition of the grasslands (Ružičková and Kalivoda 2007). Management practices also affect plant residue accumulation, nutrient cycles (Schmitt et al. 2010; Zeeman et al. 2010), and biomass production (Hejcman et al. 2010) in meadow communities. They affect the biological, chemical, and physical properties of soil (Mayel et al. 2021), which affect the composition of communities and the diversity of European managed meadow communities (e.g. Chytrý et al. 2007; Kopeć et al. 2010; Soons et al. 2017).

The impact of different types of management or their intensity on seminatural grassland communities is traditionally evaluated through views of the composition of communities and their diversity (Watkinson and Ormerod 2001; Niedrist et al. 2009; Diviaková et al. 2021). However, studies that have evaluated species richness, as well as functional richness and functional composition, have shown that the degree and extent of ecosystem processes are more consistently associated with functional composition (the presence of certain functional types or traits of plants) and functional richness (the number of different functional types of plants) than with species richness (e.g. Rusch and Oesterheld 1997). Indeed, species diversity reflecting the appropriateness or inadequacy of grassland management practices is not always consistent with functional attributes, i.e. the value and extent of the functional traits of organisms present in a given ecosystem (Díaz and Cabido 2001; Mayfield et al. 2010).

Approaches using plant functional traits (PFTs), which have been developed in recent decades, have great potential for improving the understanding of plant function in managed grassland ecosystems (Schellberg and Pontes 2012). PFTs are biological characteristics of plant species that respond to the dominant processes in an ecosystem (Kelly 1996; Lavorel et al. 1997). PFTs enable us to link morphological, physiological, and phenological plant properties to their functions (Schellberg and Pontes 2012). Knowledge of PFTs allows for a better understanding of plant adaptations to environmental conditions

(Kurtz et al. 2018), which are closely related to grassland management. They are often used as predictors of vegetation changes due to changes in management caused by mowing or grazing (Noble and Gitay 1996; Kleyer 1999), and their advantage is the ability to compare different types of vegetation and reveal general trends (Díaz et al. 2001). PFTs respond to grazing management (e.g. McIntyre et al. 1995; Dupré and Diekmann 2001; Schellberg and Pontes 2012) and to the management of mowing, mulching, burning, ploughing or abandonment (e.g. Kahmen et al. 2002; Kassahun et al. 2021). Some of the mentioned studies suggest that defoliation and soil disturbance are the main processes determining PFT responses to mowing or grazing. Differential defoliation on the vertical gradient implies an increase in small or ground layer species. Short-lived species are encouraged by soil disturbances (Kahmen and Poschlod 2008).

Several studies reported differences in the functional plant composition or various reactions of PFTs to different management practices. For example, disturbances in managed grasslands enhance seedling recruitment in small-seeded species to a greater extent than in large-seeded species (Eriksson and Eriksson 1997), managed grasslands favour graminoids, whereas abandonment encourages forbs (Pavlů et al. 2011), and management practices are known to affect distribution of plant life forms (e.g., Noy-Meir et al. 1989; McIntyre et al. 1995; Pykälä 2004; Prévosto et al. 2011).

However, in different geographical regions and under the same management changes, favoured plants are characterised by different PFTs. Therefore, prediction on a wider geographical scale is difficult (Klimešová et al. 2008). This is caused by the pressure of biotic and abiotic factors on PFTs. Nevertheless, there are several general assumptions about how different management modalities affect the representation of PFTs. On the one hand, the geographical specificities of the PFT assessment make it difficult to generalise the results of the studies. On the other hand, they highlight the need for a thorough knowledge of the impact of the management assessed by PFTs in different geographical areas.

In our study, we studied submontane Carpathian meadows with various types of management that were situated in two mountain ranges in central Slovakia. This study aimed to determine whether communities of submontane Carpathian grasslands under different types of management differ in terms of the representation of the main PFTs (plant lifespan, plant growth forms, life strategy, plant height, forbs/graminoids, reproduction type, leaf persistence, leaf distribution along the stem, duration of flowering, and seed mass). We assumed that hemicryptophytes and perennials would prevail in all the sites of the differently managed grassland biotope. We also hypothesised that grazed and mown meadows would support communities dominated by rosettes and semi-rosettes as adaptations to disturbances. At the same time, we expected that abandoned meadows would allow the occurrence of competitors with greater biomass formation, geophytes preferring sufficient nutrients, humidity, and light and competitively the strongest phanerophytes. We also expected that annuals (therophytes), species capable of spreading rapidly, would be characteristic of pasture sites in response to trampling and biomass removal.

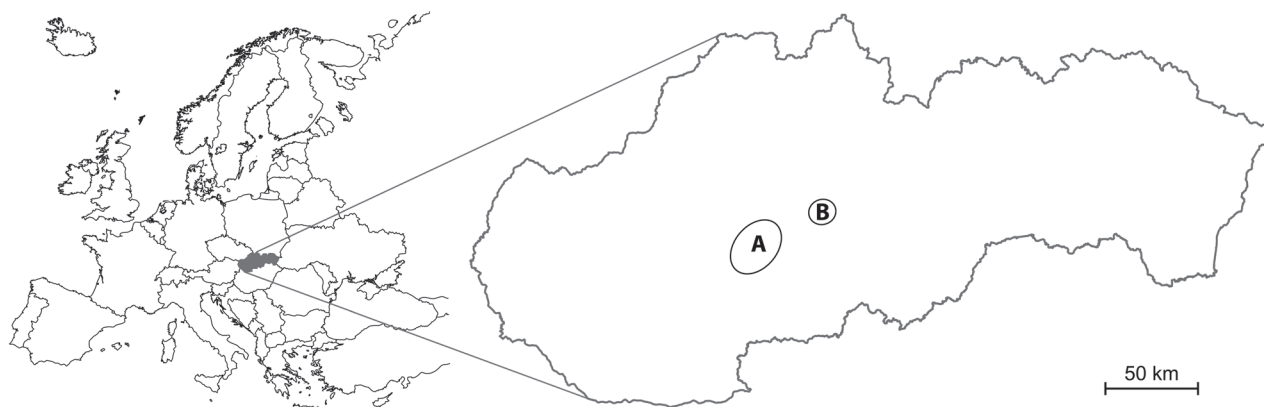
## Methods

### Study area

The study was conducted in two mountain regions in the Western Carpathians, in the central part of Slovakia: Štiavnické vrchy Mts. and Poľana Mts. (Fig. 1). Štiavnické vrchy Mts. (48°12'–48°35'N, 18°32'–19°05'E) occupy a geographical territory of approximately 776.3 km<sup>2</sup>, extending from the hills to the submontane belt. Poľana Mts. (48°35'–48°44'N, 19°18'–19°38'E) represent the highest volcano mountain range in Slovakia, with an area of approximately 183.0 km<sup>2</sup>. The study areas are typical volcanic mountains with a uniform geological substrate. Abiotic conditions led to the existence of diverse vegetation on both mountains. The dominant meadow communities include submontane mesophilic mowing meadows (*Arrhenatherion elatioris* union) and pastures (*Cynosurion cristate* union). Wet meadows of submontane and mountain areas (*Calthion palustris* union), which belong to regionally important communities, are also represented. The geological substrate of the studied sites consists of andesites. Soil types are represented by cambizems and ranchers, ranging from weathered acidic to neutral rocks. These study areas are moderately cold and very humid, with air temperatures ranging from 12 to 16 °C in July. The average annual precipitation is 800–900 mm (Landscape Atlas of the Slovak Republic 2002).

### Field work

The vegetation survey was carried out during the growing seasons of 2017 and 2018 at 30 sites. The study sites occurred at similar altitudes, ranging from 481 to 767 m a.s.l., with similar local abiotic conditions. The sites represented 3 basic types of management with more than 10 years of history (based on information from landlords), with low and medium intensities: mown meadows (hereinafter MGM, mowed once a year, usually at the end of May), grazing meadows (hereinafter MGP, seasonal pastures, or fences) and abandoned meadows (hereinafter MGA). Most of the sites have been under the same management type even for a longer time (according to historical mapping from 1957–1971). Each type of management was represented by 10 localities. Typically, there were three immediately adjacent sites, each representing one of the types of management. From a phytosociological point of view, the studied communities can be classified into the class *Molinio-Arrhenatheretea* Tx. 1937. The coverage



**Figure1.** Location of study areas **A** Štiavnické vrchy Mts **B** Poľana Mts.

of individual vascular plant species was evaluated following the Zurich-Montpellier School of Phytosociology (Braun-Blanquet 1964), using a nine-point scale of coverage and abundance (Westhoff and van der Maarel 1973), on 30 areas of 16 m<sup>2</sup>. The nomenclature of plant taxa is given in the sense of Marhold and Hindák (1998). During the field survey, the altitude was recorded using a GPS device. The slope data were drawn from the publicly accessible database of the National Agricultural and Food Centre. The original interval scale was replaced by mean percentage values for each slope interval.

## Laboratory work

To characterise the basic physical and chemical properties of the soil, soil samples were taken to a depth of 10 cm from three randomly selected locations in each area at the time of the vegetation survey. Individual samples were combined into a single sample before analysis. The analyses were carried out according to Hrivnáková et al. (2011). For a detailed description of the methods of analysis, see Diviaková et al. (2021). The basic characteristics of the study sites are listed in Table 1.

**Table 1.** Basic characteristics of the study sites. The average, minimum and maximum values are shown. Test statistics ( $\chi^2$ ) and associated probabilities (p) of the Kruskal-Wallis test for the differences among management types are displayed. Significant outputs of multiple nonparametric *post hoc* comparisons after Kruskal-Wallis testing are shown in the last column (MGM – meadow, MGP – pasture, MGA – abandoned).

Variable	MGM	MGP	MGA	$\chi^2$	p	post-hoc comparison
	Average (min.; max.)					
Altitude	626 (490; 765)	626.7 (481; 767)	616 (502; 743)	0.006	0.99	
pH	5.6 (4.9; 6.6)	5.5 (5.0; 6.3)	6.1 (5.3; 7.1)	5.46	0.07	
Electric conductivity ( $\mu\text{S}\cdot\text{cm}^{-1}$ )	207 (106; 379)	263 (172; 575)	569 (220; 1110)	15.47	<b>0.0004</b>	<b>MGM &lt; MGA, MGP &lt; MGA</b>
Phosphorus ( $\text{mg}\cdot\text{kg}^{-1}$ )	9.77 (2.9; 24.1)	11.2 (1.8; 32.5)	4.7 (1.5; 11.5)	5.14	0.08	
Nitrogen (% w)	0.37 (0.24; 0.53)	0.45 (0.38; 0.56)	0.62 (0.23; 1.17)	6.38	<b>0.04</b>	<b>MGM &lt; MGA</b>
Carbon (% w)	4.07 (2.60; 6.11)	5.13 (3.86; 6.24)	7.96 (4.00; 15.00)	11.54	<b>0.003</b>	<b>MGM &lt; MGA</b>
Slope inclination	8.5 (0.5; 14.5)	12.1 (5.0; 21.0)	2.6 (0.5; 14.5)	13.03	<b>0.001</b>	<b>MGP &gt; MGA</b>
Solar radiation input ( $10^3\text{Wh}\cdot\text{y}^{-1}$ )	1033 (917; 1132)	1020 (884; 1135)	1051 (1003; 1173)	0.71	0.70	
Species richness (E3+E2+E1) $\Sigma\text{sp.}$	36 (28; 45)	38 (30; 48)	33 (22; 42)			
Shannon diversity ( $H$ )	2.80 (2.40; 3.22)	3.00 (2.51; 3.62)	2.41 (1.44; 2.91)			

## Data analysis

We selected a set of 10 major PFTs (31 trait attributes) that were supposed to be ecologically meaningful with respect to the studied management types and that we expected to be affected by management. These PFTs included the following PFTs: seed mass, leaf persistence, reproduction type, plant height, duration of flowering, plant lifespan, plant growth form, life strategy, leaf distribution along the stem, and forbs/graminoids. Each plant species was graded for each trait according to the attributes listed in Table 2. The attributes of individual PFTs were evaluated based on 2 databases: the PLADIAS Database of the Czech Flora and Vegetation (Chytrý et al. 2021) and the LEDA Traitbase (Kleyer et al. 2008). All the PFTs monitored attributes were evaluated for each type of management. They were calculated

**Table 2.** List of the Plant functional traits and their attributes analysed in the study.

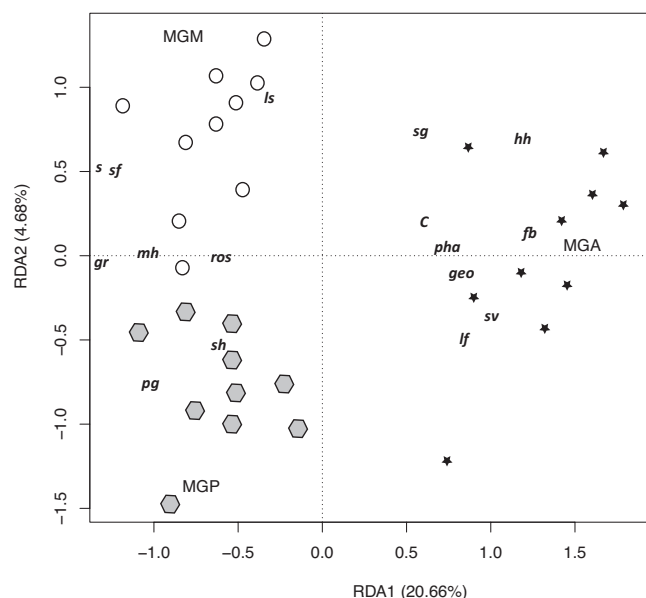
Trait	Attribute	Abbreviation	Species example
Seed mass (mg)	< 0.5 (small seed)	ss	<i>Poa pratensis</i>
	0.5–2 (medium seed)	ms	<i>Filipendula ulmaria</i>
	> 2 (large seed)	ls	<i>Arrhenatherum elatius</i>
Leaf persistence	Overwintering green	ovg	<i>Matricaria chamomilla</i>
	Persistent green	pg	<i>Festuca rubra</i>
	Summer green	sg	<i>Dactylis glomerata</i>
Reproduction type	By seed, mainly by seed	s	<i>Trifolium pratense</i>
	Vegetative and by seed	sv	<i>Carex hirta</i>
	Mainly vegetative	v	<i>Aegopodium podagraria</i>
Plant height (m)	< 0.3 (small height)	sh	<i>Viola arvensis</i>
	0.3–0.6 (medium height)	mh	<i>Cardamine pratensis</i>
	> 0.6 (high height)	hh	<i>Mentha longifolia</i>
Duration of flowering	1–2 months (short flowering)	sf	<i>Galium verum</i>
	≥ 3 (long flowering)	lf	<i>Leucanthemum vulgare</i>
Plant lifespan	Annuals	ann	<i>Rhinanthus minor</i>
	Perennials	per	<i>Ranunculus acris</i>
	Strict monocarpic bi-annuals and poly-annuals	bie	<i>Campanula patula</i>
Plant growth form	Hemicryptophyte	hem	<i>Plantago lanceolata</i>
	Chamaephyte	cham	<i>Cerastium arvense</i>
	Phanerophyte	pha	<i>Rosa canina</i> agg.
	Geophyte	geo	<i>Lilium martagon</i>
	Therophyte	the	<i>Capsella bursa-pastoris</i>
Life strategy	Competitor	C	<i>Achillea millefolium</i>
	Stress-tolerator	S	<i>Viola canina</i>
	Ruderal	R	<i>Poa annua</i>
Leaf distribution along the stem	Leaves distributed regularly along the stem	ldr	<i>Lotus corniculatus</i>
	Rosette	ros	<i>Leontodon autumnalis</i>
	Semi-rosette	sro	<i>Knautia arvensis</i>
Forbs / Graminoids	Forb	fb	<i>Cirsium rivulare</i>
	Graminoid	gr	<i>Avenula pubescens</i>
	Wood	ws	<i>Alnus glutinosa</i>

from the averages of the values, weighted using the coverage of each species of vascular plant present at the sites where the sum of all categories was 100%. Differences in basic environmental characteristics among the three management types were investigated using the Kruskal-Wallis test, as variances were not homogeneous according to the Bartlett test, which was performed prior to analysis. The relationships between the proportions of functional groups of grassland plants and the management regime were summarised using redundancy analysis (RDA) with centred response data. The functional category proportion data were square-root transformed prior to analysis. The significance of the relationship was tested with a Monte Carlo permutation test (999 permutations). All analyses were performed in R v.4.1.2 (R Core Team 2021) using the libraries vegan (Oksanen et al. 2013) and pgirmess (Giraudeau et al. 2018).

## Results

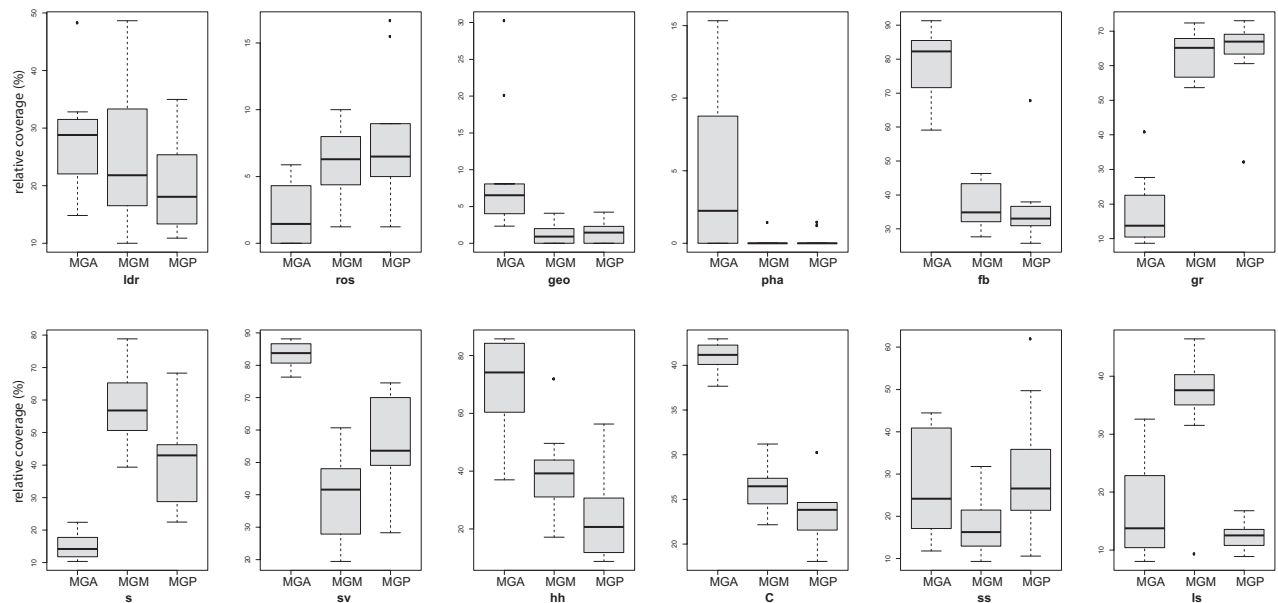
In total, 187 species were recorded at the study sites, of which 7 species are included in the Red List of ferns and flowering plants of Slovakia (Eliáš et al. 2015). The common species with high coverage at the MGM sites were *Arrhenatherum elatius*, *Dactylis glomerata*, and *Avenula pubescens*. *Festuca rubra*, *Agrostis capillaris*, and *Anthoxanthum odoratum* were often present at MGP sites. In addition to the mentioned species, the MGA sites were also characterised by the presence of more hygrophilous species, such as *Filipendula ulmaria*, *Lysimachia vulgaris*, and *Scirpus sylvaticus*. In all the studied areas, the average species richness and the Shannon index were lower at the MGA sites than at the managed MGP and MGM sites. The average number of species at all the sites was 36 in an area of 16 m<sup>2</sup> (Table 1). A more detailed description of the plant communities of the studied sites is presented in Diviaková et al. (2021).

RDA revealed a significant relationship between the proportional coverage of the studied PFTs and management type ( $F = 18.12$ ,  $p = 0.001$ ), which explained 25.34% of the total variation in coverage of the studied PFTs. The results of the RDA are summarised in the ordination diagram (Fig. 2). The MGA sites were distinct from the managed MGP and MGM sites; they featured high proportional coverages of geophytes, phanerophytes, forbs, species with reproduction type vegetative and by seed, tall species (plant height > 0.6 m), competitors, species with long flowering times (3 months or more), and species with summer green leaves, while they featured low coverages of species with reproduction by seed, mainly by seed, graminoids, and rosette species (Fig. 3). Differences between managed sites were less pronounced, and mown sites (MGM) differed from pastures (MGP), especially in coverage of persistent green species, small species (plant height < 0.3 m), and species with large seeds (seed mass > 2 mg).



**Figure 2.** RDA ordination plot showing significant differences in the coverage of the studied PFTs among sites with different management practices. Only 50% of the PFT attributes best fitted by the ordination space are displayed. For the percentage of explained variance, see the axes titles. For abbreviations of PFT attributes, see Table 2. (empty circles – MGM sites, asterisks – MGA sites, filled hexagon – MGP sites).





**Figure 3.** Box plots comparing the coverage of some attributes of selected PFTs in abandoned (MGA), mown (MGM) and pasture (MGP) meadows. For abbreviations of PFT attributes, see Table 2.

## Discussion

Grassland management affects not only total species richness but also the relative number and coverage of species with different attributes regarding anatomy, morphology and regeneration (Dupré and Diekmann 2001) and thus different PFTs. We documented significant differences in the coverage of plants with functional traits important for their competitiveness and resistance, reproduction, flowering, morphology, physiology, or plant lifespan among sites managed by phytomass removal (MGM, MGP) and abandoned sites (MGA). The coverages of the selected PFTs differed among the individual managements. Mowing and grazing had similar effects on PFTs in our study, which were different from abandoned sites. In all types of management, the dominant plant growth form was hemicryptophytes, the dominant plant lifespan was perennials, and the dominant PFT leaf distribution along the stem was semi-rosettes.

### Mowing (MGM) and Grazing (MGP) vs. Abandonment (MGA)

Compared with abandoned sites, managed sites were characterised by greater coverages of some attributes, e.g., within PFTs leaf distribution along the stem and plant height. Leaf distribution and height are important traits for plant competitive ability and persistence (Drobnik et al. 2011), ergo for plant performance under different management regimes (e.g. Garnier et al. 2007).

We found that rosette species were more successful under the two main management regimes (MGM and MGP). Erect competitor species with leaves distributed regularly along the stem were more likely to occur with higher coverage at abandoned sites (MGA). Similarly, in experimental scenarios of changes in land use, the abandonment or decreasing frequency/intensity of mowing and grazing led to decreases in rosette species and non-branched growth forms, whereas the coverages of taller species and species with leaves distributed

regularly along the stem increased (Weiss and Jeltsch 2015), indicating a shift towards stronger above-ground competition (Römermann et al. 2008).

The higher proportion of rosette species, in our study, e.g., *Taraxacum* sect. *Ruderalia*, *Bellis perennis*, *Leontodon hispidus*, *Plantago media*) due to vertical defoliation is the main response to grazing and mowing management (McIntyre et al. 1995; Dupré and Diekmann 2001; Klimešová et al. 2008). These species have lower stature, buds closer to the ground, and horizontal leaf orientation and are able to resist grazers (Noy-Meir et al. 1989; Hadar et al. 1999). The rosette species represent an escape strategy that allows plants to survive disturbances and exploit newly available spatial niches (Grime 2001). However, this issue is complicated because, in addition to the form of leaf distribution, other mechanisms may be involved in greater resistance to defoliation. Among those, e.g., low palatability (Perez-Harguindeguy et al. 2003) depending on chemical composition traits (Pontes et al. 2015), mechanisms related to phenology and dormancy (McIntyre et al. 1999), or physical defence, such as spines (Díaz et al. 2001), may be important.

PFT plant height is the trait most frequently used to assess species response to management (Klimešová et al. 2008). Height is important for competitive performance and the acquisition of carbon and is a fundamental functional trait of plants (Westoby et al. 2002). In our study, tall species (> 0.6 m) prevailed (in terms of coverage) at abandoned sites, whereas at managed sites, species of medium height (0.3–0.6 m) or small species (< 0.3 m) showed higher coverage values (cf. Peco et al. 2005; Rupprecht et al. 2016). This pattern could be explained by the higher concentrations of nitrogen observed at the abandoned sites in our study. At abandoned sites, the proportion of tall species usually tends to increase as litter accumulation increases nutrient availability and hinders seedling recruitment (Huhta et al. 2001; Rosenthal 2010). One of the main environmental filters of most plant communities is light (Crawley 1997), and increased competition for light (i.e. avoiding shade) under abandonment (Lepš 1999), especially under dense overgrowth, favours tall plants (Westoby et al. 2002; Neuenkamp et al. 2016), such as light competitors (Prévosto et al. 2011). In contrast, species with shorter heights in abandoned localities are the most susceptible to a lack of suitable microsites (Hautier et al. 2009) because of the absence of grazing animals or mowing.

In our study, the abandoned sites had greater coverage of phanerophytes than did the managed sites. Even grazed (MGP) sites showed greater phanerophyte coverage than mowed (MGM) ones probably because they remained in the area due to selective grazing (Dupré and Diekmann 2001). Phanerophytes are unpalatable to cattle and sheep because of their woody stems and the occurrence of spines (e.g., *Prunus spinosa* and *Rosa canina*). The presence of phanerophyte species and their adequate control by grazing, which prevents reforestation, can lead to greater structural or physiognomic heterogeneity in grasslands (Kun et al. 2024). In abandoned sites, succession leads logically to afforestation. The most visible consequence of land abandonment is the colonisation of previously opened land by phanerophytes, which are often of high biological and aesthetic value (Prévosto et al. 2011). However, in abandoned grasslands, there may not always be significant invasion of shrubs and trees in secondary succession, even after 60 years (Bohner et al. 2019). It is assumed that the germination and establishment of woody plants is impeded

by a virtually closed sward and by accumulated necromass, retarding further succession to these communities (Moog et al. 2002; Bohner et al. 2012). Concerning other attributes of plant growth forms, abandoned sites were characterised by greater coverage of geophytes than managed sites. This is probably because the plant storage organs are not damaged by trampling animals or oppressed by heavy mechanisms (e.g. mowing with tractors) at abandoned sites (McIntyre et al. 1995; Lavorel et al. 1999a) or simply because most of the geophytes possess the ability to regenerate vegetatively (Dupré and Diekmann 2001); i.e., they can use nutrients stored in rhizomes or bulbs to grow in the spring through the thick litter layer (Bobbink and Willems 1987).

With respect to life strategy, in our study, competitors prevailed at abandoned sites (see Rupprecht et al. 2016). Weiss and Jeltsch (2015) reported a strong increase in competitor plant types under resource (nutrient, water, and light)-rich conditions where the intensity of stress from their lack was minimal. The abandoned sites in our study were characterised by relatively high soil humidity (although not assessed exactly) and, at the same time, by the absence of disturbances, which favour long-lived plants.

Another evaluated functional trait was the reproduction type. Compared with those in the MGA, the average coverages of the species reproducing by seeds at the managed sites (MGM and MGP) were greater. Similarly, Rysiak et al. (2021) found an increasing number of species reproducing by seed on the managed plots. Seeds of numerous species can survive herbivore consumption or attach to fur, making herbivores vectors for plant dispersal (Malo and Suarez 1995; Cosyns et al. 2005). Also, increased light availability in managed sites can lead to better germination and seedling establishment (Jutila and Grace 2002).

Species reproducing by seeds possess persistent seed banks and their occurrence depends on the formation of bare ground and thus are expected to prefer grazed or mowed sites (e.g. Lavorel et al. 1999b). At the abandoned sites, species with the ability to reproduce vegetatively showed greater coverage values (cf. Pettit et al. 1995). The vegetative spread by rhizomes depends on a low frequency of disturbance (McIntyre and Lavorel 1994).

The results of our study showed that the managed sites allow the preservation of specific vegetation compositions with high coverage of graminoids. In contrast, abandoned sites are colonised primarily by perennial forbs (cf. Vannucchi et al. 2022). The high coverage values of short graminoids in managed grasslands were probably caused by better light conditions, with more opportunities to colonize the open space. Tall forbs increased their cover in abandoned meadows because, as strong competitors, they do not tolerate disturbance (Pavlů et al. 2011).

The effects of abandonment on soil properties, i.e., soil nitrogen and organic contents, confirm the key role of soil chemical properties in influencing vegetation in managed grasslands (Grime et al., 1997; White et al. 2004). Vegetation affects soil properties through a feedback mechanism (Petermann and Buzhdygan 2021). Some functional types, such as forbs, effectively increase nitrogen and carbon contents in grassland soils (Knops and Tilman 2000; De Deyn et al. 2009). Increased amounts of nitrogen and carbon in abandoned sites in comparison with managed sites (Vannucchi et al. 2022), which were also confirmed in our study, may be related to higher aboveground phytomass and increased plant litter decomposition (Gabarrón-Galeote et al. 2015; Bohner

et al. 2019). Higher aboveground plant biomass and a denser surface layer of necromass at abandoned sites can also reduce the average soil temperature and increase soil moisture (Facelli and Pickett 1991).

Our study confirmed the differences in leaf persistence between abandoned and managed sites. Summer green species prevailed in coverage at the abandoned sites, whereas persistent green species prevailed at the grazing-managed sites. Rysiak et al. (2021) reported significantly greater coverage of summer green species at mowed sites. Leaf persistence is a functional trait that is important for plant competitiveness. It depends on the climate in the distribution range of the taxon and microclimate, as well as nutrient and light availability in typical habitats of the taxon.

We also observed differences in the PFT duration of flowering between abandoned and managed sites. Long-flowering species were characterised by great coverages at abandoned sites, whereas short-flowering species were dominant at mowed sites. At managed sites, this difference can be due to management timing (as well as intensity and frequency), which is important for the phenology of seed production (Poschlod et al. 2000). Plants in managed grassland biotopes are adapted to disturbances, e.g., by mechanisms related to palatability and mechanical defence (Callaway et al. 2000), growth form (Noy-Meir et al. 1989), and reproductive phenology (Lennartsson et al. 1998). Phenology can be considered particularly important in grazing, mown, and other managed biotopes because it determines whether a plant can produce seeds before the vegetation is disturbed (Akhalkatsi and Wagner 1996). However, in abandoned meadows, some species may experience longer or delayed development due to undecomposed litter, which acts as an insulating layer and fundamentally affects the physical and chemical properties of the soil (Janišová et al. 2007).

### **Mowing (MGM) vs. Grazing (MGP)**

The similarities in the proportional coverage of the studied PFTs between the managed sites (MGM and MGP) were not surprising, as in both cases, applied management represents a disturbance to grassland habitats. In both types of management, mowing and grazing, aboveground vegetation is more or less regularly removed, but at least part of the phytomass is left (Kahmen and Poschlod 2008). This suggests that the effects of management on PFTs may operate mainly through the intensity of biomass loss or the biomass recovery rate, factors that were similar for both management practices in our study. Similar effects of mowing and grazing on PFTs indicate that different management practices may maintain communities with similar functional trait compositions and coverages, even though species compositions may differ (Moog et al. 2002; Garnier et al. 2007; Volf et al. 2016).

The grazed sites that we studied differed from the mowed ones mainly in terms of PFT plant height and seed mass. Small-height and small-seed species prevailed in the grazed sites and mowed sites hosted higher coverages of high-height and large-seed species. In contrast to mowing, grazing, as mentioned above, leads to the formation of gaps, as livestock trample and wallow within pastures (Gilhaus et al. 2017). Consequently, grazing promotes the establishment and maintenance of low-growth plants (Fleischer et al. 2013). Rysiak et al. (2021) noted that mowed and grazed sites are habitats rich in plants with relatively large

seeds. Eriksson and Eriksson (1997) or Kahmen and Poschlod (2008) reported an increase in the number and coverage of species with small seeds caused by grazing. Seed mass is a functional trait related to plant dispersal and regeneration ability. Although heavier seeds may be dispersed over shorter distances than lighter seeds, heavier seeds may enhance seedling establishment, especially when light or nutrients are in short supply (Leishman et al. 2000). In terms of seed mass, seedlings from large seed species tend to survive better under a closed canopy (Grime et al. 1997), and an increase in the mean individual seed mass was reported (Westoby et al. 2002) to be a response trait associated with an increase in canopy height (Louault et al. 2005). Plants with large and heavy seeds have difficulty colonising isolated patches (Helsen et al. 2013), and their populations may become locally extinct unless they have good vegetative propagation ability or a long lifespan (Lindborg and Eriksson 2004; Bossuyt and Honnay 2006). In contrast, isolated patches may be more easily reached by plants with low seed mass (Westoby et al. 1996). A decline in the mean seed size in response to grazing was also reported by McIntyre and Lavorel (2001) for both perennial grasses and forbs. In our study, mowed meadows were characterized by greater canopy heights without isolated patches, especially in the time before mowing.

## Conclusions

Our study aimed to determine whether the representation of plant functional groups differed among sites with different types of applied management. We identified significant differences in the coverage of some plant functional traits among sites with different management practices. Managed sites differed from abandoned sites in terms of coverage of the PFT height of the plant, the distribution of leaves on stems, the length of the flowering period, the method of reproduction, and the representation of grasses/herbs. We focused on one type of vegetation, the *Arrhenatherion* meadows. This approach allowed for sufficient clarification of the differences between management strategies in a particular place but also for generalisation for predictive purposes. *Arrhenatherion* meadows are the most widespread type of seminatural meadow in Central Europe. In this meadow ecosystem, plants are exposed to regular disturbances and, owing to their abilities, are well adapted for economic use. Currently, many grassland biotopes (especially those at relatively high altitudes, far from economic centres or in steep and sloping locations with shallow soil) are no longer managed. Here, we showed that the abandonment of the management of these valuable grasslands caused important changes in the functional structure of the communities. Several studies have assessed the impact of management on other PFTs or other types of grasslands, on which the research presented in this paper was focused. Nevertheless, many questions regarding the impact of management on grassland biodiversity, ecological stability or adaptability to climate change remain unanswered. Further research broadly focused on different types of grasslands and their management, employing detailed analysis methods, is needed to reveal general patterns of the influence of grassland management on their properties and to select optimal forms of management based on the specific identified natural conditions. This would help to preserve the natural value of these rare and currently endangered habitats.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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## Author contributions


Conceptualization: AD, SS. Data curation: HO, AD, MN. Formal analysis: AD, MN. Funding acquisition: SS. Investigation: AD, SS. Methodology: AD, MN. Project administration: AD. Writing - original draft: AD, MN, HO. Writing - review and editing: DV, SS, MN, AD, HO.

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## Data availability

All of the data that support the findings of this study are available by authors upon request.

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## Research Article

# Conservation perceptions and attitudes regarding monitor lizards in West Bengal, India

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## Abstract

Local and illegal exploitation of wildlife is particularly widespread in highly populated and bio-diverse countries such as India. In such countries, illicit utilization practices and how these are perceived among local communities are commonly not well-documented. Due to a lack of data, and environmental education measures to protect ecosystems and biodiversity, the extent of this exploitation appears immense and often goes unnoticed. Therefore, in 2019, a series of awareness workshops targeting the conservation of wildlife focused on monitor lizards (Varanidae) was conducted in five districts of West Bengal, India. The environmental awareness workshops were conducted through PowerPoint slide presentations and questionnaires collecting baseline data from the participants regarding their perceptions and relationships to the three species of *Varanus* recorded for West Bengal. Our findings reveal specific human attitudes and varying local utilization patterns of monitor lizards. This study was the first to communicate and spread awareness in the study areas about why the conservation of local wildlife such as *Varanus* species is important for ecosystems and human well-being based on the ‘Nature Contributions to People Concept’.

**Key words:** Awareness, ecosystem services, educational workshops, nature protection, *Varanidae*, wildlife exploitation

## Introduction

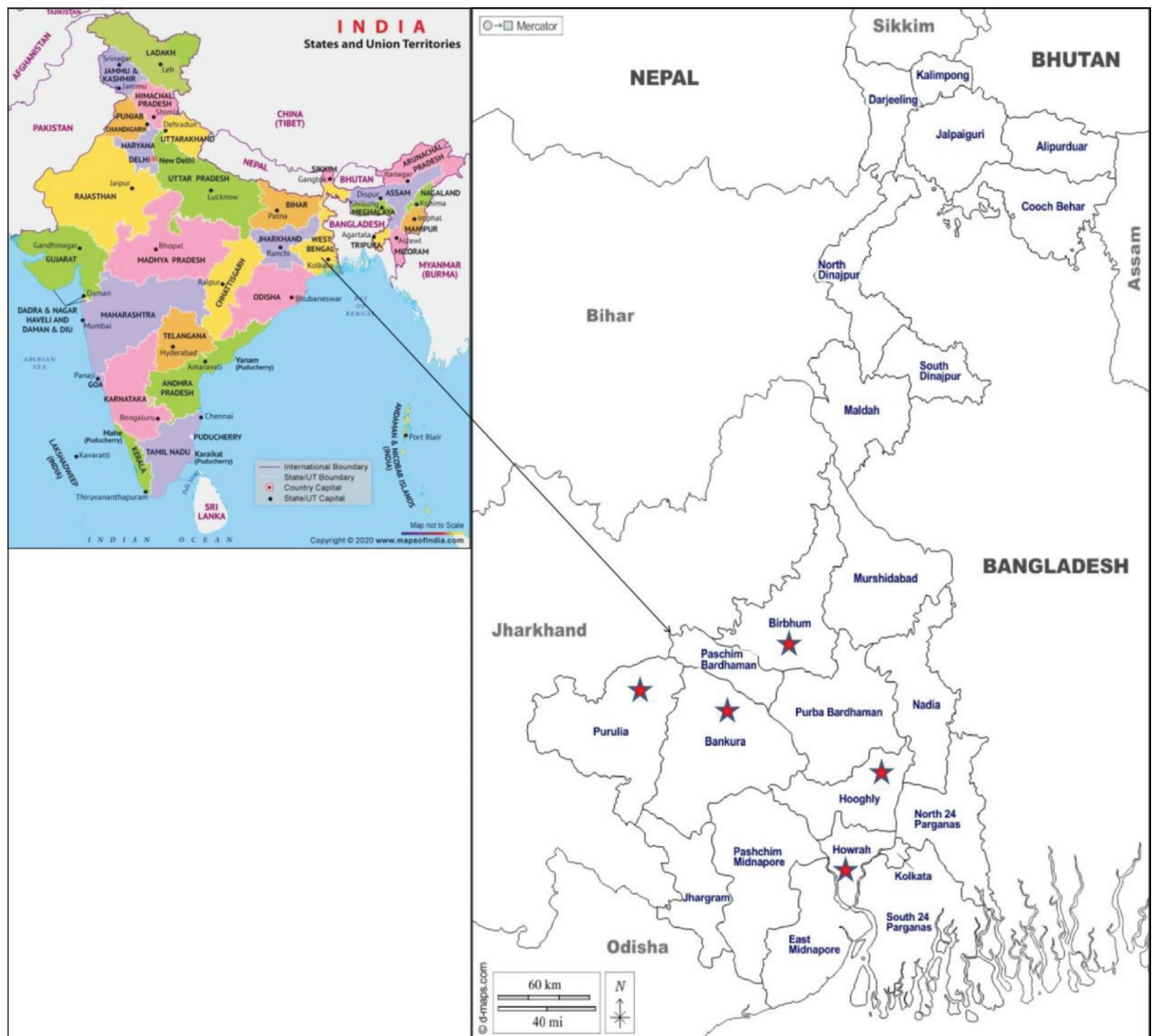
Direct drivers of biodiversity loss, destruction, and destabilization of ecosystems are the alteration of habitats, exploitation of biodiversity, climate change, pollution, and invasive species. These factors are underpinned by societal values and behaviors, such as e.g., production and consumption patterns (IPBES 2019). Accordingly, the over-exploitation of wildlife is considered the second largest threat to terrestrial wildlife (Díaz et al. 2019). Thus, human population

growth and the spread of human populations into rural areas can shift the domestic use of biodiversity from formerly sustainable to unsustainable exploitation (Dasgupta 2021).

Human-wildlife relationships have always played a crucial role in the conservation and exploitation of species and their habitats (Castillo-Huitrón et al. 2020). Traditional beliefs, ethical values, and culturally shared emotions are considered essential in shaping the attitudes of societies and communities towards their environment (Jacobs 2012; Castillo-Huitrón et al. 2020).

In India, the uses of monitor lizards (family Varanidae) and their body parts have been documented since prehistoric times as is evident from cave paintings, as well in the use of Unani medicines which dates back 2500 years before the present (Das 1989; Yuan et al. 2016). Hence, since ancient times to the present day, there is evidence of the local exploitation of monitor lizards in the Indian subcontinent (Auffenberg 1989; Das 1989; Bhattacharya and Koch 2018a, 2018b; Choudhury and Choudhury 2019; Sharma et al. 2019). Since 1972, however, that utilization is considered illegal according to the Wildlife (Protection) Act of India (The Parliament of India, 1972). Four species of monitor lizards are native to India, i.e., the Bengal monitor (*Varanus bengalensis*), the common water monitor (*V. salvator*), the yellow monitor (*V. flavescens*), and the desert monitor (*V. griseus*), of which all but the latter species occur in West Bengal (Koch et al. 2013; Chatterjee and Bhattacharyya 2015; Auliya and Koch 2020). All four species are nationally protected in Schedule I, Part II under the Wildlife (Protection) Act of India, 1972. Despite their national protection status, ongoing exploitation and illegal trade across several districts of the country are evident (Das 1989; Chakravorty et al. 2011; Bhupathy et al. 2013; Bhattacharya and Koch 2018a, 2018b; Choudhury and Choudhury 2019; Sharma et al. 2019). Besides, the active involvement and participation of local communities represent a major component of conservation efforts regarding *Varanus* species and their habitats (Sodhi et al. 2010; Bhattacharya and Koch 2018a). Hence, environmental educational workshops are important to inform local communities that a detrimental impact on wildlife not only reduces the resilience of ecosystems but disables ecosystems' services and harms human health (Oražem et al. 2019; WHO 2021; Lin et al. 2022; Ulrich et al. 2023). Several such awareness programs have previously been conducted in different parts of India such as a study on the conservation attitudes and awareness in the Kaziranga National Park, Assam (Heinen and Shrivastava 2009); an education awareness program for the conservation of the endangered medicinal shrub *Commiphora wightii* in Rajasthan (Soni 2010); community awareness programs for the conservation of endangered turtle species in north-east India (Baruah et al. 2011); surveys for identification of snake species in Kerala prioritizing the importance of awareness programs for snake conservation (Roshnath and Divakar 2019), or in gharial (*Gavialis gangeticus*) conservation (Pathak et al. 2023). Furthermore, other studies conducted in different parts of the world communicated the importance of educational awareness programs leading to positive change in attitudes toward the conservation of species, especially among the younger generation (Mutalib et al. 2013; Feilen et al. 2018; Freund et al. 2019; Kamil et al. 2020; Sánchez-Mercado et al. 2020).

The major goal of our study was to spread awareness regarding the illicit exploitation of monitor lizards in five districts of West Bengal (Fig. 1), as well as



**Figure 1.** The five districts (denoted by stars) of West Bengal, India. The awareness workshops were conducted in these districts, namely Hooghly, Bankura, Purulia, Howrah, and Birbhum in 2019. Sources: <https://www.maps.com>; <https://www.d-maps.com>.

to obtain detailed baseline information about the perception trends of the local youth, which have not been investigated before in the study area. The aims and objectives of the workshops are also described in Bhattacharya et al. (2019). We believe that the perception trends from each district will give a perspective on the presence, utilization, and trade related to monitor lizards along with the attitudes of the local people towards this particular reptile group (Bhattacharya et al. 2019).

## Materials and methods

### Study site and sampling

To address perceptions and attitudes along with the traditional beliefs and values of local people towards Indian monitor lizards, awareness workshop programs entitled “Perceptions of Wildlife Conservation of Today’s Youth in West

Bengal, India, with a focus on Monitor Lizards” were jointly organized by the Environment, Agriculture and Education Society along with the IUCN Species Survival Commission’s Monitor Lizard Specialist Group (IUCN SSC MLSG) in West Bengal, India. In the period from 29<sup>th</sup> July to 2<sup>nd</sup> August 2019, the workshops were conducted at four schools and five colleges (for details see Table 1) of five districts, i.e., Hooghly, Bankura, Purulia, Howrah, and Birbhum (Fig. 1) (Bhattacharya et al. 2019). The five districts were chosen for conducting the workshops based on our personal observations and ground surveys conducted before the workshops, since one of us (SB) lives in Kolkata (nearby to Howrah and Hooghly districts).

The target audience consisted of two age groups: (i) school students aged 12–18 years (467 participants) and (ii) college students between 18–24 years (595 participants) (Table 1).

### Structure and content of workshops

The primary objectives of the workshops were to gain an understanding of the perceptions and attitudes of the younger generation towards the fragmentation and encroachment of forest and wetland ecosystems as well as the presence, interactions, conflicts, and local and illegal utilization of monitor lizards. The workshop presentation sessions consisted of (i) the introduction of wildlife diversity, its importance and conservation; (ii) the ecological role of monitor lizards and their value as part of a biocenosis, and why they need to be conserved; (iii) the importance of protecting the varying ecosystems, such as e.g., wetlands, marshlands, forest fringes, and tropical dry deciduous forests; (iv) to introduce and make the audience aware of the Wildlife (Protection) Act of India 1972 (including the protection status and the penalties related to the illegal exploitation of Indian monitor lizards); and (v) to make the youth aware of the exploitation of protected wildlife that is an important part of their living space.

### Questionnaires

The workshops also included a survey of the participants, for which two questionnaires were developed (see Suppl. materials 1, 2). The first questionnaire was distributed to the participants before the presentation session

**Table 1.** Number of participants from schools and colleges of the five districts of West Bengal, India.

Districts	Institution names	Respondents	Total number of respondents
<b>Birbhum</b>	1. Suri Vidyasagar College	180	180
<b>Bankura</b>	1. Dhulai R.K.M. Vidyamandir H.S. School	89	
	2. Sonamukhi College	159	248
<b>Purulia</b>	1. Bamnia Vivekananda Vidyapith (H.S.)	180	
	2. Achhruram Memorial College	83	263
<b>Howrah</b>	1. Gangadharpur High School	80	
	2. Gangadharpur Sikshan Mandir	80	160
<b>Hooghly</b>	1. Itachuna Sree Narayan Institution (H.S.)	118	
	2. Bejoy Narayan Mahavidyalaya	93	211
	<b>Total Respondents from Schools</b>	<b>467</b>	
	<b>Total Respondents from Colleges</b>	<b>595</b>	<b>1062</b>



to obtain an unbiased judgement about various questions related to conservation parameters concerning wildlife in general as well as monitor lizards in particular. The second questionnaire focused on the feedback from the participants at the end of the workshop presentation session to investigate possible changes in the respondents' attitudes toward previously addressed environmental issues.

The first questionnaire comprised several questions, which were broadly categorised into ten topical areas for analysis, i.e., the perception of (i) the presence of biodiversity; (ii) the importance of wildlife conservation; (iii) the presence of wetlands; (iv) the destruction of wetlands; (v) the presence of monitor lizards; (vi) the attitude towards monitor lizards; (vii) the conflicts related to monitor lizards; (viii) the utilization of monitor lizards or their body parts other than genitalia; (ix) hunting of monitor lizards; and (x) the use of Hatha Jodi (i.e., the genitalia called hemipenes of monitor lizards) involved in illegal trade activities (Bhattacharya et al. 2019; D'Cruze et al. 2018). Questionnaires were arranged as multiple-choice questions for the convenience of the participants. Statistical graphs were used for the representation of the prominent results (see below). A comparative analysis of the categorized questions from the five districts was also conducted to gain an overview regarding the variation of the perception trend across the sampled regions. Also, as mentioned above, each categorized question consists of several sub-questions, and hence the prominent results of such individual questions are also discussed below.

For the comparative analysis of the questions from the five districts, only the multiple-choice options for the participants (such as "yes" and "no") were considered.

Results of feedback questionnaires were allocated into three main components regarding the perception of (i) a potential change in the attitude of the participants after the workshops, (ii) the protection of monitor lizards in India, and (iii) the intended future use of monitor lizards or their body parts by the participants. Thus, our analysis of the questionnaires should evaluate the overall success and efficiency of the conducted workshops.

## Results

The data obtained from both the workshops and the feedback questionnaires provided a comprehensive overview of the perceptions and trends of the participants from the five districts in West Bengal. The respective number of participants from the schools and colleges are mentioned in Table 1. In total, 1062 students took part in the workshops.

### Workshop questionnaire (Pre-presentation) data analysis

Statistics on the answers of all participants related to the questions of the nine topics are summarized in Table 2. The questions have been shortened for the sake of clarity. Also, summarized answers to a few open follow-up questions (i.e., Q4a, Q4b, Q15a, Q17a, Q19a, and Q20a) are included in Table 3. The option N.A. (i.e., not answered) refers to those participants, who did not respond to the options given in the questionnaires. Detailed answers for each district are provided in the Suppl. material 2: table S1.



**Table 2.** The overall perceptions of the participants from the five districts expressed in percentages of minimum and maximum values per district together with average (x)  $\pm$  standard deviation (S.D.) from the Pre-Presentation Questionnaire.

Topics and sub-questions	Minimum - Maximum values (x = Average % $\pm$ Standard Deviation (S.D.))				
<b>Presence of biodiversity</b>	High	Moderate	Low	Not Sure	N.A.
(Q2): How would you describe the amount of wildlife and biodiversity in your area?	4.4–11.3% (x = 7.9 $\pm$ 2.5)	23.4–45% (x = 33.3 $\pm$ 8.7)	36.2–60% (x = 43.7 $\pm$ 9.3)	2.5–15.9% (x = 8.2 $\pm$ 5.6)	4.6–7.8% (x = 6.6 $\pm$ 1.31)
<b>Perception of wildlife conservation</b>	Yes	No	Not Sure	N.A.	
(Q3): Do you think that wildlife and biodiversity are essential for the subsistence of ecosystems?	71.8–93.9% (x = 83.9 $\pm$ 9.6)	1.8–9% (x = 4.6 $\pm$ 3.1)	0.5–11.9% (x = 5.1 $\pm$ 4.2)	3.3–9.79% (x = 6.2 $\pm$ 3.0)	
(Q4): Do you think that it is important to preserve local wildlife species?	77.5–90% (x = 84.4 $\pm$ 4.9)	4.3–13.1% (x = 8.0 $\pm$ 3.7)	2.5–6.8% (x = 5.2 $\pm$ 1.6)	0.9–3.9% (x = 2.2 $\pm$ 1.3)	
<b>Perception of the presence of wetlands</b>	Yes	No.	Not Sure	N.A.	
(Q5): Do you have natural wetlands in your area?	92.2–98.7% (x = 95.5 $\pm$ 3.0)	0.6–6.1% (x = 2.9 $\pm$ 2.2)	0–2.6% (x = 1.2 $\pm$ 0.8)	0–1.1% (x = 0.3 $\pm$ 0.4)	
(Q6): Are there artificial/ man-made wetlands in your area?	70.5–90.6% (x = 82.0 $\pm$ 7.7)	6.2–27.2% (x = 15.3 $\pm$ 7.6)	0.3–1.8% (x = 1.2 $\pm$ 0.6)	0–2.8% (x = 1.3 $\pm$ 1.0)	
<b>Perception of the destruction of wetlands</b>	Yes	No	Not Sure	N.A.	
(Q7): Is a wetland in your area being destroyed or turned into an agricultural land?	29.7–63.1% (x = 41.7 $\pm$ 14.0)	28.7–56.5% (x = 47.1 $\pm$ 11.3)	5.6–11.8% (x = 8.6 $\pm$ 2.3)	0–5.9% (x = 2.4 $\pm$ 2.1)	
(Q8): Do you think wetlands should be filled up for developmental activities such as agriculture and constructions?	9.1–30.0% (x = 17.7 $\pm$ 8.7)	55.9–75.6% (x = 68 $\pm$ 9.6)	4.8–12.3% (x = 9.8 $\pm$ 3.0)	1.8–9.2% (x = 4.3 $\pm$ 2.9)	
<b>Perception of the presence of monitor lizards</b>	Yes.	No	Not Sure	N.A.	
(Q9): Do you know monitor lizards ("Gosap" or "Gorgel")?	46–75.6% (x = 58.1 $\pm$ 11.5)	12.5–42.2% (x = 30.2 $\pm$ 11.3)	1.2–9.1% (x = 6.9 $\pm$ 3.2)	1.8–8.7% (x = 4.6 $\pm$ 2.5)	
(Q10): Do monitor lizards occur in your area?	38.8–84.3% (x = 58.1 $\pm$ 19.0)	10–47.7% (x = 29.5 $\pm$ 15.1)	3.1–13.7% (x = 9.7 $\pm$ 4.0)	1.3–3.7% (x = 2.4 $\pm$ 0.9)	
(Q11): Have you ever seen a monitor lizard?	52.5–96.8% (x = 73.4 $\pm$ 18.1)	2.5–39.5% (x = 22.2 $\pm$ 15.0)	0–5% (x = 1.8 $\pm$ 1.9)	0.6–6.5% (x = 2.4 $\pm$ 2.4)	
<b>Perception of the attitude towards monitor lizards</b>	Positive	Negative	Neutral	N.A.	
(Q11a): If yes, how would you describe the interaction?	13.5–35% (x = 27.2 $\pm$ 9.6)	5–24.9% (x = 9.8 $\pm$ 8.6)	32.9–50% (x = 43.0 $\pm$ 7.4)	10–31.6% (x = 19.8 $\pm$ 10.1)	
(Q12): How is your attitude towards monitor lizards in your area?	Positive	Negative	Neutral	N.A.	
	21.6–43.1% (x = 31.6 $\pm$ 8.6)	3.7–15.9% (x = 9.1 $\pm$ 4.5)	39.1–50.5% (x = 46.4 $\pm$ 4.3)	6.2–18.8% (x = 12.6 $\pm$ 5.3)	
<b>Perception of conflicts with monitor lizards</b>	Yes.	No	Not Sure	N.A.	
(Q13): Are you aware of conflicts with monitor lizards in your area?	14.7–47.5% (x = 32.0 $\pm$ 12.6)	23–43.1% (x = 33.1 $\pm$ 8.9)	13.8–31.8% (x = 22.3 $\pm$ 7.9)	7–16.6% (x = 12.3 $\pm$ 4.2)	

Topics and sub-questions	Minimum - Maximum values (x = Average % ± Standard Deviation (S.D.))						
(Q14): What kinds of conflicts exist?	Fish ponds	Garbage	Poultry		Others	N.A.	
	6.6–26.8% (x = 14.9 ± 7.5)	7.7–15% (x = 11.6 ± 3.3)	2.2–11.1% (x = 6.4 ± 3.3)		5–21% (x = 12.0 ± 5.7)	46.8–69.4% (x = 54.9 ± 9.72)	
Perception of utilization of monitor lizards	Yes	No	Not Sure		N.A.		
(Q15): Do you or anybody you know utilize monitor lizards?	5.5–14.5% (x = 9.7 ± 3.2)	63.5–75.6% (x = 72.6 ± 5.1)	9.7–13.8% (x = 11.4 ± 1.6)		3.2–12.1% (x = 6.1 ± 3.5)		
(Q16): Have you ever seen any items or products from monitor lizard being sold in local markets?	2.2–14.4% (x = 9.0 ± 4.3)	62.0–76.6% (x = 71.0 ± 5.5)	10.5–17.7% (x = 12.9 ± 2.9)		4.1–12% (x = 6.9 ± 3.28)		
(Q16a): If yes, please specify the type of item or product.	Leather	Meat	Oil	Medicines	Astrology	Others	N.A.
	1.1–10.9% (x = 6.3 ± 3.7)	0.5–3.6% (x = 2.5 ± 1.3)	0–8% (x = 2.6 ± 3.2)	03.2% (x = 2.1 ± 1.2)	0–2.6% (x = 1.2 ± 1.0)	1.97.8% (x = 3.7 ± 2.3)	73.894%, (x = 81.3 ± 8.4)
Perception of the hunting of monitor lizards	Yes	No	Not Sure		N.A.		
(Q17): Have you ever heard of people hunting monitor lizards or participating in the hunting?	23.8–36.8% (x = 27.4 ± 5.4)	50.0–63.8% (x = 58.9 ± 5.2)	5.4–11.6% (x = 8.1 ± 2.2)		3.5–9.5% (x = 5.4 ± 2.3)		
Perception of Hatha Jodi	Yes	No	Not Sure		N.A.		
(Q18): Do you know about Hatha Jodi/Kakanashika/ Bagnakhi?	0.9–5.7% (x = 3.0 ± 1.8)	80–88.9% (x = 83.3 ± 3.9)	5.4–11.2% (x = 7.9 ± 2.7)		3.6–8.6% (x = 5.6 ± 1.9)		
(Q19): Do you know anyone who sells Hatha Jodi?	0.5–3.6% (x = 2.1 ± 1.2)	70–85.2% (x = 78.8 ± 5.7)	5.9–13.1% (x = 9.4 ± 3.3)		4.8–15.6% (x = 9.6 ± 4.1)		
(Q20): Have you or anyone you know ever bought and used Hatha Jodi?	0.5–4.6% (x = 2.1 ± 1.7)	71.2–82.1% (x = 75.3 ± 4.6)	9.6–19.4% (x = 14.3 ± 3.6)		4.6–13.1% (x = 8.1 ± 3.7)		
(Q20b): If yes, what was the source?	Market	Online	Astrologers		Other	N.A.	
	0.3–5% (x = 2.4 ± 2.2)	0–4.7% (x = 1.4 ± 1.9)	0.5–4.5% (x = 2.4 ± 1.4)		1.8–9.9% (x = 5.1 ± 3.3)	81.3–96.6% (x = 88.4 ± 6.7)	

**Table 3.** Summary Results of the Qualitative Answers in the first Pre-Presentation questionnaire.

Questions	Summary of Qualitative Answers
<b>Q4a</b>	- Maintenance of the ecological balance of the environment
	- Every species has the same right as human beings to live
	- Important for the survival of human beings to survive in the future
<b>Q4b</b>	- Note, no participant preferred to answer this open question
<b>Q15a</b>	- Meat (belly fat is considered as a delicacy among the Santhal tribes)
	- Medicinal oil
	- Leather
	- Traditional musical instruments like drums used by the Santhal tribes
<b>Q17a</b>	- In their own villages and the neighbouring villages
	- Near the ponds and marshlands of the village or the neighbouring villages
	- In nearby forests and open areas
	- In agricultural areas
<b>Q19a</b>	- In Purulia district, used for medicinal purposes
<b>Q20a</b>	- Treatment of inflammation
	- For black magic/witchcraft

Regarding the origin of the respondents (Question 1), most of the participants ( $n = 887$ , i.e., 72%) were from rural areas, but this value varied from 44.38% (Howrah) to 91.17% (Bankura). Accordingly, Howrah district has the highest proportion (55.62%) of urban participants. This proportion was lowest in Bankura and Hooghly districts with 8.49% and 11.93%, respectively. Regarding the presence of biodiversity in their area (Question 2), more than one-third of the participants ( $x = 43.7\% \pm 9.3$ ) from all districts opted for “low” (varying from 36.25% in Howrah to 60% in Birbhum), while one-third ( $x = 33.3\% \pm 8.7$ ) chose the option “moderate”, followed by a minority ( $x = 7.9\% \pm 2.5$ ), which opted for “high” (Table 2).

Most respondents from Birbhum (91.11%), Howrah (89.69%), Hooghly (85.78%), Bankura (77.85%), and Purulia (76.52%) agreed that the conservation of local wildlife species and biodiversity is important for the subsistence of ecosystems (average values of Questions 3 and 4, for details see Suppl. material 2: table S1). There were two open follow-up questions provided along with Question 4. Those participants who opted “yes” to the importance of the conservation of wildlife species were asked “why do you think it is important?” (Question 4a). Here, the answers of most participants can be summarized as “the maintenance of the ecological balance of the environment”, “every species has the same right as human beings to live” and a few stated “for the future survival of the human beings” (Table 3). Question 4b addressed those who opted for “no” for the same question (on average 8% across all districts), but none of these participants from any of the districts preferred to answer this open question.

The perception of the presence of natural and artificial wetlands, such as swamps, marshlands, and ponds, from all the districts is 95.5% and 82.0%, respectively (average values of Questions 5 and 6 in Table 2, for details see Suppl. material 2: table S1), which indicates that a considerable number of wetlands occur in the study area investigated. The perception of the destruction of these wetlands (Question 7) in the respective districts is highest in Howrah (63.13%) followed by Birbhum (48.33%), Hooghly (36.24%), Purulia (31.31%), and Bankura (29.71%). In this regard, the proportion of the participants favoring the idea of filling wetlands for developmental or agricultural purposes (Question 8) varied between 30% (Purulia) and 9.17% (Hooghly) with an average of 17.77%, while the majority (55.9%–75.6%, on average 68.06%) denied this question. Also, 9.8% of all the respondents preferred the option “Not sure” and 4.35% chose not to answer.

Question 9 tested whether participants were aware of monitor lizards (called “Gosap” or “Gorgel” in local dialects). 58.12% responded with “Yes” (varying between 46%–75.6%), while 30.28% stated they did not know monitor lizards. Participants who had no knowledge of the species from the five districts varied between 12.5% (Howrah) and 42.24% (Bankura) (see Suppl. material 2: table S1 for more details). Accordingly, 84.37% of participants from the Howrah district were aware of monitor lizards in their area (Question 10), while only 38.89% chose “yes” in Birbhum (overall average was 58.10%). Respondents who chose “no” ranged from merely 10% in Howrah to 47.78% in Birbhum (on average 29.59%). Between 3.13% (Howrah) and 13.75% (Purulia), on average 9.7%, were unsure if monitor lizards were found in their areas (Suppl. material 2: table S1).

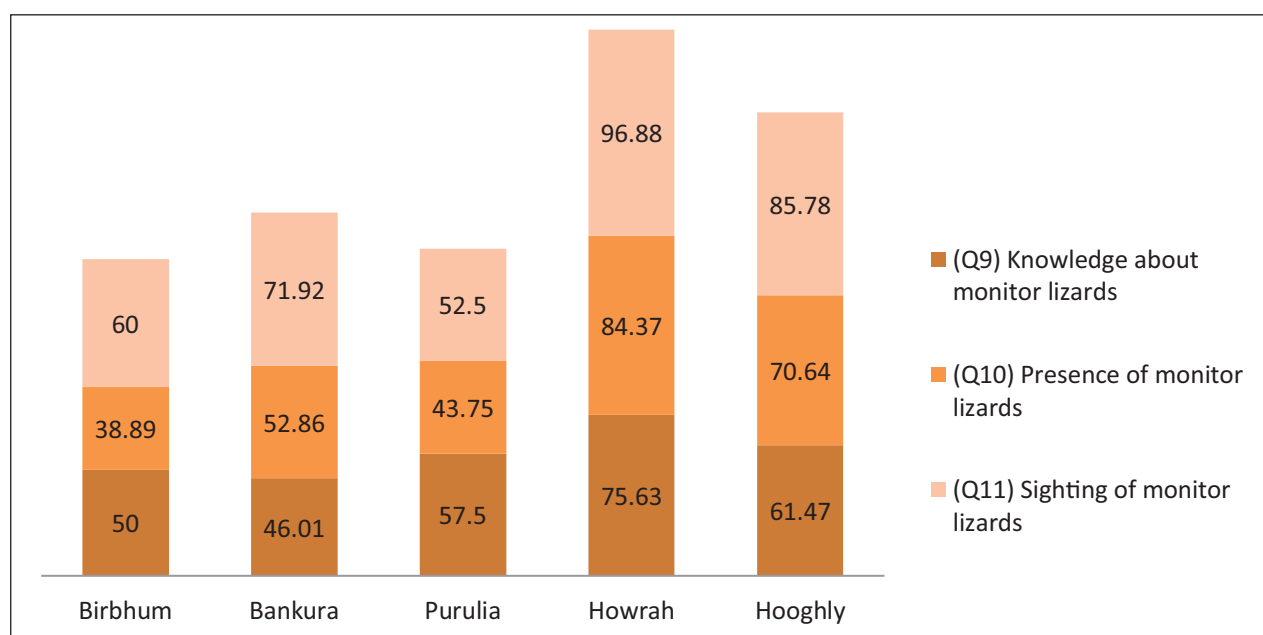
Answers to Question 11 revealed that the overwhelming majority (96.88%) of the participants from the Howrah district had sighted a monitor lizard before, whereas only 52.5% of the students from Purulia stated “yes” (the average was

73.41%, Fig. 2). Accordingly, 39.51% of the participants from Purulia, 33.33% from Birbhum, 23.5% from Bankura, 12.39% from Hooghly and only 2.5% from Howrah claimed to have never seen a monitor lizard. Out of 73.41% of all participants who had sighted monitor lizards before, an average of 9.87% described their interaction as “negative”, while “neutral” and “positive” interactions predominated on average 43.04% and 27.22%, respectively (Question 11a). Most of the negative interactions were reported from the Purulia district (24.93%) followed by Birbhum (9.44%), Bankura (5%), Howrah (5%), and Hooghly (5%) (Suppl. material 2: table S1).

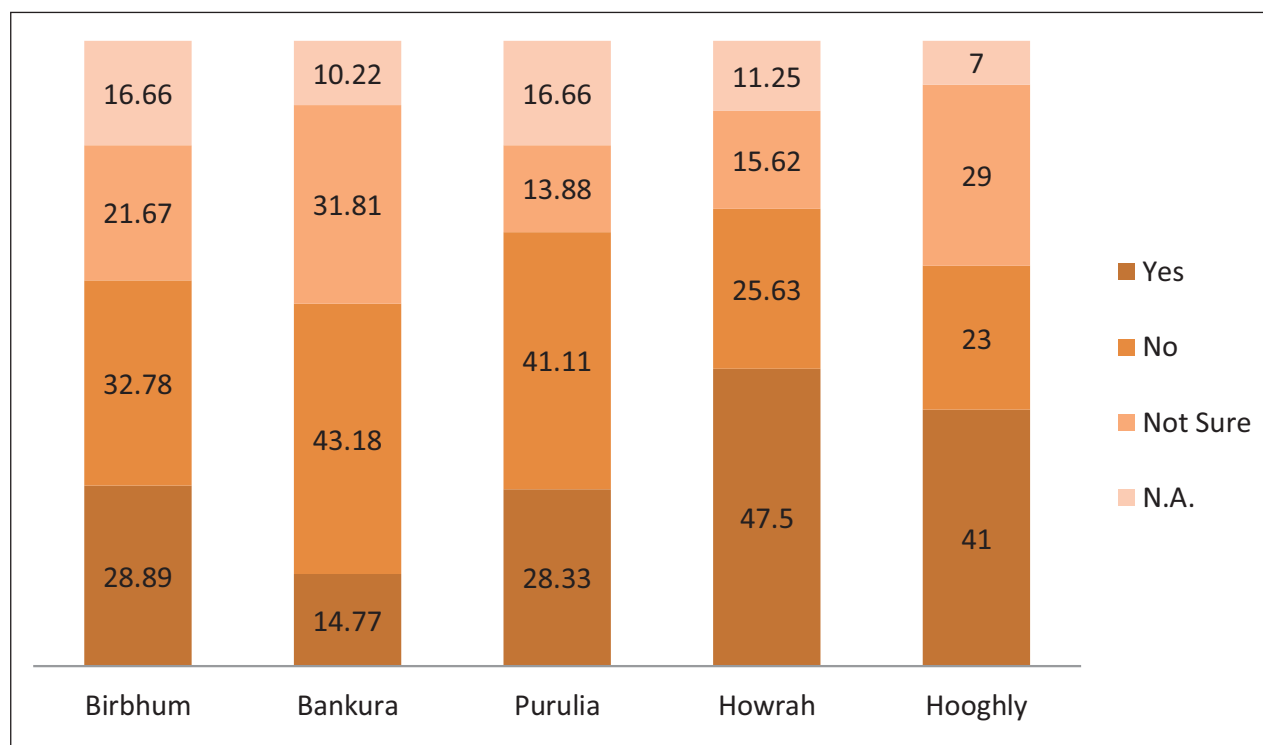
When asked about their attitude towards monitor lizards in their respective area (Question 12), the negative answers were higher in single districts (e.g., 15.96% vs. 5% in Bankura) than in the previous question, but the average was slightly lower with a value of  $9.1\% \pm 4.5\%$ . In turn, positive attitudes were higher (ranging from 21.67%–43.13%, on average 31.6%) as was the case in neutral answers ( $x = 46.45\% \pm 4.3\%$ ) (Table 2).

The knowledge of conflict situations (Question 13) was highest among the participants in the Howrah district (47.5%) followed by Hooghly (41%), Birbhum (28.89%), Purulia (28.33%) and Bankura (14.77%) (Fig. 3). One-third (32.10%) of all participants were aware of conflicts within the five districts, while about the same proportion denied this question ( $x = 33.14\%$ ) (Table 2).

Among the three potential areas of conflict provided in the questionnaires (Question 14), fish ponds received the highest scores (on average 14.97%, ranging between 26.88% in Howrah and 6.67% in Birbhum), followed by the open option “others” (on average 12.03%), to which the most common answers were the conflict situation when monitor lizards entered backyards or houses of the villagers in Howrah district. Notably, there were no answers provided from the other districts. The options “poultry” and “garbage” received on average 6.41% and 11.62%, respectively, while 54.95% did not answer this question.



**Figure 2.** Comparison of the participants' perceptions of the three questions. The questions include the knowledge (Q9), presence (Q10), and sightings (Q11) of monitor lizards found in the five districts expressed in percentages of those who answered “yes”. Note therefore, that the sum is not 100% in each column.



**Figure 3.** The graph shows the knowledge of conflict situations with monitor lizards among the respondents. This is the result from the five districts expressed in percentages (Question 13).

The knowledge about utilization of monitor lizards (Question 15) was found to be highest among the respondents from Purulia (14.51%) followed by Hooghly (10.55%), Howrah (9.38%), Bankura (8.55%), and Birbhum (5.56%) with an average of 9.71%, while the majority (on average 72.7%) from all districts denied this question (Table 2). The open follow-up question (Question 15a, Table 3) was to specify the purpose of utilization of monitor lizards for those participants who had opted for “yes”. Here, the common answers were that the species or their body parts were used for meat (especially the flesh near the belly fat is considered delicious), oil, leather, musical instruments (drums), and medicines. Noteworthy, a high number of participants specifically mentioned that local tribes, such as the Santhals, traditionally consume monitor lizard meat.

Also, the proportion regarding the sightings of the sale of monitor lizards and their parts (Question 16) was highest in Purulia (14.44%), while 2.22% in Birbhum stated “yes”. The majority of participants, on average  $71.01\% \pm 5.5\%$  (ranging from 62.01% - 76.61%), denied this question (for more details see Suppl. material 2: table S1).

Among the five districts that provided information on the illegal utilization purposes of monitor lizard parts (Question 16a), the usage as leather was highest (on average 6.32%) followed by oil (2.65%), meat (2.57%), medicines (2.10%), and astrology or witchcraft (1.21%). The perceptions of the use of monitor lizard skin for leather products were found to be highest in Purulia (10.97%) and lowest in Birbhum (1.11%). In comparison, the usage of monitor lizard oil was revealed to be highest in the Bankura district (8.05%) and lowest in Birbhum (0%). The perceptions of the utilization of monitor lizard meat were at almost the same level in all the districts investigated (on average 2.5%). Perceptions of the usage of the species for medicinal purposes were found to range from

Hooghly (3.21%) to Bankura (2.76%), Purulia (2.56%), Birbhum (2%), and Howrah (0%). The utilization in astrology or for witchcraft and mojo purposes such as the use of Hatha Jodi in black magic resulted in 2.63% (Purulia) and 0% (Birbhum) (for details see Table 2, Suppl. material 2: table S1). There was also the open answer option “others” in the question, which was chosen on average by 3.77% of the youth (the highest value was detected in Hooghly with 7.8%) and thus received the second highest percentage of all provided answer options. Here, the most common answers of the participants (who chose “others”) were the utilization of the monitor lizards’ fat and claws (see questionnaire).

The district with participants who have seen or have participated in the hunting of monitor lizards (Question 17) was highest in Howrah (36.88%) followed by Hooghly (27.06%), Purulia (25.27%), Bankura (23.95%), and Birbhum (23.89%). The majority, however, on average 58.99%, denied this question. For more details about the five districts, please refer to Suppl. material 2: table S1. The open follow-up question (Question 17a, see Table 3) asked those participants who opted “yes” to specify the place or location where they have heard of people hunting the species. The common answers were “in their villages or the neighbouring villages”, “near ponds and marshlands of the villages”, and “in the nearby forests, open grounds”.

The knowledge of Hatha Jodi (also known as Kakanashika or Bagnakhi), a plant root used for Ayurveda medicinal purposes with the newly emerged illegal practice of monitor lizard genitalia being sold as alleged plant roots (Bhattacharya and Koch 2018b; Sharma et al. 2019), was found to be almost negligible among the respondents in the five districts (Question 18). On average, just 3.0% of the students knew about Hatha Jodi, the highest value was from the Purulia district (5.76%), followed by the districts of Bankura (3.83%), Birbhum (2.22%), Howrah (2.5%), and Hooghly (0.92%). The open follow-up question (Q18a) about the personal opinion of Hatha Jodi was answered by only five students, of which four participants from Purulia considered it as a plant root and one respondent from Bankura regarded Hatha Jodi as an animal.

Consequently, very few participants from the districts were aware of persons selling Hatha Jodi (Question 19). On average, just 2.12% of all respondents answered “yes”, while the vast majority, on average 78.8%, opted for “no.” To the subsequent follow-up question concerning the selling places of Hatha Jodi (Q19a), there was just a single response from a pupil from the Purulia district, who wrote “near my house – used for medicinal purposes.”

Whereas, for the question regarding the usage of Hatha Jodi (Question 20), an average of 2.13% decided on the option “yes”. On the contrary, 75.37% opted for “no”, 14.36% were not sure and 8.11% chose not to answer. There were no prominent differences in the percentages found between the various districts. The next follow-up open question regarding the purpose of the use of Hatha Jodi (Question 20a) had two common answers: “for the treatment of inflammation” and “for black magic or witchcraft.” Among those few who were aware of the Hatha Jodi practice, of the three provided categories of potential selling places (Q20b), local marketplaces and astrologers were almost equally chosen by the respondents with, on average, 2.48% and 2.46%, respectively, followed by the online sale (on average 1.43%). The majority of the respondents from all the districts (on average 88.42%) chose not to answer this question. Similar to some earlier questions, online sources were found to be the highest in Purulia district



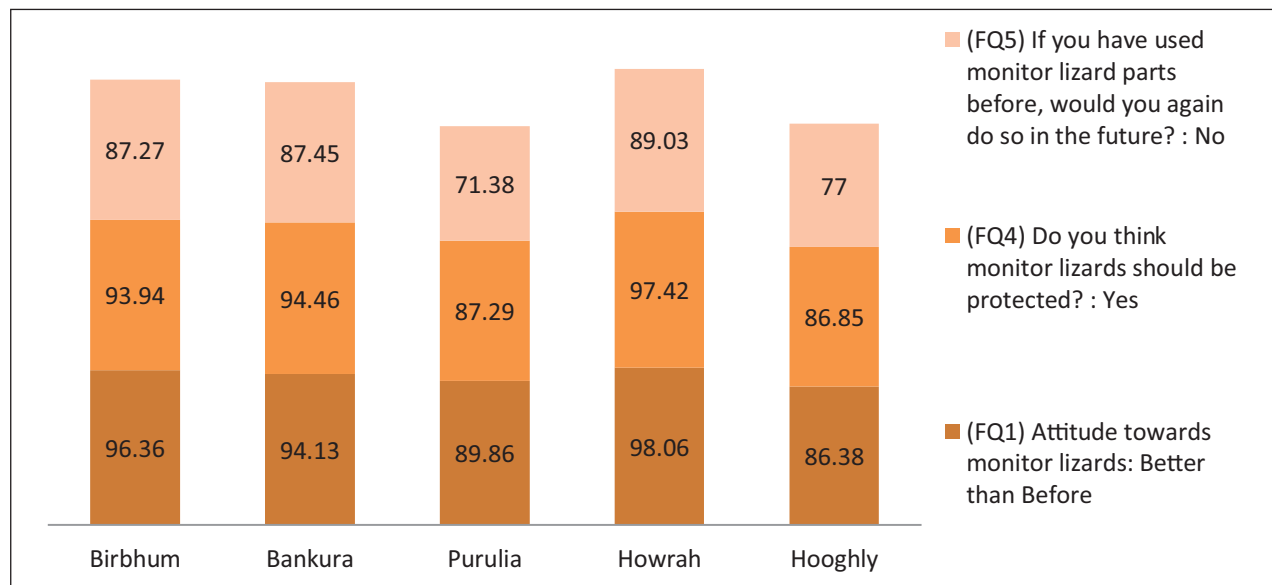
(4.79%), followed by Hooghly (1.38%) and Bankura (0.98%), but this answer was not opted for by the participants from Birbhum and Howrah. In turn, participants from the Hooghly district favoured astrologers, fortune tellers, and shamans (4.59%) as selling places of the Hatha Jodi. The common responses of the participants for the option “others”, which was chosen, on average, by 5.19% were from individual sellers, tribes’ men, and locals, who can acquire Hatha Jodi on personal orders. Here, the highest value of 9.99% resulted from the participants of Bankura district (for more details please refer to Suppl. material 2: table S1).

### Feedback questionnaire data analysis

The feedback questionnaires were distributed among the participants after the workshops in order to receive some information about the effectiveness of our awareness-raising campaign. The answers per district to the three non-open questions are provided in Table 4. In general, the participants’ attitude towards monitor lizards was, on average, 92.96% better than before the workshops (FQ1, see Fig. 4). This overwhelming positive change in attitude was most pronounced in Howrah (98.06%), followed by Birbhum (96.36%), Bankura (94.13%), Purulia (89.86%), and Hooghly (86.38%). Only a very small proportion (on average 0.46%) had an attitude worse than before. Of all participants, 52 students (4.81%) recognized no change in their attitude towards monitor lizards and 1.76% did not answer this question.

**Table 4.** Summary Results of the open questions from the feedback questionnaire.

Questions	Qualitative answers
<b>QF2</b>	<p>Yes:</p> <ul style="list-style-type: none"> <li>- Learnt from the workshop presentation about the extent and impacts of wildlife utilisation and trade across the world</li> <li>- Monitor lizards are nationally protected in India under the Wildlife (Protection) Act of India, 1972 and it is a punishable offence to hunt them</li> <li>- Monitor lizards are important for the ecosystem</li> <li>- Monitor lizards are used in illegal wildlife trade (hatha jodi) and their numbers are declining globally</li> <li>- Monitor lizards feed on venomous snakes</li> <li>- It is our responsibility to promote the conservation of wildlife - Hunting festivals are illegal and is a threat to wild species</li> </ul>
<b>QF3</b>	<ul style="list-style-type: none"> <li>- Monitor lizards are nationally protected in India under the Wildlife (Protection) Act of India, 1972</li> <li>- Hatha Jodi are actually the hemipenes of monitor lizards and not a plant root</li> <li>- Hunting monitor lizards is a punishable offence</li> <li>- Monitor lizards feed on snakes</li> <li>- Monitor lizards are not a snake species</li> <li>- There are four different species of monitor lizards in India</li> <li>- Monitor lizards can survive in deserts, but we see them mostly near water bodies</li> <li>- There is no scientifically proven evidence about the healing success of the medicinal use of monitor lizard oil</li> <li>- Monitor lizards can be of various sizes</li> <li>- Local utilisation of monitor lizards is an unsustainable practice and can significantly impact their population</li> </ul>
<b>QF6</b>	<ul style="list-style-type: none"> <li>- We did not get any answers mentioning that anybody would like to use monitor lizard body parts again. For the Option “Would not use again” was answered as follows:</li> <li>- It is a punishable offence and violation of the Wildlife (Protection) Act of India, 1972</li> <li>- There is no scientifically proven evidence about the healing success of the medicinal use of monitor lizard oil</li> <li>- The monitor lizards’ population is decreasing due to local utilization</li> <li>- The utilization can contribute to illegal trade, for e.g., Hatha Jodi</li> </ul>



**Figure 4.** The feedback responses of the participants from the five districts to the three non-open questions. Note that the values are expressed in percentages.

The common opinion concerning the change of attitude of the participants after the workshops (FQ2) was that monitor lizards were previously considered mere pests and were chased away, or in some instances even killed without the presence of any conflicts or reasons. However, after knowing about the habitat fragmentation, threats, and ecological importance of these large reptiles, and how it is ultimately the responsibility of the local inhabitants, especially the youth, to bring about a positive change in the societal norms and rituals, their attitudes and opinions have changed towards the species (see also FQ4).

Most participants mentioned that they had no previous knowledge that the Indian monitor lizards are protected under the Wildlife (Protection) Act of India, 1972, and that utilizing the species or any of their parts is a punishable offense (FQ3). Also, some of the students were surprised to know that monitor lizards can also occur in deserts (i.e., *V. griseus*), as they were only used to observing these reptiles in or near the village water bodies.

Accordingly, after the workshops the majority of participants opted for the protection of monitor lizards (FQ4). Values ranged from 86.85% (Hooghly) to 97.42% (Howrah), (mean: 91.99%). Thirty-one students (2.90%) denied this question, while 2.35% (Bankura) to 6.10% (Hooghly) were indecisive (see Fig. 4 for details). Participants who had used monitor lizards and their body parts before and who did not favor the future use (FQ5, see Fig. 4) ranged between 71.38% (Purulia) and 89.03% (Howrah), (mean: 82.43%), while 5.97% (Purulia) to 0% (Howrah) (mean: 3.04%) were willing to continue the use of monitor lizards after the workshops. Between 6.06% (Birbhum) and 16.52% (Purulia) (Suppl. material 2: table S2), (mean: 10.29%), stated being not sure.

The most common answer to the question (FQ6), why they would or would not utilize monitor lizards or their body parts in the future, was that, after the workshop presentations and discussion session, they had learned that if the species are further exploited for their body parts, then they might get locally extinct. Also, another opinion was that there is no scientific evidence on the medicinal efficacy of monitor lizard oil, which is locally used for the

cure of rheumatism and muscle sprains. There were no written responses regarding the students' willingness for the future utilization of monitor lizards or their parts.

## Discussion and conclusions

Data obtained from the questionnaires provided a clear understanding of the attitudes and perception trends of the young participants from the five districts studied in West Bengal. The "rural" and "urban" areas refer to the specific localities of the participants from each district. The baseline data regarding the various categorized questions treated above can be used to recognize the presence, sightings, interactions, local utilizations, hunting pressures, conflicts, and the illegal trade of monitor lizard species in human-dominated landscapes of eastern India.

Based on our study findings, the perception of the presence of wetlands (Q5+6) can be correlated with that of the presence of monitor lizards (Q10). The presence of wetlands was found to be the highest among respondents of Howrah and the least from Purulia. In this regard, it is interesting to note that Howrah exhibited the highest percentage (55.62%) of urban respondents, and considering the comprehensive perception data, such as the presence of monitor lizards (84.37%), it may be interpreted as an indication for the ecological adaptability of monitor lizards to human-influenced environments (Uyeda 2009; Bhattacharya and Koch 2018a). Similarly, perceptions of the knowledge (Q9), presence (Q10), and sightings (Q11) were found to be the highest in Howrah and the least in Purulia (Fig. 3), which suggests that most respondents from Howrah were aware of the presence of monitor lizards in their region. This interpretation can be reconciled with the highest percentage regarding conflict situations (Q13+14) with monitor lizards attracted by fish ponds.

The perceptions of the use of monitor lizard skin as leather products and the knowledge of Hatha Jodi were found to be the highest in Purulia. These trends may indicate that although the perception of the presence of monitor lizard species was the least in Purulia, their local illicit use and trade might be the highest. Similarly, sightings of products made from monitor lizard parts were significantly higher (14.44%) in Purulia compared to the other districts, which might suggest the more frequent presence of local markets selling illegal wildlife products in the district. In this context, it is noteworthy to mention that one of the participants from Purulia stated in the open questionnaire that monitor lizard products, such as oil, belly fat, and claws were openly sold in some of the local marketplaces.

Interestingly, perceptions of the hunting of monitor lizard species were similarly categorized in all five districts (See Suppl. material 2: table S1, Q17). This trend may indicate the equally high extent of illegal hunting and local utilization of monitor lizards irrespective of the perception of their presence in the five districts. However, the highest proportion was observed in Howrah (36.88%), potentially due to the high perception values related to the presence (84.37%), sightings (96.88%), as well as conflicts with the monitor lizards subjected to fish ponds (26.88%) compared to the other districts. Despite the above facts, the perception data values regarding the utilization of the monitor lizards (see Q15, Q16, Q16a from Suppl. material 2: table S1) and with reference to Hatha

Jodi (see Q18, Q19, Q20, Q20b; Suppl. material 2: table S1) were comparatively found to be lower than the majority of the districts. This observation would imply that although monitor lizards are being hunted, infrastructure (e.g., local markets) in Howrah is absent or negligible to encourage trade in illegal wildlife, as compared to Purulia.

Besides, during the workshop presentation session, we asked the participants about the Wildlife (Protection) Act, 1972, concerning the protection of monitor lizard species in India. The majority of school and college students were unaware of the existence of such national laws. Therefore, a simple measure to disseminate information about nature conservation laws and to improve local knowledge about protected wildlife species could be the installation of information boards near suitable and possibly protected habitats. Also, to simultaneously emphasise the importance of protecting ecosystems and biodiversity to generate ecosystem services considered essential to human health as well as to sustain livelihoods for local communities (Keane et al. 2011; Redford et al. 2022).

Knowledge regarding the Hatha Jodi trade was negligible among the students queried in the five districts. Only a few participants from Purulia, Bankura, and Birbhum appeared to have been confronted with these items and their trade. However, since it is not known whether the information about these illegal activities has already been passed on in the five districts, this could be an explanation for the clear reticence of the respondents. Besides, another interpretation is that the young respondents from the schools and colleges were not yet the direct consumers of Hatha Jodi. The answers suggested that the selling places of Hatha Jodi in these districts were mostly in markets, from astrologers, and through online shopping sites. A crucial observation is that the illegal sale of such items is often easier through commercial online sites (Lavorgna 2014; Harrington et al. 2021). In contrast, in the outskirts the sale of Hatha Jodi is considered suitable in open marketplaces or through astrologers, likely a result of lacking or inappropriate legislation, enforcement, and the failure to provide information and awareness on national legislation in these remote regions.

The feedback data analysis indicates the success and effectiveness of our workshops. A considerable proportion of participants (mean: 92.95%) recognized a positive change in their attitude towards monitor lizards after the workshop sessions. Similarly, the majority of the participants (mean: 91.99%) from all five districts supported the protection status of all Indian *Varanus* species. Likewise, an average of 82.42% of all participants were against the future use of the monitor lizards or their parts, which gives reason for confidence in future compliance with the existing laws for the protection of these giant reptiles in West Bengal. However, there might be biases due to the social desirability/undesirability of the answers provided (Gordon 1987).

Nevertheless, awareness workshop programs are an integral part of species conservation measures as a result of, and in conjunction with, scientific research (Ardoin et al. 2020). As stated by Castillo-Huitrón et al. (2020), perceptions, beliefs, and experiences help to shape the structure of a society. Besides, in human-dominated landscapes, to address the issue of species conservation in general, it is equally essential to consider the attitudes of local communities as that of the ecological parameters of the species occurring in the region

(Mascia et al. 2003). The results of a similar study on the awareness program of the Komodo dragon (*V. komodoensis*) in Indonesia were found to have positive attitudes among elementary school students concerning the diet, habitat, and conservation efforts of the species (Kamil et al. 2020). These results can be compared to our study, wherein in the feedback question (FQ1) the majority of the respondents (92.95%) agreed that their attitude towards monitor lizards has considerably improved after the awareness workshops had taken place (for details see Suppl. material 2: table S2).

Similarly, a study about the perceptions and conservation awareness of local citizens towards the endangered marine green turtle (*Chelonia mydas*) in Malaysia was found to be more positive among the younger generation when compared to the older ones. Often, it is more difficult for the older generation to break away from their traditional beliefs and adapt to alternative behaviors (Mutalib et al. 2013). Furthermore, the study also revealed that the younger respondents were more sensitive toward educational programs which have prominently increased their awareness towards the conservation of sea turtles in Setiu, Malaysia (Mutalib et al. 2013); a joint finding with this study. A study on the conservation of the cotton-top tamarins in Columbia, found valid evidence of the engagement of youth following in the conservation of the critically endangered primates in the region, and that the knowledge of the students significantly increased ( $p < 0.01$ ) after conservation education programs were performed (Feilen et al. 2018). Another study by Salazar et al. (2024) found a significant positive increase in the local knowledge of rural school children aged 10–13 years in India as a result of conducting educational programs in the areas affected by human-wildlife conflicts.

Thus, from the few results of the above-mentioned studies, it is obvious that the youth can be motivated and inspired to develop a positive attitude toward the conservation of a certain species (Mutalib et al. 2013; Feilen et al. 2018; Kamil et al. 2020; Salazar et al. 2024).

Findings of Sánchez-Mercado et al. (2020), concerning the illegal trade of the yellow shouldered Amazon (*Amazona barbadensis*) in Venezuela, were found to be linked to the perceptions and attitudes of the local communities. Subsequently, the evaluation of the perception trends of the communities were used to design effective conservation programs in the study (Sánchez-Mercado et al. 2020). In addition, the baseline perception data obtained from our workshops can be used to analyze ecological factors, threats, utilizations, and conflicts related to the monitor lizards among the respondents of the five districts.

In conclusion, once the negative perception trends are analysed in a particular area, they could be further addressed and gradually altered with more such workshop programs over a specific period. Over-exploitation of natural resources commonly results from the lack of enforcement and regulations being ignored (in this study the interviewees learned about the protected status of the monitor lizards for the first time). However, the involvement of local people as stakeholders, e.g. leading such educational campaigns, could reduce exemplified violations (Secretariat of the Convention on Biological Diversity 2004).

Therefore, awareness workshops (which are to be continuously adapted and improved according to gained experience) should be regularly conducted in areas where protected wildlife species such as monitor lizards (threatened and yet to be nationally protected) are illegally exploited for domestic use and trade.

This approach will further help to promote and encourage local communities and the young generation to develop a positive attitude and responsibility towards conservation of vulnerable wildlife and their ecosystems in human-dominated landscapes. Based on these findings, it can be deduced how changes in ecosystems affect human well-being and poverty reduction and why the sustainable protection of ecosystems and their biotic communities contributes to human well-being (Millennium Ecosystem Assessment 2005). The benefits that nature provides are determined by the maintenance of resilient ecosystems, their services, and values (TEEB 2010). Nature Contributions to People (NCP) are intertwined with the non-anthropocentric value of nature and the anthropocentric value that is conducive to a good quality of life (Pascual et al. 2017). These essentials need to be disseminated in local communities as part of subsequent educational campaigns.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

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### Author contributions

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## Data availability

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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## Supplementary material 1

### Questionnaires of the awareness workshops

Authors: Shreya Bhattacharya, Syeda Zuboor Zia, Santanu Mahato, Ravi Kumar Gangwar, Neha Singh, Mark Auliya, André Koch

Data type: pdf

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## Supplementary material 2

### Supplementary tables

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Data type: pdf

Explanation note: **table S1**. Workshop questionnaire (Pre-Presentation) results of the multiple-choice questions from respondents of the five districts. **table S2**. Results of the feedback questionnaire for the three non-open questions.

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## Research Article

# The old good landscape maps: New interpretations enabling ecosystem services assessment of conservation potential at a national scale

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## Abstract

The ecosystem services (ES) approach has been widely accepted in environmental policies and management as an adequate platform that can serve as a link between nature and society. Many ES are influenced by the landscape structure. Thus, national-scale landscape mapping can potentially contribute to nature conservation management. However, there are no attempts to directly link the ES assessment with the landscape units at a national level. In this paper, we propose an approach for the transformation of paper copy information from old landscape maps to enable the assessment of ES conservation potential at the national landscape scale. The conceptual scheme of the approach contains three main elements: (i) data acquisition; (ii) landscape and ES assessment data processing; and (iii) mapping of ES potential at a landscape level. The results reveal the landscape heterogeneity based on landscape classification and mapping at a national level and the ES conservation potential based on the analyses of the Natural Heritage (NH) in the country to provide ES. The assessment of ES conservation potential using the national scale landscape mapping allows us to analyze the spatial relationships between the landscapes with high conservation value and the existing nature protection network. The conceptual scheme of the study demonstrates how the results of the ES potential provided by the NH at a national level can be combined with the landscape units from the traditional landscape classification schemes to produce various spatial and statistical metrics that reveal how the national system of protected areas coincides with the areas of high ES conservation value.

**Key words:** Bulgaria, digitization, GIS, landscape classification, landscape heterogeneity, mapping, nature conservation, spatial data

## Introduction

Conservation and natural resource management have been dominated by approaches that tend to focus on a single sector and a narrow set of objectives that often ignore the wider set of consequences of decision-making (Tallis and Polasky 2009). The ecosystem services (ES) approach has been widely accepted in environmental policies and management as an adequate platform that can serve as a link between nature and society (Burgos-Ayala et al. 2020). The mapping of ES is



a crucial activity because the conservation benefits cannot be identified unless ES can be quantified and valued and their areas of production mapped (Naidoo et al. 2008). The importance of the spatial aspects of ES is stressed also in the EU biodiversity strategy by the requirements for the member states to map and assess the state of ecosystems and their services in their national territory (Maes et al. 2013). The methodological framework for Mapping and Assessment of Ecosystems and their Services (MAES) provides a set of indicators for the assessment of ecosystem condition and mapping of ES that have been developed both at the European level and at the national level in the EU countries. The experience from the different approaches developed through the activities of its implementation is a valuable source of information for developing assessment processes further, especially with regard to their uptake into policy and decision-making (Vári et al. 2024).

Natural heritage (NH) as an outstanding universal value at a national level, refers to the importance of ecosystems, biodiversity, and geodiversity for their existence value. The ecosystems can be considered as the spatial units for its mapping and assessment and the ES concept provides an appropriate basis that can be used as a platform to find solutions to the problems related to the conflicts between conservation and the use of the NH (Nedkov et al. 2021a). However, many ES are influenced by the landscape structure such as the position of ecosystems, functional traits, landscape elements, or land use units in the space (Bastian et al. 2014). Willemen et al. (2012) propose the use of the concept of 'landscape services' as the flow of ES to society provided within a landscape. In general, the landscape approach to the assessment of the benefits from nature to people and the mapping and assessment of ES at the landscape level has also been proposed and applied by some other authors (Termorshuizen and Opdam 2009; Willemen et al. 2010; Müller et al. 2010; De Groot et al. 2010; Prager et al. 2012; Lei et al. 2016; Kivinen et al. 2018; Bezák et al. 2020).

Landscape definitions differ according to the context or type of application (Mücher et al. 2009) and the approaches used to map the landscapes differ in different countries and even within a country. In Germany, the national-level landscape mapping was based on data on national boundaries and current land use while each landscape was assigned to different landscape types and geographical regions (Gharadjedaghi et al. 2004). However, the regional mapping in Saxony was based on classification based on physical-geographic features and landscape units (microgeohores) defined from various sources (Bastian 2000). In the UK, the mapping is based on the concept of a landscape character area (LCA) which is defined as "a distinct recognizable and consistent pattern of elements in the landscape different from another, rather than better or worse" (Somper 2002). The national landscape typology based on LCA for England has 79 generic landscape types (Mücher et al. 2009). In Bulgaria, landscape works are predominantly based on the geosystem concept which defines the landscape as "a specific geographical area forming a system of natural components (rock, soil, air, water, vegetation, and animals), which is changing in time under both natural factors and human activities" (Nedkov and Gikov 2014).

Assessing the ecosystem services using the national scale landscape mapping is a research question that can potentially contribute to nature conservation management. The results of such an assessment could enable us to define whether the rich natural heritage landscapes with high ES conservation value are well protected by the national system of protected areas. The long-term

experience in the mapping of landscapes at a national level (Tzvetkov 2021) and the recent development of the ES mapping and assessment practices in Bulgaria (Nedkov et al. 2024) are a good basis to test such a hypothesis.

The tradition of landscape mapping in Bulgaria is based mainly on the assumption that the landscape is a system of interacting components. This approach is developed particularly in Russia (former USSR) and Eastern Europe (Kondracki 1960, Richling 1984, Romportl and Chuman 2012), and is based on the geosystem paradigm, on soil science, physical geography, and geology, which is not well known to the international readership due to the lack of publications in English (Bastian et al. 2015). The landscape maps are based on hierarchical classification which vary in the number and characteristics of the taxa. The first Bulgarian map at a national level was developed by Petrov (1979) for his doctoral dissertation in 1974 who utilized the five-level classification system proposed by Gvozdetski (1972). The second national scale landscape map was developed by Nikola Todorov for his dissertation in the mid-80's and published in co-authorship with his advisor Velchev et al. (1989, 1992) who utilized the four-level classification system proposed by Beruchashvili (1986). Both maps are prepared using traditional cartographic methods and are available only as paper copies. The lack of digital copies of the landscape maps at a national level is the first research gap to be solved by this study. Furthermore, the hard copies contain distortions in some of the landscape contours that need to be corrected.

The mapping of ES at a landscape scale is a popular topic in the ecosystem research. Bastian et al. (2014) argue this with the assumption that many ES are influenced by the landscape structure, e.g. the position of ecosystems, functional traits, landscape elements or land use units in the space in question. The mapping of ES at a landscape level is argued by Willemsen et al. (2010) with the assumption that the landscapes are spatial systems formed by the interaction between human and environment and the specific geographic context is important for both ES supply and demand. The importance of landscape structure on ES provision through multiple landscape-level processes and their influence on ES supply and demand is studied by Metzger et al. (2021). However, there are no attempts to directly link the ES assessment with the landscape units at a national level.

The main objective of this study is to develop an approach that enables the assessment of ES conservation potential at a national landscape scale based on spatial data from old landscape maps. The specific tasks are: (i) to explore the quality of the old landscape map of Bulgaria and transform its content into a GIS database; (ii) to assess the potential of the NH at the national level to provide ES for the needs of nature conservation; (iii) to analyze the spatial relationships between the landscape units, ES conservation potential and the existing nature protection network at the national level.

## Materials and methods

### Methodological approach

Our study deals with two types of initial data (landscape mapping and ES assessment data), which were generated at different times and using different research approaches. The landscape mapping in Bulgaria at a national scale held in the 80s and 90s of the 20<sup>th</sup> century used traditional multicomponent analysis and

paper-copy maps. This necessitates preprocessing of the available data, georeferencing, vectorization and validation. The ES assessment data for Bulgaria are available from various studies but only a few of them deal with the entire country at a national level. In this case it was a matter of selecting the most appropriate ES assessment study and adapting the data to the needs of the current study. This means, in particular, reprocessing procedures and analyses related to the conservation purposes of the study. Finally, the two sources are integrated to achieve the main objective directed to exploring the linkages between ES provision at a landscape scale. Therefore, we developed a methodological approach that incorporates different GIS techniques and spatial analyses. The conceptual scheme of the approach contains three main elements: (i) data acquisition; (ii) landscape and ES assessment data processing; (iii) mapping of ES potential at a landscape level (Fig. 1). Each of them is described in more detail in the following subchapters.

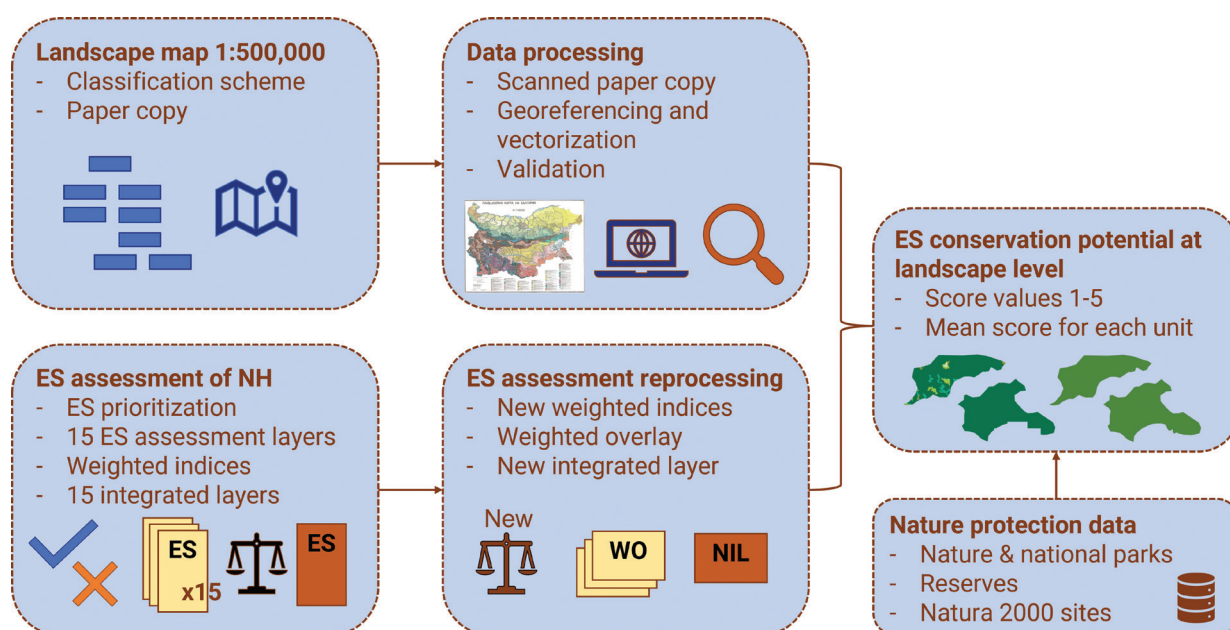


Figure 1. Conceptual scheme of the study.

## Initial data

### Landscape map of Bulgaria

In this study, we used spatial data for the potential landscapes in Bulgaria. The data were produced based on the second edition of a paper copy map of the landscapes in Bulgaria at a scale of 1:500,000 (Todorov et al. 2004 after Velchev et al. 1989, Velchev et al. 1992). This second edition map was published as a supplementary map in a learning book for students titled “Natural Geography of Bulgaria” (Todorov 2004). The paper map represents the spatial distribution of potential landscapes in the country according to the classification of Velchev et al. (1989, 1992). It consists of four hierarchical levels (class, type, subtype and genus) determined by different factorial criteria including main types of relief and geomorphological forms, hydro-climatic conditions, and natural vegetation. According to the map legend and landscape classification, the map represents two classes (level 1), 17 types (level 2), 29 subtypes (level 3), and 82 genera (level 4).

## ES assessment data

For this study we choose the dataset developed under the project “Conceptualization, flexible methodology, and a pilot geospatial platform for access of the Bulgarian natural heritage to the European digital single market of knowledge and information services” which aimed to promote the sustainable use of NH in Bulgaria using the ES as a conceptual background (Nikolova et al. 2021). The conceptual framework used in the project is based on the assumption that the generation of NH for the needs of specific activity can be presented as the linkages between the natural systems and this activity in the form of ES potential, flow, and demand (Nedkov et al. 2021a). The mapping and assessment procedures are fully developed for application at a national level and the dataset from the mapping are available in the form of ES assessment database. The identification and mapping of ecosystem types was made following the MAES topology of ecosystems (Maes et al. 2013) which is organized in two main levels and its structure enables CORINE Land Cover (CLC) data to be applied for spatial delineation. A third level of this typology was developed for Bulgaria (Bratanova-Doncheva et al. 2017; Zhiyanski et al. 2017). The CORINE classes were correlated to the ecosystem subtypes (third level of the MAES typology) to develop a relevance table (Hristova and Stoycheva 2021). The ecosystem services were selected as a results of prioritization procedure (Nedkov et al. 2021b). The application of the methodological framework resulted in generation of 15 GIS layers each of them corresponding to one of the 15 priority ES assessed by various methods at multiple tiers (Table 1). At tier 1, are the services with no uniform data at the national level, which were assessed by expert judgement. The services at tier 2 were provided with statistical data or biophysical parameters at the municipality level that could be interpolated using GIS spatial analyses at the national level. The services at tier 3 were selected for more detailed analyses by different modelling methods. The importance of the different ES for recreation and tourism is not equal and the results from the prioritization were used to define weight indexes that represent these differences (Nedkov et al. 2022). The generation of the GIS layer for each ES was made individually using different spatial unit according to chosen method for indicators quantification (Table 1). The results for each ES were normalized to the 0 to 5 relative scale to be comparable for the overall assessment.

## GIS processing and development of a landscape database

To create a digital version of the landscape map of Bulgaria, we used a scanned copy of the original paper map. The paper map was scanned on a large format scanner with a resolution of 300 dpi (Prodanova and Petrova 2020). The scanned copy of the map was then geo-referenced in GIS and used afterward as a basis to create a vector layer by reproducing the contours of the landscapes. The GIS processing was done manually using the “heads-up digitizing” process, meaning that work is being done while looking at the image on the computer screen. A polygon shapefile of the territory of Bulgaria with an

**Table 1.** Assessed ecosystem services at different tiers and methods. Methods abbreviations: E.A. – expert assessment; Stat. – analysis of statistical data; Sp. Pr. – spatial proxy model; Mod. – modeling methods. Spatial unit abbreviations: Ec. – Ecosystem subtypes; Mun – municipality; Var. – various. (after Nedkov et al. 2022).

Nº	High priority ES	N indicators	Tier	Method	Weight index	Sp. unit
I	Cultivated plants and animals used for nutrition	1	1	E.A	0.6	Ec.
II	Wild plants used for nutrition	1	1	E.A	0.7	Ec.
III	Animals reared to provide energy	1	2	Stat.	0.6	Mun.
IV	Surface water for drinking	3	3	Sp. Pr.	0.8	Var.
V	Regulation of pollution and other harmful impacts	1	1	E.A	0.7	Ec.
VI	Regulation of natural hazards	1	3	Sp. Pr.	0.6	Var.
VII	Maintaining populations and habitats	2	3	Sp. Pr.	0.8	Var.
VIII	Local climate regulation	1	1, 3	E.A, Mod.	0.6	Ec.
IX	Conditions for recreation by biotic systems	2	3	Mod.	1	Var.
X	Science and education value	2	1, 2	E.A, Stat.	0.8	Ec.
XI	Cultural heritage	1	1	E.A	1	Ec.
XII	Aesthetic experiences	2	1, 3	E.A, Mod	1	Ec.
XIII	Symbolic and spiritual value by biotic systems	1	1	E.A	1	Ec.
XIV	Conditions for recreation by abiotic systems	2	3	Mod.	0.9	Var.
XV	Symbolic and spiritual value by abiotic systems	1	1	E.A	1	Ec.

increased % transparency was used for both georeferencing and drawing polygons of the landscapes. The Projected Coordinate System was WGS\_1984\_UTM\_Zone\_35N in both cases. The landscape polygons were extracted one by one from the national territory with the application of the ArcMap editing tool “Cut polygon”. Each of the newly cut polygons was given a letter index following the legend of the original map. Another two layers representing major rivers and cities in Bulgaria were used as a reference. During the digitizing process, we noticed an approximate 30–40 km mismatch between the rivers and the riparian landscapes as originally mapped on the scanned and georeferenced copy. Herewith the contours of the Hydromorphous and Subhydromorphous landscapes were corrected where possible.

As a result of the landscape map digitization, new vector data were obtained for the lowest mappable units of the landscape classification at level 4, namely landscape genera. From this layer data, three new layers were subsequently exported to generate the spatial information about class, type and subtype landscapes (levels 1–3). We did this by merging all individual polygons belonging to the same taxa of the upper level. The procedure was repeated three times while data from the previous layer was used for the next one. All GIS layers contain attribute data for landscapes in the respective classification level, index and area calculated in km<sup>2</sup>. Indices were included to be used as short version labeling on map. They represent letter and number combinations following the original classification at each level. Letter indices were transliterated from Cyrillic to Latin and alphabetically ordered according to Bulgarian alphabet as published by Todorov et al. 2004. The final result at this stage of digitization was a GIS database (Suppl. material 1) containing four separate layers and one integrated layer of spatial data about the landscape diversity in Bulgaria according to the four-level landscape classification and map of Todorov et al. 2004.

## ES assessment data reprocessing

The ES assessment that produced the data described in previous section was carried out for tourism activity. The overall ES supply map was produced by weighted overlay of the 15 ES layers. For this study, we followed the same approach. First, the results from indicators' quantification (in vector polygon format) for each of the ES were integrated into a single layer. Then, all vector layers were converted into 50 m raster layers using the ArcGIS "Polygon features to raster data" tool. This ensures the correct spatial overlay between the ES layers. Thus, 15 layers with 50 m resolution representing the priority ES were generated. A weight index representing the significance of each of the 15 ES was calculated. For this study the weight indices were recalculated for the conservation activities. Some of the experts who assessed the ES for the prioritization procedure in the NH assessment were asked to grade the relevance of the selected ES to nature conservation. The values of the weighted indices are given in Table 2. The map of the overall ES potential of the NH to provide ES at the national level was generated using the ArcGIS map algebra tool which enabled us to apply the weighted overlay of the 15 ES raster layers.

**Table 2.** Weighted indices for mapping of the overall ES conservation potential.

Nº	High priority ES	Weight index
I	Cultivated plants and animals used for nutrition	0.6
II	Wild plants used for nutrition	0.9
III	Animals reared to provide energy	0.7
IV	Surface water for drinking	0.8
V	Regulation of pollution and other harmful impacts	0.8
VI	Regulation of natural hazards	0.9
VII	Maintaining populations and habitats	1
VIII	Local climate regulation	0.9
IX	Conditions for recreation by biotic systems	0.7
X	Science and education value	1
XI	Cultural heritage	0.8
XII	Aesthetic experiences	0.8
XIII	Symbolic and spiritual value by biotic systems	0.8
XIV	Conditions for recreation by abiotic systems	0.7
XV	Symbolic and spiritual value by abiotic systems	0.8

## Analyses of the NH potential to provide ES at a landscape level

For the analyses of the NH potential at a landscape level, we need to integrate the spatial data for three sources (ES assessment, landscape map, and protected sites). The NH potential to provide ES is represented by the integrated GIS layer generated at the previous stage. The digital landscape map contains four levels of heterogeneity corresponding to four taxonomic levels of the landscape classification. The first one is too coarse, while the third and fourth are too detailed for the analyses at a national level, therefore we choose the second level (landscape types). The classification criterion at this level is a hydro-climatic condition which led to the delineation of 17



landscape types (Todorov et al. 2004; Velchev et al. 2011). The contours of these landscape units were extracted in a separate layer to be used for the integration of the two sources by using the spatial overlay procedure. The potential of the landscapes to provide ecosystems services is assessed using the 0 to 5 relative scale proposed by Burkhard et al. (2009, 2012). In our case, the matrix contains the 15 ES selected at the previous stages but instead of CORINE Land Cover classes we used the GIS layers for each of the 15 selected ES. Two types of spatial data analyzing the NH potential for conservation purposes were produced at this stage. One is a layer representing detailed information about the scored conservation potential of all ecosystem types within a concrete landscape type. Second is a layer representing the mean scored potential for a single landscape type.

Second part of the NH potential for nature conservation was to analyze the spatial coverage of different landscape types within the existing nature protection network in Bulgaria. Data for protected sites was downloaded from the Environment Executive Agency. Originally, it consisted of six types of protected areas including national parks, reserves, nature parks, natural monuments, maintained reserves, and protected sites. We adapted this data for the purposes of our study by deleting the natural monuments and protected sites feature classes, and combined maintained reserves with reserves in one class. Additional data for Natura 2000 sites in Bulgaria was downloaded from the official website. First, the two layers for both directives for habitats and birds were combined into one by spatial overlay. Since many of the Natura sites fall within the national or nature parks and reserves territories, data for Natura sites was erased from the first layer representing other protected territories (national and nature parks, and reserves) while they have higher conservation level than Naturas.

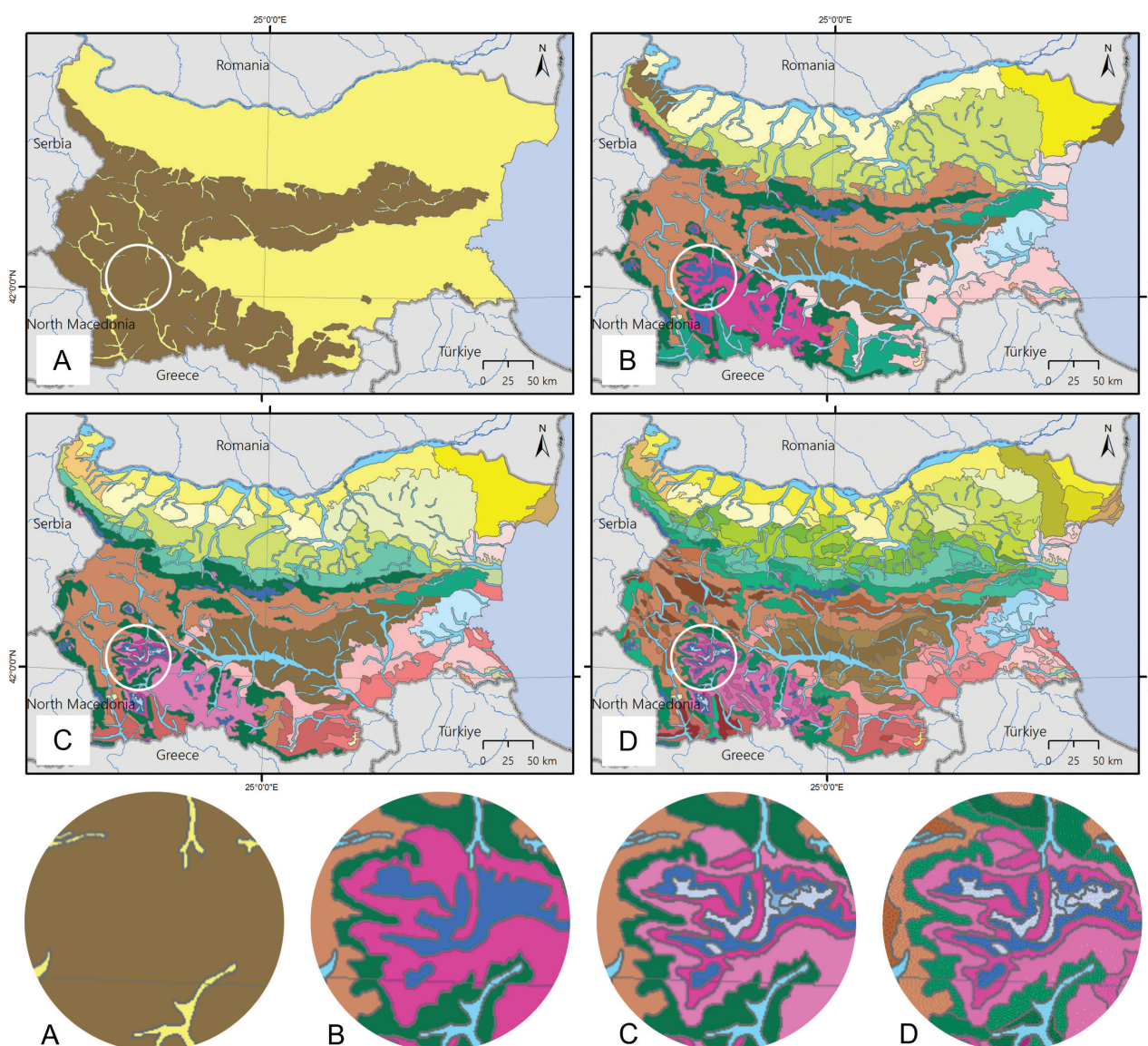
The newly erased layer was then combined with the parks and reserves one. This last layer consists of all four major types of protected territories in one single layer that is used for spatial and statistical analysis of the landscape types. By overlapping the landscape types with the protected areas' data we generated important information for the spatial distribution of landscapes with highest conservation potential, their characteristics, and showed the real % of nature protection. That allowed us to record the most represented type of protection within a particular landscape, and to highlight both the landscapes with least and most % protected territories.

## Results

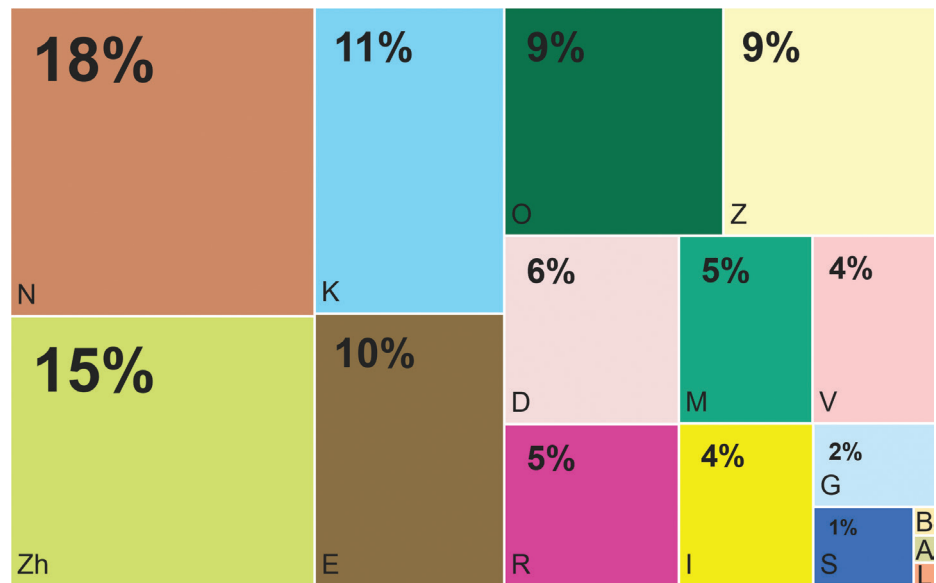
The application of the proposed approach enabled us to generate three main outputs which correspond to the three main tasks of the study. Firstly, the GIS database developed from the old landscape map enabled us to explore the spatial distribution of the landscape units in the country. Secondly, the reprocessing of the NH potential to provide ES enabled us to develop a map of the ES potential for conservation needs. Thirdly, the spatial analyses of the relationship between the landscape units, the ES potential and the nature protected areas enabled us to identify the landscapes with high potential to provide ES and how they correspond to the existing nature protection network in Bulgaria. The following subchapters present these results in more details.

### Spatial distribution of the landscape units in Bulgaria

The developed GIS database enables us to explore the landscape heterogeneity in Bulgaria at different classification levels. The inventory of the digital landscape map shows two classes, 16 types, 28 subtypes, and 82 genera. The landscape heterogeneity at the first level (class) shows a rather simple pattern with two relatively compact areas, which is especially valid for the plain landscapes (Fig. 2A). At the next level (type) the 16 taxa are unevenly distributed throughout the country with total areas ranging from 18% to 1% (Fig. 2B, 3). The highest share of the country is occupied by Mountain Warm-temperate Semi-humid (18%) and Foothills Hilly Warm-temperate Humid (15%). The first one is distributed as a compact area from the central-western to the middle part of the country. The second one is distributed also as a compact area in the northern part of the country. A similar spatial pattern with a slightly lower area has the Plain and Hilly semi-arid (10%) and the Plain and Hilly Temperate Semi-humid (9%).



**Figure 2.** Spatial distribution of the landscape units in Bulgaria at different levels of the landscape classification **A** level 1 **B** level 2 **C** level 3 **D** level 4. Spatial data is available in Suppl. material 1.



**Figure 3.** Distribution of the areas covered by the landscapes at level 2 of the classification. Indices are transliterated from Cyrillic to Latin letters and alphabetically ordered according to the original classification. Description of the landscape types **A** (A) hilly subtropical humid **B** (Б) plain and hilly mediterranean semi-humid **V** (В) plain and hilly submediterranean semi-humid **G** (Г) plain submediterranean semi-arid **D** (Д) plain and hilly warm-temperate semi-humid **E** (Е) plain and hilly semi-arid **Zh** (Ж) foothills and hilly warm-temperate humid **Z** (З) plain and hilly temperate semi-humid **I** (И) plain semi-arid **K** (К) hydromorphic and subhydromorphic **L** (Л) mountain subtropical humid **M** (М) mountain submediterranean **N** (Н) mountain warm-temperate semi-humid **O** (О) mountain temperate humid **R** (Р) mountain cold-temperate humid **S** (С) high-mountain grassland.

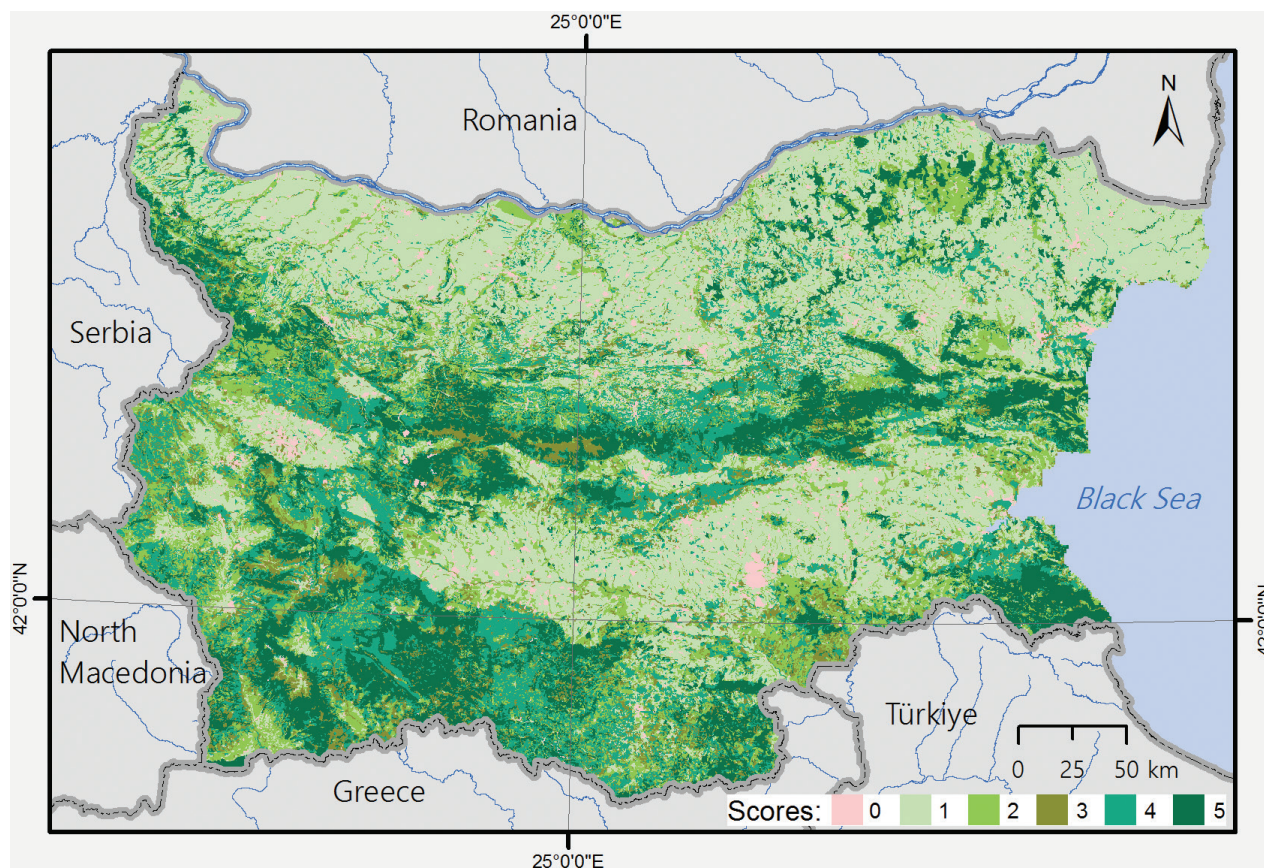
The Hydromorphic and Subhydromorphic landscapes occupy about 11% of the country. Still, their pattern is totally different from the previous as their areas have linear shapes with scattered distribution throughout the country. Another type with a relatively high share (9%) and scattered distribution is the Mountain Temperate Humid. The rest have a lower extent and three of them occupy less than 1% of the country's territory (Fig. 3).

The third level (subtypes) represents more detailed differentiation of 28 landscapes within the landscape types. Three of them (D, E, and S) are divided into three subtypes each, six (V, Zh, Z, M, N, and R) are divided into two subtypes each, while the remaining eight have only one subtype each. The landscape heterogeneity at this level is relatively higher, which is more pronounced in the mountain areas (Fig. 2C). At the fourth level of the landscape classification the heterogeneity is the highest with 82 mapping units delineated (Fig. 2D). Their distribution among the landscape subtypes varies between one and six.

### Mapping of the NH potential to provide ES for nature conservation needs

The results of the reprocessing of the ES assessment data for the needs of nature conservation enable us to generate a map representing the NH potential to provide ES at a national level (Fig. 4). The map represents the potential of the NH to provide ecosystem services at national level based on the overall score for the 15 selected ES. The values of the 15 ES were normalized





**Figure 4.** Potential of the natural heritage to provide ES for nature conservation.

to the 0–5 relative scale. It shows that the areas with very high potential are relatively evenly distributed across the country with a pronounced dominance in the mountain areas. They are presented by relatively small patches rather than compact extended areas. However, several clusters with a concentration of very high potential areas could be outlined. The largest one is located in the southwestern part of the country within the high mountain areas of Rila, Pirin, and the Western Rhodopes. It covers predominantly the altitude range between 1000 and 2000 m where the forest habitats (both deciduous and coniferous) are best preserved. The second one covers Central Stara Planina (Balkan Mountain) and Sredna Gora Mountain with similar altitude ranges and predominantly forest habitats. However, the coniferous forests are less present there. Three other clusters can be outlined in the western and eastern parts of Stara Planina Mountain. Two clusters are formed in the low mountains and hilly areas of the Eastern Rhodopes and Strandzha. The last one is located in the lowland-hilly area of the Eastern Danube plain. The areas with very high potential cover 19,118.6 km<sup>2</sup> which is about 17% of the country. The areas with high potential cover 19,010.3 km<sup>2</sup> (17%). The areas with moderate and low potential cover about one third of the country distributed again predominantly in the mountain areas but mainly in the highest peaks with bare rocks and no forest cover. The areas with very low potential (about 36%) are located primarily in the plains of the northern and southeast parts of the country. In general, the results show that the whole country has some potential for ES provision and the areas with no potential amount to only 3%.

### ES potential of the NH at a landscape level

The potential of the landscapes in Bulgaria to provide ES at the second level of the classification varies between 2.39 and 3.67 (Fig. 5). The highest scores are calculated for the Mountain Subtropical Humid (L), Plain and Hilly Mediterranean semi-humid (B), Hilly Subtropical Humid (A) and Mountain Cold-temperate Humid (R) landscapes. All of them are located in the southern part of the country (Fig. 6). Five landscape types have moderate potential with scores from 2.75 to 3.25. They are located predominantly in the mountains of central, western, and southern parts of the country. The landscapes with low potential have

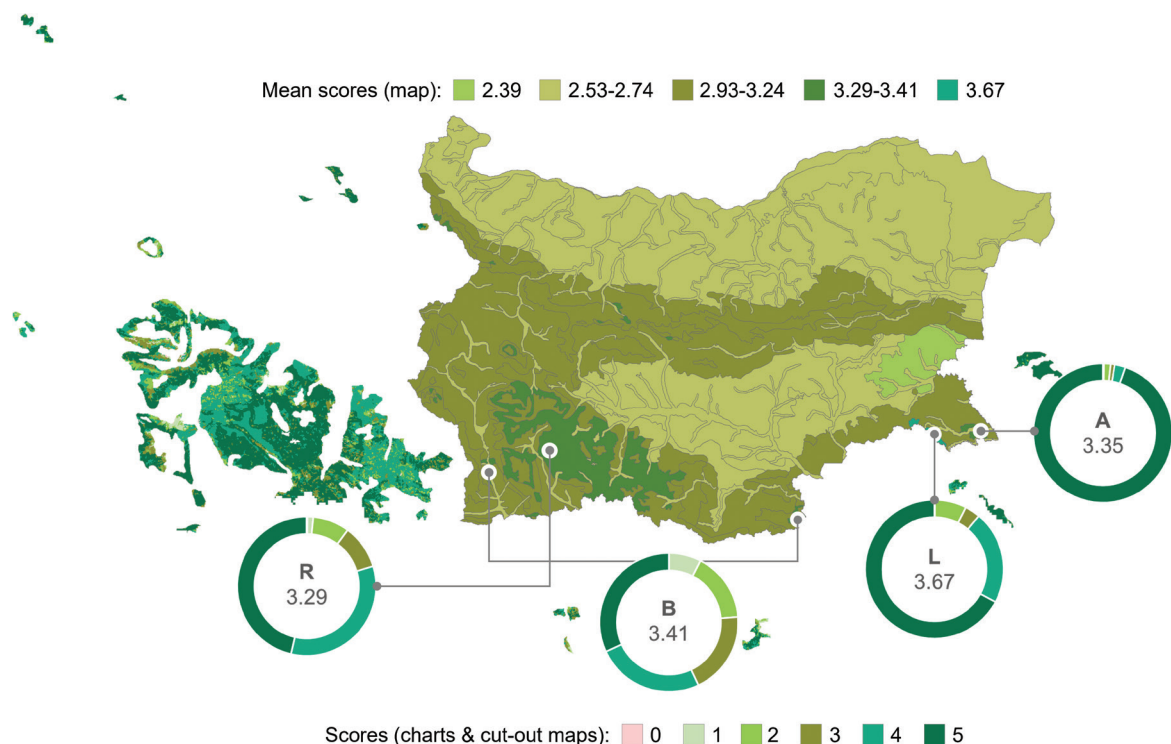


Figure 5. ES potential scores of the landscape types (the names of the indexes are given in Fig. 3).

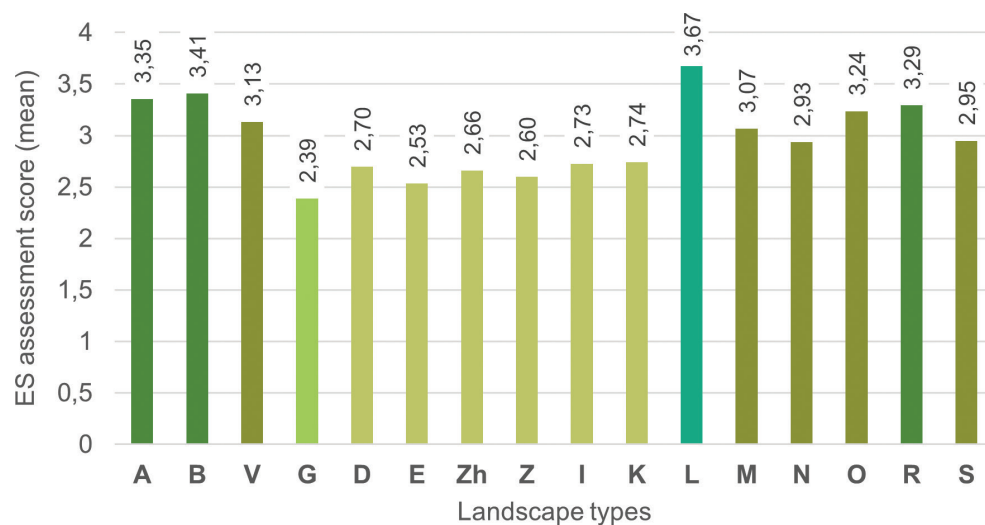
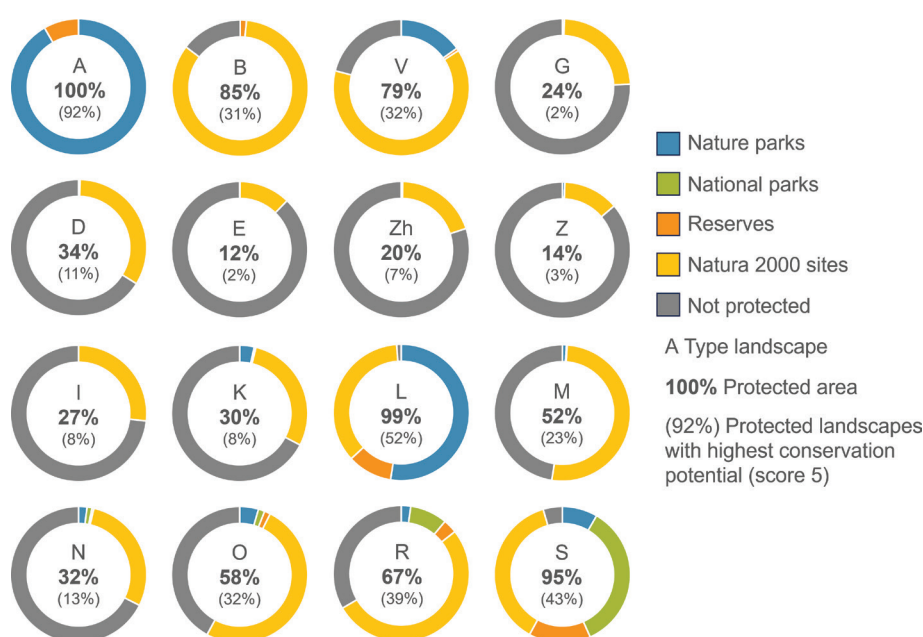


Figure 6. Potential of the landscapes to provide ES at a national level (the landscapes with highest potential are highlighted).

scores varying between 2.25 and 2.75. They cover the lowlands and hilly areas of northern (Danube Plain and Predbalkan) and southeastern (Upper-Thracian and Burgas Plains) part of the country. The lowest scores (2.39) are calculated for the Plain Submediterranean semi-arid landscapes.

The landscape with the highest level of nature protection is the Hilly Subtropical Humid (A), which falls entirely within protected areas (Fig. 7). Its protection is ensured predominantly by the Nature Park regime and partly by Reserve regime. This type is also in the group of landscapes with the highest ES potential. The other two landscape types from this group (B, and L) have high levels of nature protection (85% and 99% respectively) and similar protection patterns dominated by Natura 2000 sites. The fourth landscape type from this group (R) has a lower share of nature protection (67%), but more diverse protected regimes including Nature Park which is missing in the previous landscapes. Five landscape types (G, E, Zh, Z, and I) have a low level of nature protection (below 30%) presented only by Natura 2000 sites. Therefore, there is no IUCN category of protected area in these landscapes. Most of them also have lower scores of ES potential. For the rest of the landscapes, the share of protected area varies between 30% and 65%. A specific case is the landscape type S which has a very high level of protection (95%) and moderate ES potential.

A more precise indicator for conservation purposes is the percentage of the areas with the highest conservation potential (score 5) within particular landscapes under a protection regime. Only the Hilly Subtropical Humid landscape (A) has a very high percentage of such areas under protection. The Mountain Cold-temperate Humid landscape (L) has 52% protection for the areas with very high ES potential while the rest are below 50%. The highest contrast between the average ES score and the percentage of the protected areas with the highest potential is for the Plain and Hilly Mediterranean semi-humid Landscape (B). They have 85% protected landscapes but only 31 of them are with very high ES potential. Some landscapes such as G, E, and Z have an extremely low share (2–3%) of the protected areas with very high ES potential.



**Figure 7.** Distribution of the protected areas per landscape and those with highest conservation potential.



## Discussion

The geosystem-based landscape works from the 1980s and 1990s contain valuable information that may improve ES supply assessments by strengthening their scientific foundation and elaborating ES in a spatial context (Bastian et al. 2015). However, some of them are still in the paper archives which makes them inappropriate for contemporary research methods. This study proves the need to implement approaches that transform the landscape information from the paper archives to GIS data. The main problem in this transformation is the correction of the inaccuracies of the paper maps which necessitate searching for more precise digitization methods and verification with different data sources. The complex character of the landscape maps makes this verification even more difficult as some of the criteria for differentiation are based on the delineation of potential landscapes that cannot be verified by the existing data. The application of regression models based on the relationship between hydro-climatic indices and the topography is one possible solution (Prodanova 2022; Prodanova et al. 2024).

The landscape map of Velchev et al. (1989, 1992) which was used in this study, also has some shortcomings that should be discussed concerning the uncertainty of the results. Firstly, there is a difference between the taxa at the level type of the classification and the mapping units at the landscape map. The landscape type P (Mountain Temperate Semi-arid and Semi-humid) from the classification is missing in the original paper map as a polygon and consequently in the database. This affects also the results at the lower level of the classification. For instance, at the level subtype according to the classification, there are 29 landscapes, but in our database, there are 28. Secondly, the position of the Hydromorphic and Subhydromorphic (K) landscapes at the second level (type) is contradictory as the main criterion at this stage is the zonal distribution of the hydro-climatic conditions but their formation is determined by the azonal geomorphological conditions (Nedkov and Gikov 2014). Their spatial pattern is totally different from the other types as their areas have linear shapes with scattered distribution throughout the country (Fig. 2), which affects the whole landscape pattern at a national level. Further studies such as fragmentation analyses could be highly affected by this problem as the results with and without this landscape type would be totally different.

The results about the ES potential for conservation provided by the NH at the national scale show some similarities but also differ from the results about the tourism ES potential presented by Nedkov et al. (2022). The main similarity is in the distribution of the areas with very high potential which form similar clusters in both maps. However, the areas with very high ES conservation potential are twice as large. The main difference is in the distribution and share of the areas with low and very low potential. The areas with very low potential (score 1) are much larger on the map of ES conservation potential. The respective areas on the map of ES tourism potential have slightly higher score (2) that corresponds to low potential. Consequently, the areas with very low ES conservation potential (score 1) are more than twice higher than those with very low tourism potential. These differences concern mainly the lowland areas which have a predominance of cultivated lands and cultural landscapes. Their underestimation is a matter of further development of the methodology as there is a growing

interest in the conservation of cultural landscapes based on the awareness that they may be biologically rich (Eriksson 2018).

The landscapes with high conservation potential in Bulgaria are relatively well preserved in terms of coverage by protected areas. This is especially valid for three types (A, B, and L) which have limited extent in areas with very well-preserved natural habitats that facilitate their conservation. However, the share of the strictly protected areas there is low and they remain outside the national parks which are the best managed protected areas in the country. The fourth landscape type with high ES conservation potential (R), has a lower share of protected areas which is due to its larger extent. However, there are more diverse types of protection regimes (including two national parks) that ensure better options for nature conservation management. Natura 2000 network is the only nature protection option for many landscape types such as G, D, E, Zh, Z, I, and M. This protection network seems a good option for areas with a mixture of highly cultivated areas and small natural habitats. A study in the Czech Republic reports low overall effectiveness of the Natura 2000 network but the critically endangered habitats receive maximum protection (Pechanec et al. 2018). Therefore, these small natural habitats can have good protection if the respective Natura sites are well managed. For instance, landscape ecological principles could be applied for comprehensive landscape protection (Janík et al. 2024). The landscapes with lower ES conservation potential have also lower share of protected areas. A specific case is the High Mountain Grassland type (S) which has relatively low ES conservation potential but a higher share of nature protection regimes. One possible explanation could be the ES assessment approach which generally favors the forests and underestimates the low forested landscapes. These analyses provide initial data for the conservation options at the landscape level. However, further analyses are needed to propose better options for nature conservation at the landscape level. The integrated approach for landscape contrast analysis, proposed by Hou and Walz (2016), which integrates both ecotones and small habitats to obtain a detailed and comprehensive description of landscape pattern, is a good option.

Mapping and assessment of ecosystem services can be applied at different levels of scale and complexity which depends very much on the spatial units used for the initial mapping. One of the most convenient spatial units are the CORINE Land Cover classes which ensure timely and easily available data comparable for most European countries. However, CORINE data has its limitations and when more detailed and high resolutions sources are available, they are preferred. Specifically for the matrix approach besides CORINE Land Cover, the EUNIS habitat classification was used for marine and benthic habitats, as well as across different ecosystem types (Campagne et al. 2020). In our case, we combine various data sources (including CORINE) to develop spatial units for each individual ES. They are used for more precise assessment and mapping of ES. The landscape map represents another level of spatial organization and the integration of the ES assessment data into the landscape units ensures different view on the nature conservation pattern.

## Conclusion

In this study, we developed and applied an approach that enable us to transform the paper copy information from old landscape maps to GIS data that is appropriate for assessing the ES at a landscape scale. The assessment of

ES conservation potential using the national scale landscape mapping allows us to analyze the spatial relationships between the landscapes with high conservation value and the existing nature protection network. The conceptual scheme (Fig. 1) of the study demonstrates how the results of the ES potential provided by the NH at a national level can be combined with the landscape units from the traditional landscape classification schemes to produce various spatial and statistical metrics that reveal how the national system of protected areas coincides with the areas of high ES conservation value.

The results of the ES potential assessment at the landscape scale and the consequent analyses of the nature protection network enabled us to draw four main conclusions. Firstly, the landscapes with high conservation potential at the national level in the country are relatively well preserved by the existing nature protection network. Secondly, the character of this protection varies between the four landscape types with high conservation value, which is predefined by their size and location. Thirdly, the Natura 2000 network is the only nature protection option for many landscape types, which appears a good option for areas with a mixture of highly cultivated areas and small natural habitats. Fourthly, the landscapes with lower ES conservation potential are less presented in the protected areas, but in some cases, the lower ES potential does not fully correspond to low protection. The latter is a sign of the need for further improvement of the methodology, especially in its ES assessment part.

## **Additional information**

### **Conflict of interest**

The authors have declared that no competing interests exist.

### **Ethical statement**

No ethical statement was reported.

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### **Author contributions**

Conceptualization: HP, SN. Data curation: HP, SN. Formal analysis: HP, SN. Funding acquisition: HP, YY. Investigation: HP. Methodology: HP. Resources: HP, SN. Supervision: SN. Validation: HP, YY. Visualization: HP. Writing - original draft: HP, SN. Writing - review and editing: YY, HP, SN.

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## Data availability

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

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## Supplementary material 1

### Spatial data for the potential landscapes in Bulgaria

Authors: Hristina Prodanova

Data type: rar









Explanation note: Digitized after Todorov et al. 2004: Landscape map of Bulgaria at a scale of 1:500,000. This rar file contains spatial data (.shp, .lyr), and legend (.pdf).

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## Research Article

# Ritualistic hunts: exploring the motivations and conservation implications in West Bengal, India

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## Abstract

Ritualistic hunts are illegal, large, organised cultural events which are a prevalent concern in West Bengal from both an animal welfare and conservation perspective. We carried out a socio-economic survey with 112 individuals in the districts of Jhargram and West Medinipur to better understand the characteristics of these hunts, the species impacted, and the drivers and motivations of the communities that engage in these types of illegal activity. Specifically, we asked which wild animals were most desirable, which were most profitable, what derivatives from hunted animals were used for, and which wild animals were perceived to have increased most in rarity. We found that these events involve both indiscriminate and targeted killing of a wide variety of wildlife, including at least 93 inferred species (seven of which are categorised on the IUCN Red List as threatened (i.e. either Vulnerable, Endangered or Critically Endangered); in 34 the population trend has been categorised as declining, and 25 are listed on Schedule I of India's Wildlife Protection Act). We found that wild meat/food was the most frequently stated personal use of hunted wildlife in addition to belief-based use, traditional medicine, and decorative use. Engagement in the onward commercial sale of wildlife was also reported by more than a third of hunters. However, personal enjoyment was identified by most hunters as their main motivation for taking part in ritualistic hunts. Despite widespread engagement in ritualistic hunting, we found that the majority of hunters also expressed a willingness to engage in legal non-consumptive alternatives if they were made available. As such, we recommend that in addition to effective law enforcement, further research to identify viable non-consumptive alternatives and inform associated human behaviour change initiatives could help deliver a positive transformation for both wildlife and people in West Bengal.

**Key words:** Animal welfare, illegal wildlife trade, protected species, Shikar Utsav, traditional hunting



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## Introduction

Hunting is a threat to wildlife across the tropics (Bennett et al. 2002; Milner-Gulland et al. 2003), and over-exploitation (harvesting at a rate that cannot be compensated for by reproduction or growth), together with agriculture, is one of the biggest drivers of biodiversity decline globally (Maxwell et al. 2016; Caro et al. 2022; Challender et al. 2023). Hunting, in various guises, is a feature of most human communities, at least historically (e.g. Alves et al. 2018), and remains a common practice amongst tribal people, and other community groups, in many countries to this day, including among the various tribal groups residing in India (Dutta and Mondal 2020; Selvan et al. 2013). In West Bengal, tribal communities have a longstanding relationship with forest ecosystems and the wildlife and resources they encompass (Bhattacharya et al. 2016). Hunting wild animals remains deeply ingrained in their cultural practices, reflecting their historical dependence and connection with these environments (Sarkar and Modak 2022). Changing socio-economic circumstances, such as the availability of alternative sources of protein, improved job prospects, awareness of declining wildlife abundance, and increased enforcement of wildlife protection laws, has meant that the need for, and interest in, hunting has declined in some communities over recent years, particularly amongst younger people (Dutta and Mondal 2020). However, engagement in organised ritualistic hunts during village festivals persists, reportedly driven by multiple driving factors including traditional and cultural reasons (Aiyadurai et al. 2010; Ghosh et al. 2013; Dutta and Mondal 2020; Sarkar and Modak 2022).

Ritualistic hunts typically involve large numbers of people who engage in group hunting of various wildlife species on certain days of the year, usually coinciding with a day of cultural or religious significance (Ghai 2017; HEAL 2020; Sarkar and Modak 2022). Undertaken by several different tribes<sup>1</sup> across India, different names are used in different places, and by different tribes; for example, in Jharkhand's Dalma Wildlife Sanctuary, ritualistic hunting is known as "Vishu Shikar", "Sendra", or "Jani Shikar" (Ghai 2017). In West Bengal, ritualistic hunts are called "Shikar Utsav" (or "hunting festival") and typically take place between January and June (Ghai 2017; HEAL 2020). Participation is largely limited to men in the community, who reportedly hunt whatever animals they encounter, with traditional weapons. The impact of ritualistic hunts on local forest biodiversity is difficult to determine. However, the number of participants, and the diversity of species targeted raise concerns about the sustainability of the associated offtake, potential risks to vulnerable and threatened species, and the long-term possibility of contributing to "defaunation" (cf. Poulsen et al. 2023) and "empty forests" (Redford 1992). The methods used also raise additional concerns over animal welfare. For example, several hunters simultaneously chase animals with wooden spears, bows and arrows or hit them with bamboo clubs until they are dead (HEAL pers. comm.).

<sup>1</sup> Although this study considers the definition of tribe in a legal and conventional manner, it should be noted that some researchers have attributed tribal status to all humans. This includes Desmond Morris in 'The Human Zoo' in 1969 and David R Samson in 'Our Tribal Future,' in 2023. So whilst this article refers to the identities that the respondents provided, and those provided by law, the authors wish to highlight the wider on-going discussion on tribal characteristics surrounding all humans, and to state unequivocally that there is no inference of discrimination by the usage of the word, 'tribe'.

Hunting has been illegal in India since 1972 when the Wildlife (Protection) Act (WPA) was originally enacted. Updated in 2022, the WPA remains a comprehensive piece of legislation, under which hunting of all forms of wildlife in India is prohibited, as is the possession and trade in wild animal derivatives (including trophies) without previous permission in writing from the Chief Wildlife Warden or the Authorized Officer (Legislative Department, Ministry of Law and Justice, Government of India). Although traditional community rights to wildlife and forest resources are granted under Section 3(l) of the Scheduled Tribes & Other Traditional Forest Dwellers (Recognition of Forest Rights) Act, 2006 or Forest Rights Act (FRA), the FRA specifically excludes the use of forest animals as a right (i.e., hunting or trapping or extracting a part of the body of any species of wild animal). India has been a signatory to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) since 1976. CITES aims to regulate international legal trade to safeguard certain listed species from overexploitation ([www.cites.org](http://www.cites.org)). Species, such as tigers and pangolins are currently listed in Appendix I of CITES, and as such, international commercial trade in these animals, their parts, or derivatives is essentially prohibited (Gomez et al. 2023). Despite these legislative deterrents, illicit hunting still continues in parts of India for recreation, subsistence, trade, in retaliation to human-wildlife conflicts, and as part of traditional ceremonies, especially of tribal communities (Aiyadurai et al. 2010; Aiyadurai 2011; Gubbi and Linkie 2012 and references therein; Bhupathy et al. 2013).

In relation to the situation in West Bengal, the continuation of these festivals recently prompted a decision by the Calcutta High Court in early 2023 which equated “indiscriminate wildlife hunting” with murder under Section 302 of the Indian Penal Code (Kaul et al. 2023). Furthermore, it accused the Chief Wildlife Warden of West Bengal of non-compliance with a court directive issued in 2019 to curb hunting festivals in southern West Bengal (Kaul et al. 2023). To address the issue, the Calcutta High Court proposed the creation of “Humane Committees” to ensure the implementation of the 2019 ban and prevent further indiscriminate animal killings for five districts (including Jhargram and West Medinipur) where hunting rituals were prevalent (Kaul et al. 2023). These committees would need to comprise a district judge, district magistrate, police superintendent, representatives from the forest department and railways, civil society representative, and a tribal community representative (Kaul et al. 2023).

## Study aims

Despite substantial media coverage in recent years (e.g. Dasgupta 2016; Sarkar 2016; The Statesman 2019; Nibedita 2021), comparatively little academic research has focused on trying to develop a deeper understanding of the drivers and potential impacts of ritualistic hunting in West Bengal (as compared to research carried out elsewhere in parts of Northeast India, see, for example, Aiyadurai et al. 2010; Aiyadurai 2011; Velho et al. 2012). To help address this situation, and to better understand the drivers and motivations of communities in West Bengal that engage in ritualistic hunting, we carried out a socio-economic study in two neighbouring districts (Jhargram and West Medinipur) where

ritualistic hunting is prevalent. We used in-person questionnaire surveys and asked what species respondents are hunting and why, how often they hunt, and what their captures are used for. Ultimately, our aim was to provide an evidence base upon which local and national level interventions can be designed to help shift communities away from illegal hunting activity and towards sustainable non-consumptive alternatives. This would also reduce the risk of negative impacts on the welfare and conservation status of the species involved.

## Methods

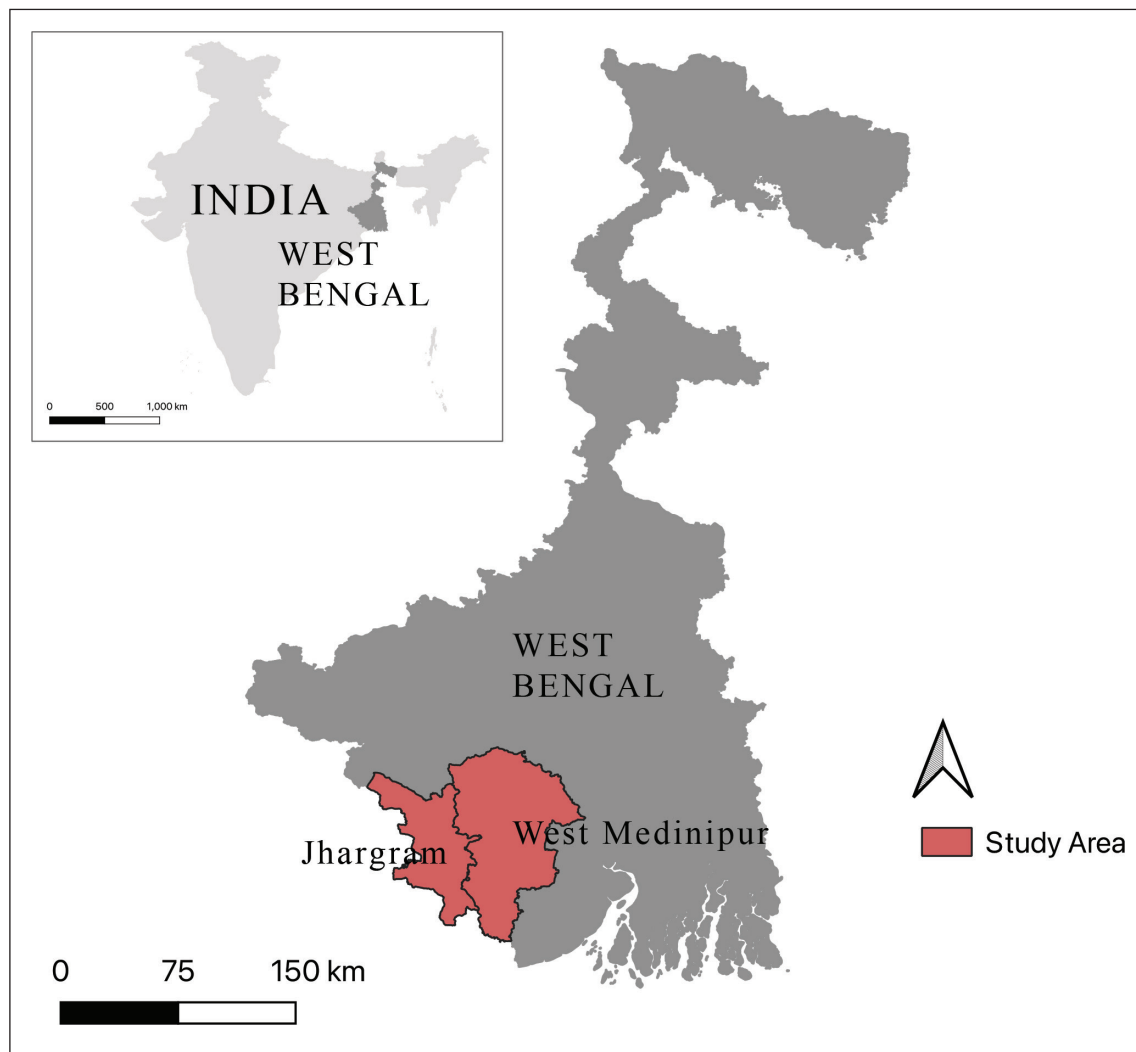
### Study site

The study was carried out in West Bengal (Fig. 1), the thirteenth largest state in India in terms of area (88,752 km<sup>2</sup>) and the fourth most populous state (population density: 1,000 per km<sup>2</sup>), located in the eastern region of India (latitude: 27°13'15"N to 21°25'24"N, longitude: 85°48'20"E to 89°53'04"E, [wb.gov.in](http://wb.gov.in)). West Bengal stretches from the Himalayas in the north to the Bay of Bengal in the south and is bounded in the north by Sikkim and Bhutan, in the south by the Bay of Bengal, in the east by Assam and Bangladesh and in the west by Odisha, Bihar and Nepal. Questionnaire surveys were carried out in villages in the districts of Jhargram and West Medinipur (Fig. 1), located between the Chota Nagpur Plateau and the Gangetic Plains in the south of West Bengal. These two districts form the southern part of the former Junglemahal (translated as “jungle estates”; the term has no formal meaning but is still commonly used, Das 2021). Jhargram has an area of 3,042 km<sup>2</sup> and, in the 2011 census, a population of 1.14 million ([jhargram.gov.in](http://jhargram.gov.in)), whilst West Medinipur has an area of 9,295 km<sup>2</sup> and a population of 5.94 million ([paschimmedinipur.gov.in](http://paschimmedinipur.gov.in)).

In both districts, a large proportion (94% and 88%, respectively) of the population live in rural areas in relatively small, closely spaced villages (average distance between neighbouring villages = 1.17 km, Pal 2019). The landscape is primarily agricultural land interspersed with patches of tropical dry, deciduous forest, a large proportion of which is classified by the Forest Department as Reserved (c. 59% across West Bengal as a whole) or Protected Forests (c. 32%), where felling/burning trees and clearing land is prohibited (Indian Forest Act, 1927, [nbaindia.org](http://nbaindia.org)). In Jhargram there has been significant forest regeneration since the 1980s, with almost 7,000 km<sup>2</sup> of new forest land area generated between 1985 and 2015 (Mandal and Chatterjee 2021). In Jhargram (as of 2011) 29.4% of the population was tribal (the highest proportion of tribal people in West Bengal, Bera and Roy 2022, [jhargram.gov.in](http://jhargram.gov.in)), and in West Medinipur 14.9% of the population was tribal ([paschimmedinipur.gov.in](http://paschimmedinipur.gov.in)).

### Data collection

The questionnaire survey was conducted in August and September 2022 by local field staff who asked a set of 26 predetermined questions (Suppl. material 1) that included open-ended, closed, and multiple-choice questions. The questionnaire focused on hunter demographics, the drivers and socio-economic dynamics of ritualistic hunting, the attitudes of participants towards ritualistic hunting, the species involved, and perceived impacts of the hunt on the forest



**Figure 1.** Map showing the location of Jhargram and West Medinipur districts in southern West Bengal, India. Administrative boundaries data source: <https://www.diva-gis.org/gdata>; mapping software: QGIS ([www.qgis.org](http://www.qgis.org)). The boundaries shown, and the designations used on this map may not be correct and do not imply any official endorsement and/or acceptance by the authors or their respective institutional affiliations.

and its biodiversity. Key questions related to the nature of the hunting event, personal and commercial use of hunted animals, the significance of ritualistic hunting to people, and willingness to engage in potential non-consumptive wildlife-friendly alternatives. For this study, we define “non-consumptive wildlife-friendly alternatives” as activities which do not involve the deliberate killing of animals (in contrast to consumptive practices such as hunting) or have a negative impact on species conservation or individual animals’ welfare.

We interviewed participants who self-identified as having engaged in ritualistic hunting (either in the past or present) and were willing to participate in the study, through a process of chain referral (Newing 2011), whereby participants recommended other potential participants, or persuaded others to take part. This snowball sampling approach (Babbie 2004) is useful when researchers are interested in the opinions of a particular hidden population (Potgieter et al. 2017) and, in this case, ensured that participants who could provide information pertinent to the study were selected as representatives of the ritualistic hunting community. Our



aim was not to extrapolate our results to the wider community but to thoroughly understand what drives the hunters we interviewed to take part in ritualistic hunts.

Participants were initially asked some non-hunt related questions such as what pets they keep before being asked about their involvement in ritualistic hunting in an attempt to create a comfortable and relaxing environment (Newing 2011).

Specifically, participants were asked to identify the 10 wild animals (using local common names) that they currently considered to be the most profitable (as opposed to most valuable per item or most commonly traded, with wildlife body parts and live animals considered separately), the 10 wild animals that they considered to be the most desirable (i.e. attractive and useful), and the 10 wild animals that they considered to have most increased in rarity (and therefore inferred reduced availability) over the past five years (corresponding to the period 2017–2021). Common names relating to taxonomic class (e.g., bird and mammal) or below were included in the analysis of the survey responses but were excluded when considering conservation status. Participants were also asked to provide additional information including the wildlife body parts sold per unit, price paid per unit, where the body parts were sold (locally / nationally / internationally), and their intended purpose categorised as “food” (wild meat and other nutritional use), “spiritual” (e.g., items to invoke magic, good luck and prosperity), “medicinal” (ingested or topical treatments for illness), “clothing and decoration” (for fashion and aesthetic purposes), and “unknown” (which included unknown purposes).

Interviews were conducted in Bengali and later translated into English. In accordance with the British Sociological Association Statement of Ethical Practice (BSA 2017), informed consent was obtained verbally from every survey participant prior to the interview. The objectives of the study were explained to participants, and they were made aware of their rights to voluntarily participate or to decline. No identifying participant or household data were collected, and the database collated was entirely anonymous. In addition, villages were coded in the database and village names not reported to further protect study participants from harm or discrimination (St John et al. 2016). Ethical approval was obtained via Manchester Metropolitan University on 06/09/2022, EthOS Reference Number: 43711. When asked about the species involved, participants gave common names in their local language. Scientific names were inferred where possible, otherwise common names are reported. For species whose scientific name could be assigned, its current conservation status and population trend was obtained from the IUCN Red List of Threatened Species (<https://www.iucnredlist.org/>).

## Data analysis

We used descriptive statistics, frequency histograms and pie charts to describe, and to illustrate, patterns in the data. Chi-squared tests of association were used to describe the distribution of age groups and the education level of participants, and to test for differences in perceived trends in the number of animals in the forest, and among rankings for proposed drivers for taking part in hunting rituals. Spearman's rank correlation was used to assess the relationship between the species that were stated as becoming increasingly rare and the declared most desirable and most profitable species. Word clouds were used to summarise and to illustrate the answers given to open-ended questions where there was sufficient text available (excluding the terms “activity” and “animals”); otherwise,

we described the key words used by hunters to describe their actions or feelings. Data descriptions and statistical analyses were carried out in R (version 4.2.3; R Core Team 2023); word clouds were based on a data frame containing the most frequently used words and their respective frequency of occurrence, obtained using the text mining package “tm” (Feinerer et al. 2008; Feinerer and Hornik 2018), and drawn using the “wordcloud” package (Fellows 2018). All interviews were included in the analysis even if they contained missing data. Monetary values were reported in Indian rupees (INR) and converted to US dollars (USD) using 1 INR = 0.0121 USD (conversion rate as of 10.08.23, xe.com).

## Results

### Hunter demographics

We interviewed a total of 112 people (hereafter respondents) from 93 villages in two districts of West Bengal: Jhargram ( $n = 59$ ), and West Medinipur ( $n = 53$ ). Ninety-nine respondents identified themselves as hunters; 13 respondents self-identified as “non-hunters” but provided information on someone else who engages in hunting. All but one of the respondents were male (one of the non-hunters was female). Respondents ranged in age between 18 and 76, with an approximately even distribution across age groups (grouped as 18–25, 26–35, 36–45, 46–55, 56–65, > 66:  $\chi^2 = 2.92$ ,  $df = 5$ ,  $p = 0.712$ ) and an average age of about 40 (median = 38.5, mean = 41.6,  $n = 56$ ; where respondents gave an age range rather than a precise number of years we used the mid-value so the average may be slightly underestimated). Where sample size does not equate to 112, the remainder did not answer the question. Most ( $n = 81$ , 72.3%) respondents were married (15 were unmarried and one was widowed). Most reported owning one house ( $n = 90$ , 80.4%, two reported owning two houses), and most had lived in the village since birth ( $n = 98$ , 87.5%). Others had lived in the village for between 5 and 50 years ( $n = 7$ , 6.3%), “since marriage” ( $n = 1$ , 0.9%), or described themselves as “resident” ( $n = 3$ , 2.7%) or “migrant” ( $n = 3$ , 2.7%) but did not say how long they had lived there. Households reportedly comprised between two and 14 people (mean = 5.9,  $n = 78$ ) and included between one and three children, mostly ( $n = 20$ ) two children (mean = 1.8,  $n = 39$ ). Two (1.8%) respondents reported, respectively, “10–15” and “12–20” people in the household.

The education level of respondents varied and was split relatively evenly amongst those that had reportedly not received any formal education ( $n = 20$ , 17.9%), educated to primary level ( $n = 27$ , 24.1%, included one who was only “nursery” educated), or to secondary level ( $n = 17$ , 15.2%, including 9 at “secondary” level and 8 at “higher secondary” level), but only five (4.5%) respondents were educated to college level ( $\chi^2 = 9.10$ ,  $df = 3$ ,  $p = 0.028$ , education level grouped as “no formal education”, “primary”, “secondary” and “college”). When asked for their ethnicity, most respondents ( $n = 72$ ) reported only that they were “Adivasi” (a term usually used to refer to Scheduled Tribes in official records, Paliwal 2023), an additional eight respondents stated that they belonged to the Santal tribe and twelve belonged to the Mahato community. Others reported that they belonged to the Bauri ( $n = 1$ ), Kurmi ( $n = 1$ ), or Majhi ( $n = 1$ ) community. Two respondents referred to themselves as “General” (i.e., a person that does not self-identify as belonging to any of the government categories for Indian citizens, Wankhede 2021). When

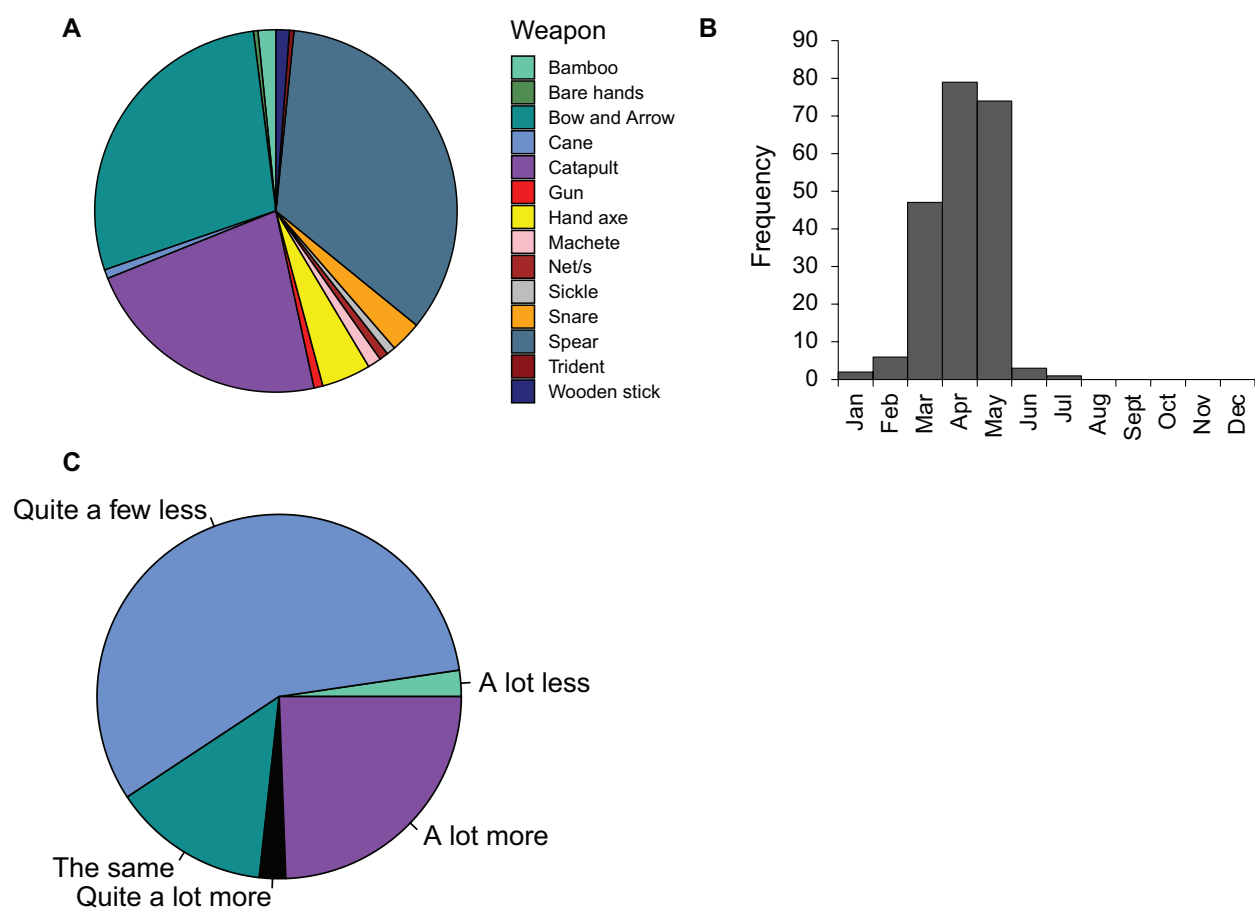
asked about their main job, 96 respondents reported “cultivation” (predominantly of rice;  $n = 95$ ), three reported “contract labour” or “contractual work”, five reported both cultivation and contract work; one was a government employee, one a retired government worker, one “collected raw materials from the forest”, one was unemployed, and two were students. Collectively, 98 (87.5%) respondents reported that agriculture was their primary source of income. One man who reported collecting raw materials said that his primary source of income was selling plates made from Sal tree (*Shorea robusta*) leaves. Annual income ranged from 10 to 150 thousand rupees (equivalent to approximately 121–1,815 USD), although for this question only 30 respondents provided an answer. The following results focus only on the responses of the 99 respondents who self-identified as hunters.

### Characteristics of the hunt

When asked about how frequently they had been involved in ritualistic hunting (hereafter ‘hunting’/ ‘hunts’) in the last 12 months, the majority (82.7%) of respondents who answered the question ( $n = 75$ ) said that they took part in hunts only once or twice (“once”:  $n = 55$ , “1–2 times”:  $n = 7$ ). A small number of hunters ( $n = 10$ ) reported that they were involved in more frequent hunts: seven took part in hunts between two and five times in the last 12 months, and three reported taking part, respectively, seven times, 13–14 times, and “many” times. Three respondents reported that they had not taken part in a hunt in the last 12 months (although two of these confirmed that they had taken part in hunts 2–3 or 3–4 times in the last 5 years); 24 did not answer the question. Accordingly, the majority of hunters reported taking part in between one and 15 hunts over the last five years, most ( $n = 48$ , 77.4% of the 62 respondents who gave numeric answers to this question) stated five or fewer; two respondents reported taking part in, respectively, 25 hunts, and 35–40 hunts.

All but one of the survey respondents (who did not answer this particular question), described “thousands” of people collectively taking part in a ritual hunt, in hunting parties of 20–40 people (overall minimum 10, maximum 200 or 300), using spears (Ballam/ Kencha;  $n = 87$ ), bows and arrows (Kaar baansh/ Teer dhonukh;  $n = 71$ ), catapults (Batul/ Gulti;  $n = 56$ ) and a range of other methods including hand axes (Tangi) and wooden sticks (Lathi; Fig. 2A). Seven respondents reported setting snares (“lasso’s”, Faand) alongside other ‘direct’ methods; only two respondents reported using guns (Fig. 2A). One respondent reported only using snares. Most respondents ( $n = 79$ , 79.8% of all respondents) estimated that the total number of people involved in the hunt was between 4,000 and 5,000, a small number of respondents suggested that the overall total might reach 6,000 ( $n = 4$ ), 7,000 ( $n = 1$ ) or as high as 10,000 ( $n = 5$ ). Two respondents reported that only tribal people participated in the hunt; but, with the exception of one respondent who did not answer the question, most ( $n = 96$ , 97.0% of all respondents) stated that the “majority” of people taking part in the hunt were tribal, and 91 of these explicitly stated that a few of the people taking part were non-tribal.

The best time for hunting was most commonly reported to be between March and May ( $n = 73$  respondents suggested one, or a range of months, within this period), although some hunters suggested that hunting could start as early as January or February, and others suggested that it could take place later in the year (starting in April/May and extending to June/July) (Fig. 2B).



**Figure 2.** **A** pie chart showing proportion of hunters using different hunting/killing methods during ritualistic hunting in West Bengal. In most cases, hunters reported using more than one method (five hunters said they only used spears, and one only used snares) and so the number of methods exceeds the number of survey respondents ( $n = 99$  hunters,  $n = 252$  methods reportedly used; 10 survey respondents did not say which methods they used) **B** bar chart showing the months reported to be best for hunting. Where hunters suggested a range of months, we counted each month within the specified range, and so the total frequency of months being specified as 'optimal' exceeds the number of survey respondents ( $n = 99$  hunters,  $n =$  total frequency of months specified; 13 survey respondents did not answer the question) **C** pie chart showing perceived trends in the number of animals in the forest compared with 5 years ago (based on the answers of 75 hunters), five-point scale: a lot less / quite a few less / the same / quite a lot more / a lot more.

Estimates of the number of animals captured and killed, by the hunting party, in the last 12 months, varied between 1–2 and, in most cases, up to 20 (with most respondents answering towards the lower end of this range: mean = 4.1, median = 2.5,  $n = 61$ , excluding one outlier); three respondents reported that the hunting group captured and killed 20–25, 25–30, and 100–200 animals, respectively. Accordingly, estimates of the number of animals captured and killed by the hunting party over the last five years were predominantly ( $n = 43$ ) between 10 and 50; 11 respondents estimated that the hunting party had captured and killed fewer than 10 animals in the last five years. Six hunters suggested that the numbers captured and killed over the last five years exceeded 50: four estimated 50–100, one > 100, and one 1,000–2,000 (one hunter reported that they had “lost count”).

Fifty-four survey respondents stated specifically that the animals captured are “slaughtered at home” (some that they are taken to the “leader’s” house

where they are slaughtered and divided), 32 described “slaughtering” and cooking animals in the forest (three of these also said that the animals were eaten in the forest). Two respondents explained that if they are hunting far away, and stay overnight in the forest, the animals are slaughtered there but otherwise they bring them home, and six refer to slaughtering animals at home or in the forest (three also said that the “excess” is brought home). Respondents referred to transporting the animals killed by carrying them on their shoulders (in some cases with their feet tied to sticks), on bikes, or in jeeps or other 4-wheelers.

### Species diversity

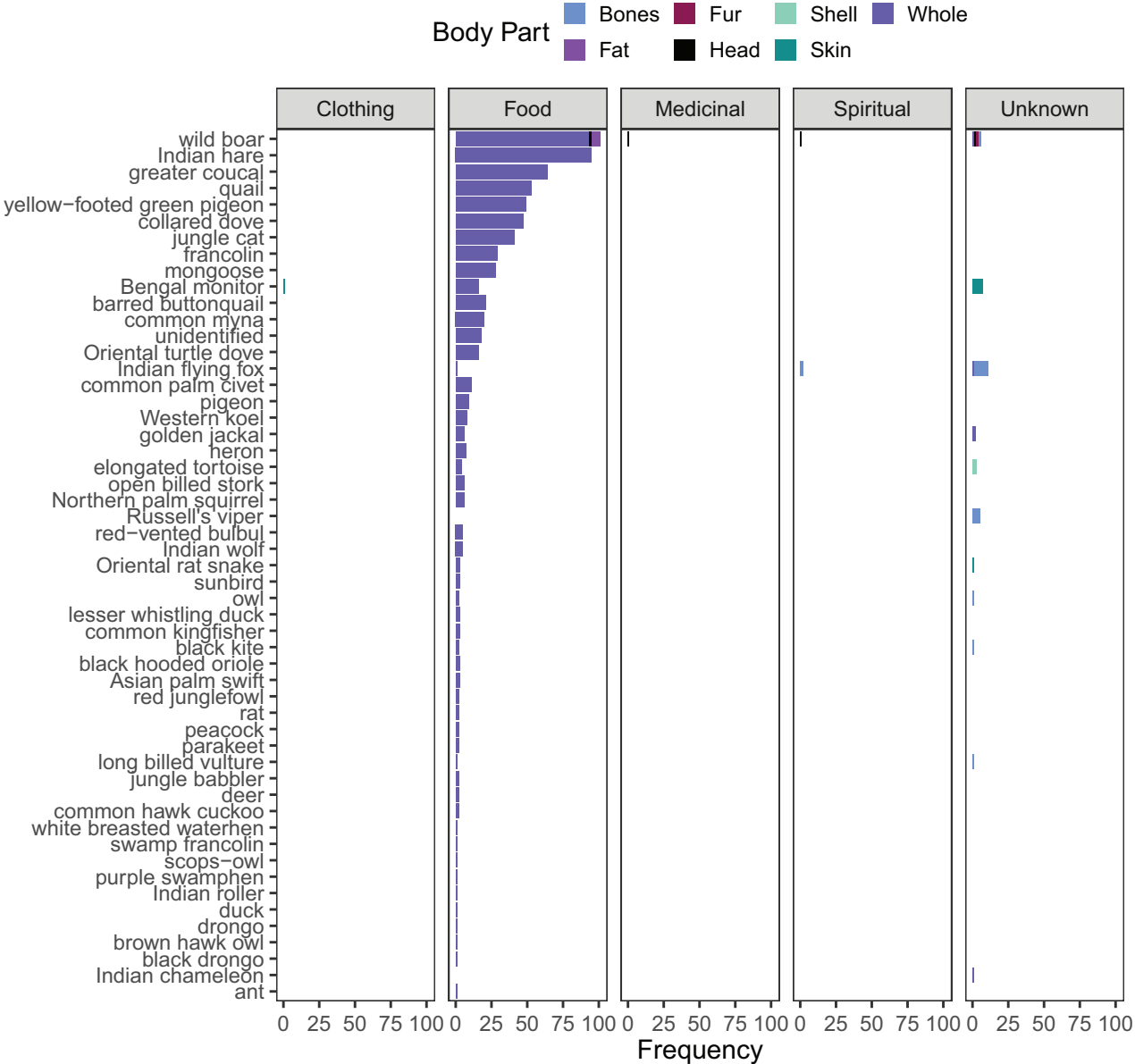
Overall, during our survey, the 99 respondents who self-identified as hunters used 74 different local names to refer to 53 distinct different common names of the most desirable and profitable wildlife species that they currently hunt (plus at least four unidentified animals), comprising 36 common names referring to birds (67.9%), 11 to mammals (20.8%), five to reptiles (9.4%), and one insect (ants) (Suppl. material 2). Overall, we estimate that the 53 distinct common names provided by hunters to refer to the most desirable and profitable wildlife species hunted (excluding invertebrates i.e., ‘ants’ [Formicidae]) potentially refer to at least 93 species within the study area in West Bengal, including 73 birds (Aves), 15 mammals (Mammalia) and 5 reptiles (Reptilia). This is assuming that a common name could refer to multiple species of the same taxon; for example, the local name ‘titir pakhi’ meaning ‘francolin’ could potentially refer to two francolin species found in West Bengal: the grey francolin (*Ortygornis pondicerianus*) or black francolin (*Francolinus francolinus*); see Suppl. material 2.

### Most desirable species

A total of 52 unique common names were given when participants were asked to list the ‘ten most desirable wildlife species or body part from ritualistic hunting’. All respondents (n = 99) provided answers, and respondents each listed 2–15 species. The total number of species mentioned was highest for birds (n = 35 species) followed by mammals (n = 11), reptiles (n = 5) and insects (ants, n = 1). The most frequently mentioned common names (scientific names are inferred) for the ‘ten most desirable wildlife species hunted’ were wild boar (*Sus scrofa*, n = 99 mentions, 100% of hunters), followed by Indian hare (*Lepus nigricollis*, n = 96, 97.0%), greater coucal (*Centropus sinensis*, n = 64, 64.6%), quail (Phasianidae, n = 53, 53.5%), collared dove (*Streptopelia decaocto*, n = 47, 47.5%), yellow-footed green pigeon (*Treron phoenicopterus*, n = 47, 47.5%), jungle cat (*Felis chaus*, n = 41, 41.4%) and Bengal monitor (*Varanus bengalensis*, n = 22, 22.2%) (Fig. 3). In addition, two local names mentioned in relation to the most desirable species could not be identified to a single species level: francolin (c.f. grey francolin or black francolin; n = 29, 29.3%) and mongoose (c.f. Indian grey mongoose (*Urva edwardsii*) or small Indian mongoose (*Urva auropunctata*); n = 28, 28.3%). Five local names given for the most desirable species were unidentified (Suppl. material 2).

A total of eight different body parts related to the 52 most desirable species that were mentioned at least once, with ‘whole body’ being most frequently cited (94% of a total of 757 species and body part combinations mentioned. Note: each respondent listed up to 15 most desirable species) (Fig. 3). With the excep-

tion of one respondent who reported consuming ants alive, all other desirable species were used as dead animals. Across all respondents, the most frequently cited purpose of hunting the most desirable species was for 'consumption' i.e., food (94%, n = 713 mentions), three respondents cited 'spiritual' use (e.g., bones of Indian flying foxes (*Pteropus medius*) used to "ward off odd/evil spirits" (n = 2), and wild boar head used "for new born kids to ward off evil spirits" (n = 1)), one referred to 'medicinal' use (for the body and head of wild boar), and one referred to the making of 'clothing and decoration' items (Bengal monitor skin used "for preparation of belts"). Seven respondents explicitly stated that some of the most desirable species were "not consumed" (in relation to whole dead golden jackal (*Canis aureus*; n = 2), whole dead animal and bones of the Russel's



**Figure 3.** Breakdown of most desirable species by body part and purpose. Frequency refers to the number of times a respondent reported each purpose of use of desirable species' body parts. Note: the responses were categorised as "clothing and decoration", "food", "medicinal", and "spiritual" based on their inferred meaning (see text for full description of items within each category). The full list of common names provided along with local and inferred scientific names is given in Suppl. material 2.



viper (*Daboia russelli*;  $n = 1$ ), and the bones of Indian flying foxes ( $n = 4$ ). The purpose of hunting of the most desirable species was unknown or could not be determined for 5.1% ( $n = 39$ ) of responses. For example, the Indian chameleon (*Chamaeleo zeylanicus*) was reportedly used for “other uses”, eight hunters who listed Bengal monitors as a desired species used their skins or hunted them exclusively for their skins, but the purpose of the skins was unknown, and two of the eight respondents who reportedly desired catching golden jackals said that they did not consume them but did not say what they used them for.

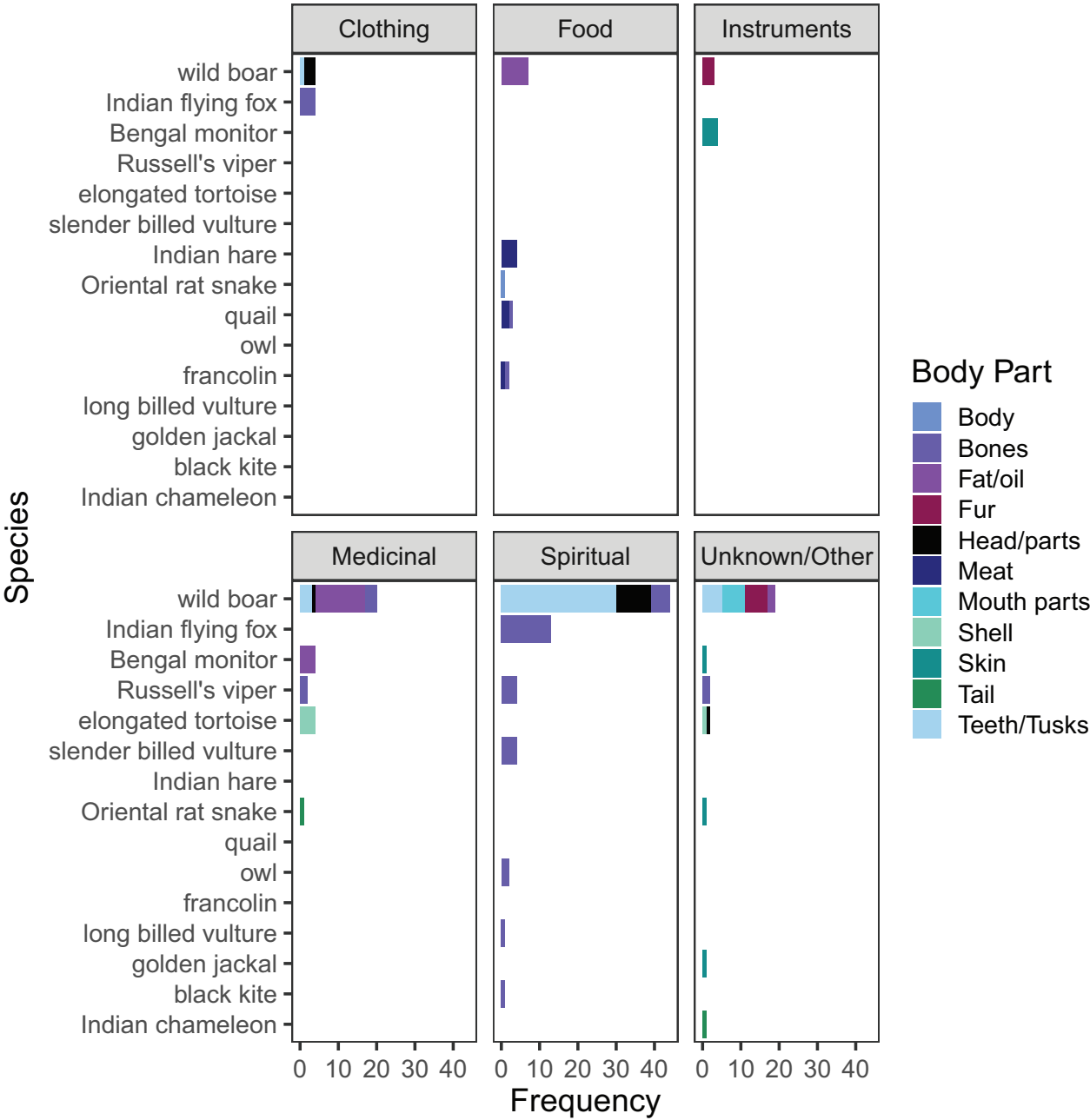
Only six respondents provided sale prices for the most desirable species; the most expensive items were reportedly derived from red junglefowl (*Gallus gallus*, Rs 2200–2300 (~26–27 USD) per whole animal for consumption) and Indian hare (Rs 500 (~6 USD) per animal for consumption) (Suppl. material 3).

### Most profitable species

Sixteen unique common names (6 birds, 5 mammals, and 5 reptiles) were given when participants were asked to list the ‘ten most profitable wildlife species or body part from ritualistic hunting’ ( $n = 58$  provided answers, and respondents each listed 1–5 species). The most frequently mentioned common names were wild boar ( $n = 53$ , 91.4%), followed by Indian flying fox ( $n = 13$ , 22.4%), Bengal monitor ( $n = 8$ , 13.8%), Russell’s viper ( $n = 6$ , 10.3%), and elongated tortoise (*Indotestudo elongata*,  $n = 6$ , 10.3%) (Fig. 4, Suppl. material 3).

Eleven different body parts related to the 16 most profitable species mentioned with ‘bones’ being most frequently cited ( $n = 43$ , 27% of a total of 159 species and body part combinations mentioned), along with ‘fat/oil’ ( $n = 26$ , 16.4%), and ‘head/head parts’ ( $n = 14$ , 8.8%) (Fig. 4). Across all respondents, the most frequently cited purpose for hunting of the most profitable species was for ‘spiritual’ use ( $n = 69$ , 43.4%). This included wild boar teeth, head parts, tusks and bones ( $n = 34$ ), as well as the bones of Indian flying fox ( $n = 14$ ), vultures (*Gyps* sp.;  $n = 5$ ), owls (*Strigidae*;  $n = 2$ ), Russell’s viper ( $n = 4$ ), and black kite (*Milvus migrans*;  $n = 1$ ), all believed to “ward off ill-omen” or “evil spirits”. The second most frequently cited purpose was ‘medicinal’ ( $n = 31$ , 19.5%). This included wild boar teeth and bones ( $n = 1$ ) and head ( $n = 1$ ) used “for preparation of medicines” or “consumed as a medicine during fever” ( $n = 2$ ), wild boar bones used as “a medicine for back pain” ( $n = 1$ ), and fat used for “body pain” ( $n = 2$ ), “joint pains” ( $n = 10$ ), or “massage” ( $n = 2$ ). Additionally, fats of the Bengal monitor were used “for preparation of medicine” ( $n = 1$ ), “to treat joint pain” ( $n = 1$ ), or “as an ointment to cure swelling and blisters” ( $n = 2$ ). Tortoise shell was used “in combination with oil to cure body pain” ( $n = 4$ ), Russell’s viper bones used “to make medicines” ( $n = 2$ ), and Indian rat snake (*Ptyas mucosa*) tails were “cooked with spinach and consumed to treat ear pain” ( $n = 1$ ). Other purposes cited included ‘consumption’ (i.e. food;  $n = 17$ , 10.1%), ‘clothing and decoration’ ( $n = 8$ , 5%), which included wild boar head parts “worn as bangles”, and bones of Indian flying foxes “worn over the waist”) and ‘manufacturing goods’ ( $n = 7$ , 4.4%), such as Bengal monitor skin used for “making musical instruments” and wild boar fur used “as a bicycle brush”. For 16.9% ( $n = 27$ ) of responses, the purpose of use of the most profitable species was unknown or could not be determined.

Seventeen respondents provided prices for the most profitable species; the most expensive individual items were derived from the Bengal monitor (Rs



**Figure 4.** Breakdown of most profitable species hunted by body part and purpose. Frequency refers to the number of times a respondent reported each purpose of use of profitable species' body parts. Note: categories were inferred during analysis ("Unknown/Other" includes unknown purposes (n = 21) and wild boar "mouth parts" used to "stop children crying" (n = 6); see text for full description of items within each category).

3000–4000 (~36–48 USD) per whole animal skin), Indian hare (Rs 700–800 (~8.4–9.6 USD) per kg or Rs 500 (~6 USD) per animal for consumption), and Russell's viper (Rs 500–600 (~6–7.2 USD) per 'piece' of bones) (Suppl. material 3).

**Hunting for personal use**

When asked specifically if they hunted for personal use, while all 99 hunters interviewed reported that they used the animals hunted for personal 'consumption' (i.e. food), 41 of these also referred to "other uses". Other uses were most often

associated with wild boar – specifically, the fat oil (used for “massage for joint pains”, cooking, or traditional medicine;  $n = 18$ ), the teeth, tusks, or “head parts” (to ward off “bad omens”, “evil spirits” or to avoid “bad luck”;  $n = 33$ , one also said that they kept the head as a souvenir after they had sold the body for meat), the fur (used as a brush for a bicycle;  $n = 11$ ), or the bones (used for medicine;  $n = 1$ ). Respondents also mentioned using the bones of Indian flying fox, birds, owls, or Russell’s vipers, primarily to “ward off bad omens” or “evil spirits”, or (occasionally) for medicine ( $n = 14$ ), tortoiseshell to treat pain ( $n = 1$ ) or the skins of Bengal monitors, Indian rat snakes, or golden jackals for unknown purposes ( $n = 3$ ). Thirteen respondents in this set of questions referred to “other people” using these body parts, or that they were “not sure of the purpose” suggesting that they did not necessarily do these things themselves and were not particularly familiar with these types of use. Three respondents mentioned the personal use of giving wild boar teeth or “mouth parts” to small children (to stop them crying or eating mud).

### Hunting for commercial use

When asked specifically if they hunted for commercial use, approximately a quarter ( $n = 27$ , 27.3%) of respondents said that they sold meat from the hunt at local markets, and 17 said that they sold the body parts “locally”. Meat and body parts were reportedly sold for local consumption (one said that they sometimes gave the meat to relatives in different districts). Of the 27 respondents that sold the meat, 24 specifically stated that the meat was sold only when there was excess available. Hunters reportedly received between Rs 150 and 200 (~1.8–2.4 USD) per kilogram of meat sold (either ‘meat’ of an unspecified species or ‘wild boar’); two hunters suggested as much as Rs 300 (~3.6 USD) per kg for wild boar meat, one hunter suggested Rs 150 (~1.8 USD) per kg for golden jackal meat, and two suggested, respectively between Rs 250 and 300 (~3–3.6 USD) for a “rabbit”, or Rs 500 (~6 USD) for a whole Indian hare. One hunter suggested that francolin and quail were also sold live for Rs 200–300 (~2.4–3.6 USD). One respondent reported that Indian rat snake meat was eaten (but no price was given).

When asked what the body parts were used for when sold, the answers given were similar to those for personal use: respondents most often referred to the use of wild boar teeth, head, or “mouth parts” ( $n = 33$ ; often as a “garland” (mala)) to ward off bad omens (or sometimes to “stop children eating mud”), or the use of wild boar oil or fat ( $n = 19$ ) for joint pain, cooking, or tooth ache. The bones of owls, birds (black kite, slender billed vulture (*Gyps tenuirostris*)<sup>2</sup> and long billed vulture (*G. indicus*) were mentioned specifically), and Indian flying foxes were also reportedly used for “warding off evil spirits” ( $n = 11$ ). Bengal monitor fat was sold for medicine and their skins for musical instruments (dug-dugi and behala). Other body parts sold included wild boar fur, tortoise shell, and the skins of Indian rat snakes and golden jackals.

A pair of wild boar teeth were reportedly sold for Rs 30–40 (~0.4–0.5 USD), a wild boar head for Rs 50–60 (~0.6–0.7 USD), wild boar fur for Rs 100–200 (~1.2–2.4 USD) (per 250 g), a whole snake for Rs 40–50 (~0.5–0.6 USD) (although the bones of Russell’s viper were reportedly sold in local markets for Rs

<sup>2</sup> It is possible that this species was an incorrectly identified long-billed vulture or Himalayan griffon (*Gyps himalayensis*).

50–60 (~0.6–0.7 USD) per snake, Rs 150 (~1.8 USD), or Rs 500–600 (~6–7.2 USD)), and Rs 200 (~2.4 USD) for a pair of birds' legs. One hunter reported that golden jackal skins sold in local markets for Rs 400–500 (~4.8–6 USD). The highest prices reported were Rs 2200–2300 (~26.5–27.8 USD) per bird for a junglefowl and Rs 3000–4000 (~36–48 USD) for a monitor lizard.

### Perceptions of increased rarity

Hunters' perceptions of the trend in the number of animals in the forest compared with 5 years ago differed significantly from what might be expected under an equal random distribution ( $\chi^2 = 38.71$ ,  $df = 4$ ,  $p < 0.001$ ): of the 75 hunters that answered this question, 51 (68%) believed that there were fewer animals in the forest than there were 5 years ago ( $n = 49$  that there were "quite a few less", and 2 that there were "a lot less", Fig. 2C). Nevertheless, opinions varied: 23 (30.7%) believed that there were more ( $n = 2$  "quite a lot more", and 21 "a lot more") and 12 (16%) believed that there were the same number (Fig. 2C). One interviewee stated that there had been an increase in the number of wild boars in the forest. 24 interviewees did not answer the question.

When asked which (if any) wild animals had become rare and/or unavailable in the last 5 years, respondents ( $n = 50$ ) collectively listed 10 species that they believed had become extremely rare (including tigers (*Panthera t. tigris*), sloth bears (*Melursus ursinus*), and vultures), 21 that they believed had become slightly rare (mostly bird species, but also golden jackals, jungle cats, and wild boar), and four that were reported to be either extremely or slightly rare by different interviewees (deer [Cervidae], Indian wolf (*Canis lupus pallipes*), barred buttonquail (*Turnix suscitator*), and peacock (*Pavo cristatus*)) (Suppl. material 2). Forty-nine interviewees did not answer the question and those who did answer listed 1–4 species.

The most frequently mentioned common names for the 'ten species which have become rare or unavailable in the last five years' were 'deer' ( $n = 11$ , 12.8% of a total of 86 mentions), followed by 'slender billed vulture' (which may or may not have been misidentified, see footnote 2,  $n = 8$ , 9.3%), 'Indian hare' ( $n = 7$ , 8.1%), 'crow' (Corvidae;  $n = 6$ , 7.0%) and 'tiger' ( $n = 6$ , 7.0%) (Suppl. material 2). Twenty-five species that were perceived as having become 'rare or unavailable in the last five years' were also listed among the ten most desirable animals hunted, including 'wild boar', 'collared dove', 'yellow-footed green pigeon', and 'jungle cat'. Eight species that were perceived as having become 'rare or unavailable in the last five years' were also listed among the ten most profitable animals hunted, including 'wild boar', 'Indian hare', and 'slender billed vulture' (see footnote 2, Suppl. material 2). When considering all common names ( $n = 59$ ) provided by respondents, no correlation was found between the species that were stated as becoming increasingly rare and the declared most desirable species (Spearman's  $\rho = 0.126$ ,  $p = 0.345$ ) or most profitable species (Spearman's  $\rho = 0.038$ ,  $p = 0.776$ ).

Only 24 respondents suggested reasons why animals had become rare – of these, 11 (45.8%) suggested that it was due to hunting and 8 (33.3%) suggested it was due to deforestation or burning the forest (sometimes by hunting parties). Other suggested reasons included: irregular rainfall, disease, animals migrating to different areas, and the ignorance of the forest department. Two respondents reported that the number of animals/birds had increased, one due to hunting, and one because people were not able to enter the forest due to elephants.

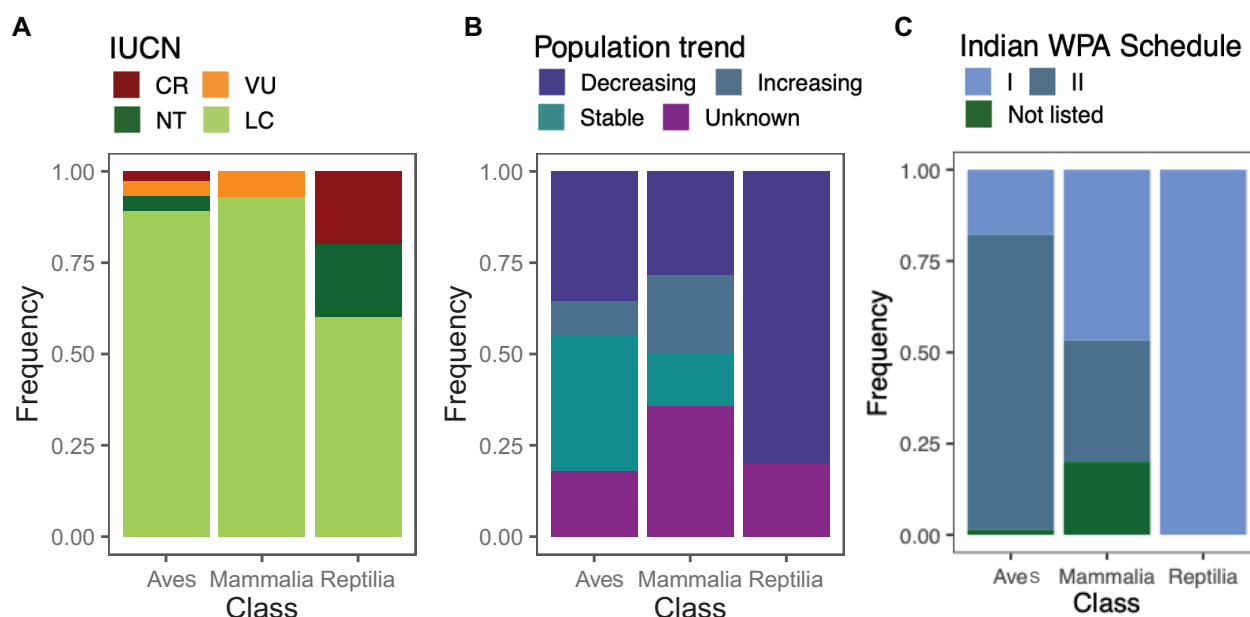
### Species conservation status

With regards to international conservation status, three of the 93 inferred hunted species (3.2%) are currently categorised on the International Union for Conservation of Nature (IUCN) Red List as Critically Endangered (elongated tortoise, long billed vulture, and slender billed vulture [see footnote 2 regarding the latter]), four (4.3%) as Vulnerable (common pochard (*Aythya ferina*), swamp francolin (*Ortygornis gularis*), pale capped pigeon (*Columba punicea*), and Sambar (*Rusa unicolor*)), four (4.3%) as Near Threatened, 81 (87.1%) as Least Concern, and one sub-species (Indian wolf) is Not Evaluated (Fig. 5A, Suppl. material 2).

With regards to their population trends, 10 (10.8%) inferred species have wild populations considered to be increasing, 29 (31.2%) have wild populations that are stable, 34 (36.6%) that are decreasing, 19 (20.4%) have an unknown population status, and for one sub-species (Indian wolf) the population trend has not been assessed (Fig. 5B, Suppl. material 2). An additional 28 inferred species (27.7%) that are not currently considered as threatened (according to the IUCN Red List i.e. they are listed as Near Threatened, Least Concern or have not been assessed), were believed by hunters to have increased in rarity (Suppl. material 2). With regards to their status under the Indian Wildlife Protection Act 1972, 25 (26.9%) inferred hunted species are listed under Schedule I of the Act, 64 (68.8%) are listed under Schedule II, four species are not listed (Fig. 5C, Suppl. material 2).

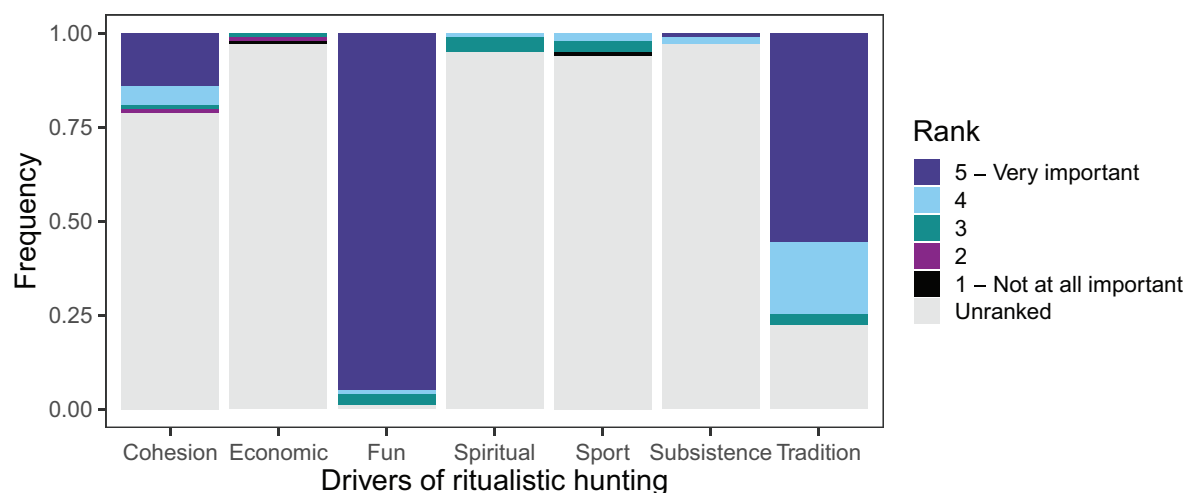
### Motivations and alternatives

When asked how they felt about ritualistic hunting, all but one of the hunters reported that they strongly liked it (one exception amongst the hunters said that they quite disliked hunting). The 13 non-hunters also reported that they 'strongly liked' ritualistic hunting when asked how they felt about the practice. The seven potential drivers for taking part in hunting rituals presented to hunters dif-



**Figure 5.** Breakdown of **A** IUCN Red List status **B** population trend, and **C** Indian Wildlife Protection Act 1972 Schedule status for the species listed as either the most desirable or most profitable species hunted.

ferred in their reported importance with 'Fun' and to a lesser extent 'Maintaining a traditional practice' statistically significantly more often highly ranked (ranks 4 or 5; ranked on a scale of 1–5, where 1 is not at all important and 5 is very important) than low or unranked (fun:  $\chi^2 = 50.72$ ,  $df = 1$ ,  $p < 0.001$ ; traditional practice:  $\chi^2 = 11.88$ ,  $df = 1$ ,  $p < 0.001$ , ranks 4 and 5 combined and ranks 3, 2, 1 and unranked combined, tested against an even distribution of responses between "important" and "unimportant" ranks, Fig. 6). Whereas hunting as a 'Sport', 'Economic opportunity', or for 'Subsistence benefits', or 'Spiritual benefits' were significantly more likely to be unranked or ranked low (not important) than ranked high (important) (sport:  $\chi^2 = 56.75$ ,  $df = 1$ ,  $p < 0.001$ ; economic:  $\chi^2 = 63.36$ ,  $df = 1$ ,  $p < 0.001$ ; subsistence:  $\chi^2 = 53.66$ ,  $df = 1$ ,  $p < 0.001$ ; spiritual:  $\chi^2 = 59.98$ ,  $df = 1$ ,  $p < 0.001$ , Fig. 6); these drivers were ranked as important or very important by

**A****B**

**Figure 6. A** importance of eight potential drivers for taking part in ritualistic hunts. Drivers were proposed by interviewers and ranked by respondents on a scale of 1–5, where 5 was “very important” and 1 was “not at all important”. Data given are the number of respondents giving a particular rank (we assume that a hunter that did not rank a particular driver – categorised here as “unranked” – did not consider it to be important) **B** wordcloud showing words used to describe why hunters felt the way they did regarding benefits from hunting to themselves or to the forest. (NB. “paves” was used in the context of “paves the way for...”) (based on responses from 66 hunters).



between 0 and 3 respondents. 'Social cohesion' was ranked as important but only by 19 of 99 interviewees; overall, social cohesion was more often unranked or ranked low (unimportant) than important ( $\chi^2 = 19.42$ ,  $df = 1$ ,  $p < 0.001$ , Fig. 6A).

Of the 99 hunters, 87 (87.9%) said that they did not currently engage in animal-friendly alternatives to ritualistic hunting. All but two of the 88 interviewees that answered this question suggested that they would engage in potential animal-friendly alternatives if they were available, that they would like to see such alternatives, and that awareness programs should be "organised about birds and animals within the forest, and awareness about snakes, which would not involve hunting" (Note that several respondents gave the same responses to these questions using the same words). Responses to this question were the same for the non-hunters: 10 of 13 did not currently engage in alternatives but gave the same answer about awareness programs (3 did not answer the question).

When asked if hunting provided benefits either to themselves or to the forest, 58.6% ( $n = 58$ ) answered that hunting benefited them, one answered that it benefited the forest (and did not mention personal benefits), four answered that it provided no benefit to either (36 did not answer the question or were not sure). Of the 58 hunters who reported a personal benefit, 56.9% believed that hunting either did not benefit ( $n = 29$ ) or was harmful ( $n = 4$ ) to the forest, 27.6%



**Figure 7.** **A** adult and juvenile barking deer killed in Purulia district in 2019 **B** 2 injured monitor lizards (*Varanus salvator*) (including a pregnant individual) captured by hunters in Panskura railway station in East Medinipur district in 2018 **C** hunting weapons, along with hunted wild animals – rose-ringed parakeet, lesser golden-backed woodpecker, white-throated kingfisher, Indian palm squirrel and Indian grey mongoose **D** mongoose meat being cooked in a vessel on a railway platform. Copyright HEAL.

believed that hunting benefited ( $n = 14$ ) or did not harm ( $n = 2$ ) the forest (nine did not mention the forest specifically or did not specify a benefit or disbenefit). Personal benefits were attributed to celebration, fun, providing food for their family, and showing off their skills to other hunters, whilst forest benefits were attributed to the numbers of animals in the forest (keeping a “check”, and “pav[ing] the way for younger animals”) and chopping down old trees (Fig. 6B).

## Discussion

### Scope and scale

Hunters confirmed that there are a wide range of species across a number of taxonomic groups that would be considered either desirable or commercially valuable captures during ritualistic hunting festival events in West Bengal (Fig. 7). These included mammals such as wild boar and Indian hares, birds such as greater coucals and yellow-footed green pigeons, and reptiles such as Bengal monitor lizards and elongated tortoises. The number of different vertebrate wildlife species reported by hunters (53 common names that infer to potentially as many as 93 species) represents a longer list than that reported in other recently published studies on this issue. For example, Dutta and Mondal (2020) documented 36 wild animal species following interviews with 56 tribal hunters in the Bankura district of West Bengal. The additional 17–74 inferred species reported by hunters in our study include up to four mammals, 66 birds, and four reptile species respectively, bringing the maximum total number of inferred species across both studies to potentially as many as 112 different species of wild vertebrates (Suppl. material 2). Notably, 19 species documented by Dutta and Mondal (2020) were not reported by hunters during our study, these include four mammals (porcupine, Indian civet, fox, small bat), nine birds, four reptiles, and two amphibian species. These differences highlight the value of case studies such as this to garner a more complete understanding of the diversity of species that may potentially be impacted during these types of ritualistic hunts.

With regards to scale, hunters also confirmed that hunting festivals are periodic large collective events involving “thousands” of participants that operate in groups that typically range from 20 to 40 (and up to a maximum of 300) people per “hunting party”. This aligns with observations made in other recently published reports. For example, Ghai (2017) described ritualistic hunts typically taking place between January and April every year when men, each of whom are assigned a specific role, go into the forest “en masse” to celebrate the post-harvest season. Similarly, HEAL (2020) estimated that the hunting festivals of South Bengal attract approximately 50,000–60,000 hunters every year, with the number of participants ranging from 1,000 to 15,000 hunters on each occasion, organised into between 50 to 500 members per hunting party. Although the exact number of hunts and participants is not possible to determine, due in part to their illegal nature, it is clear that the number of wild animals that are illegally hunted as a result of these hunting festivals each year can be substantial. For example, based on hunter responses from our survey, even a conservative estimate of 5,000 wild animals per year is reached based on the assumption that 50,000 hunters might take part in hunting festivals over one year, organised into hunting parties of 40 people, each of which captures an average of four individual wild animals.

## Consumption and culture

All 99 of the participants in our survey who self-identified as hunters confirmed that the wild animals captured during ritualistic hunts were used for personal consumption of wild meat as part of celebratory feasts and meat-sharing in the village (Ghai 2017). This is not unexpected as the cultural components of ritualistic hunts have been highlighted by previous authors; Ghai (2017), for example, describes spiritual leaders of the tribe determining hunt schedules, victorious return marches, and slaughtered animal offerings to deities prior to food preparation. Moreover, hunting for food has previously been reported as one of the main motivations for hunting among other tribes in Northeast India (e.g., Aiyadurai et al. 2010). Dutta and Mondal (2020) also refer to a preference for Indian wild rabbit for its “tasty meat” among communities residing in the Bankura District of West Bengal and occasional hunting of reptiles and amphibians for their meat. Hunters in this study, in the districts of Jhargram and West Medinipur, also considered Indian “wild rabbit” (i.e., the Indian hare) to be particularly desirable, although wild boar was their most commonly stated and preferred species in this regard. In contrast to Dutta and Mondal (2020), no amphibian species were mentioned by any of the hunters that participated in our study. However, although several hunters mentioned providing food for their family when asked about the benefits of hunting, few (only three) ranked subsistence as an important or very important element of the hunt (or indeed ranked subsistence benefits at all). This finding is also in accordance with Dutta and Mondal (2020) who suggest that there is little need for local communities to hunt for meat these days in the study landscape (see also Kazmi 2021).

In addition, although most of the species perceived by hunters to be “desirable” were reportedly consumed, some had additional or alternative uses – for example, almost 40% of hunters who listed Bengal monitors as desirable species also used their skins, with personal uses described suggesting that this might have been for belts. More broadly, wild animal parts have historically been sold as jewellery, souvenirs and for medicinal/spiritual purposes by tribal communities in India for millennia (Jugli et al. 2020a). To this day, with regards to traditional medicine, villagers often still rely on local village priests and healers who recommend the use of wildlife derivatives due to inadequate health-care facilities in these types of remote areas (D'Cruze et al. 2018a; Jugli et al. 2020a). Sarkar and Modak (2022), similarly describe how animals have been traditionally targeted during hunting festivals in the Purulia district of West Bengal to provide a rich source of essential nutrients which help to restore the tribe and maintain a healthy reproductive life. Likewise, hunters are also known to use body parts from wild animals that are otherwise consumed for food for spiritual and aesthetic purposes. For example, Dutta and Mondal (2020) noted how the skulls of hunted animals were hung in front of the house as a symbol of prestige, and the colourful feather of the birds to decorate their room in the Bankura district of West Bengal. Ghosh et al. (2013) also refer to birds' feathers as ornaments commonly worn by members of the Santhal tribe during festivals, and similar uses of wild animal parts are described for tribal communities elsewhere in India (Jugli et al. 2020b). Several hunters in this study confirmed that body parts of the most profitable wild animals (particularly wild boar but also Bengal monitor, Indian flying fox, and Russell's viper) were sought for several different cultural purposes, such as traditional medicine, belief-based



practices, and decoration, although it was not clear to what extent hunters used body parts for these purposes themselves as opposed to simply being aware that they might be used in this way.

### Economics and enjoyment

Beyond the dominant personal consumption of wild meat and the enduring cultural aspects, our analysis of hunter responses regarding the most profitable wildlife species suggests that these ritualistic hunts also comprise a commercial component (albeit for a relative minority of hunters and largely limited to occasions when there was excess meat available). Notably, approximately half of respondents were able to provide information on the profitability of particular species, but not much more than a quarter said that they sold them themselves (i.e. hunters seemed to be aware of the potential value of the species captured and their body parts but, for the most part, did not personally engage in selling them). Moreover, although some hunters also sold wild animal body parts - including wild boar derivatives, Russell's viper bones, Bengal monitor fat, elongated tortoise shells, Indian rat snake tails, golden jackal skin, and Indian chameleon tails - many were unable to provide information relating to their intended end use. Other recent studies have described cash income as being an important driver for hunting among other tribes in India. For example, Aiyadurai et al. (2010) reported how certain species that have valuable commercial parts, such as otter species, musk deer species, and Asiatic black bears (*Ursus thibetanus*), are hunted primarily for commercial sale. Likewise, previous research has also documented how musk pods, bear gall bladders, and pangolin scales are sold in towns in the neighbouring state of Assam, and in some cases then prepared for export as part of illegal international wildlife trade chains, often without any knowledge of what is responsible for driving consumer demand (e.g. Aiyadurai et al. 2010; D'Cruze et al. 2018a). The growing online component of trade in wildlife products is also a particular conservation concern related to illegal wildlife trade in India. The '*hatha jodi*' trade, for example, originating from India, targets CITES Appendix I monitor lizards (including the Bengal monitor and yellow monitor (*Varanus flavescens*)) for their hemipenes that are dried and falsely sold online as rare and 'sacred' Himalayan plant roots purported to have spiritual properties (D'Cruze et al. 2018b). In our study, however, "economic opportunities" were not considered an important element of ritualistic hunts by any of the hunters surveyed, and it is important to note that, even for those hunters that do sell some of the meat or body parts obtained, this type of wildlife trade activity appears to function as a supplementary source of income. The majority of hunters surveyed stated that agriculture was their main source of income.

When hunters were questioned more broadly about their motivations for participating in these types of hunting festivals, our study uncovered a consistent positive sentiment toward ritualistic hunts, even among those who identified as non-hunters, despite the illegal status of the hunts (and the fact that many respondents were aware of their illegality). The majority of hunters ranked "engaging in a fun activity" and "maintaining a traditional practice" as important elements of ritualistic hunts, and some (n = 19) also ranked "social cohesion" as important. Personal enjoyment as a primary motivating factor (even above personal use for wild meat) may also help to partly explain recent observations

made by a number of conservation-focused NGOs, which have reported that the hunting festivals of south-western West Bengal have recently attracted an influx of hunters from surrounding areas including West Medinipur, Jhargram, Bankura, Murshidabad, Purulia and Jharkhand (HEAL 2020). These groups appear to involve individuals from “various walks of life” beyond those involved in local agriculture including students, teachers, shopkeepers, factory workers, and even government employees (Kazmi 2021).

### Animal welfare concerns

Our research also draws attention to the animal welfare concerns associated with ritualistic hunts. A growing body of research continues to demonstrate the cognitive capacity of wild animals and their ability to experience a range of emotions and states including excitement, fear, frustration, and pain (Lambert et al. 2019). In this context, ritualistic hunts raise significant concerns not only due to the relatively high number of individual animals involved, but also due to the intensity and duration of suffering that they can experience throughout the different stages of the trade chain including capture, transport, and slaughter (Baker et al. 2013). For example, our study revealed that hunters reported employing 13 different primarily traditional killing methods during capture, with bows, catapults, and spears being the most commonly used. These findings align with similar studies; for instance, Aiyadurai et al. (2010) also documented the use of various indigenous capture methods like stone-fall, trigger-and-release, canopy, spring-pole, gum, metal noose, hanging stone, pitfall, box, log-fall, and rodent traps. Additionally, Dutta and Mondal (2020) reported the use of nylon nets for catching wild hare, iron wire for rats, sticky gum from various plants for capturing water birds and other birds near water bodies, and lancets with pointed tips for killing larger mammals. All these methods can lead to a slow and painful death, characterized by considerable pain and suffering. Moreover, in describing “memorable experiences” associated with taking part in ritualistic hunting, when hunters did refer to the animals directly, they talked about “chasing” and being “chased”, it being “funny” when two hunters tried to kill the same animals, and their friends laughing as they tried or failed to hunt or kill an animal. In some cases, hunters referred to hunted animals (boar or in one case, a tiger) biting or attacking the hunters ( $n = 4$ ), hurting the hunters ( $n = 3$ ), or hunters being killed by the animals ( $n = 3$ ). Few hunters ( $n = 24$ ) provided answers to these questions, so it is not possible to determine to what extent they are representative of all ritualistic hunts and all hunters but, in addition to being potentially dangerous to the hunters, the scenarios described suggest an experience that is highly stressful for the animals involved.

Although the wild animals are often slaughtered, skinned, and cooked at the hunting site, they may also be taken away for slaughter elsewhere (HEAL 2020). For example, hunters have been observed slaughtering, skinning, and cooking wild animals openly on the railway platforms (HEAL 2020). In these cases, the captured wildlife remains alive during transport that can involve physical restraint before their meat, skins and other body parts are consumed or prepared for onward sale. This raises particular animal welfare concerns given that some researchers have reported how villagers in other locations have described that they travel longer distances ( $>5$  km) to hunt than they have done a decade ago (presumably due to wildlife population declines around villages) (Aiyadurai

et al. 2010). These animal welfare concerns also pose a risk to public health, particularly in relation to the spillover of zoonotic diseases in scenarios where animals have endured debilitating conditions that compromise their immune systems and that promote disease transmission (Shivaprakash et al. 2021).

### Conservation concerns

Our study reiterates potential conservation concerns associated with ritualistic hunting in India (e.g., Aiyadurai et al. 2010). According to the IUCN Red List, a considerable proportion of the wild animals that are targeted by hunters in the districts of Jhargram and West Medinipur, are already considered as threatened (7.5%) (Suppl. material 2). In particular, some common names provided by respondents as either most desirable or most profitable species that could be captured include three species which are currently classified as Critically Endangered (the elongated tortoise, long billed vulture, and slender billed vulture [see footnote 2]), and four species (the common pochard, swamp francolin, pale capped pigeon, and sambar) that are classified as Vulnerable (Suppl. material 2). For these species, already considered at risk of extinction, even relatively low-level trade (as reported for long billed vulture) can have highly detrimental impacts on their conservation status. However, the risk of local extirpation to species that are currently considered as non-threatened should not be ignored especially given that these types of hunting festivals often take place in remote regions where law enforcement is challenging to implement, and where wildlife populations may already be in decline - a perception reported by 51 (68%) of the hunters that participated in our study.

Specifically, 28 of the species inferred on the basis of local and common names given by hunters as most desirable or profitable species are not currently listed on the IUCN Red List as threatened but have wild populations that are considered to be either in decline, not assessed or unknown (Suppl. material 2). This includes the Bengal monitor which is currently considered as Near Threatened but is listed on CITES Appendix I, and several mammal species (including the jungle cat, wild boar and Indian hare) which are currently classified as Least Concern but which have been identified to be at risk of local extirpations due in part to unsustainable hunting for their body parts for personal use and commercial sale (IUCN 2023). All of these species were named specifically by hunters. Hunters also referred to 23 species that have greatly increased in rarity from the hunters' perspective, such as the yellow-footed green pigeon, western koel (*Eudynamys scolopaceus*), Indian peafowl (*Pavo cristatus*), golden jackal, and Indian wolf (Suppl. material 2). Some species (such as the wild boar, Bengal monitor, red junglefowl, Indian hare, and Russell's viper) may also be of particular local conservation concern despite their current non-threatened status as they were found to be particularly expensive and among the species perceived to have increased most in rarity in recent years (Suppl. material 2). Relatedly, it is also worth highlighting that IUCN Red List assessments for a number of the species identified in this study, such as the small Indian mongoose, chital (*Axis axis*), sambar, Asian chameleon and jungle cat, were undertaken more than 10 years ago and therefore may not fully reflect the conservation status of these species.

Ritualistic hunting in contemporary West Bengal appears to have departed significantly from its original cultural components as described by Ghai (2017) and Sarkar and Modak (2022). Sarkar and Modak (2022), for example, describe



how the main purpose of the festival is not killing animals, and suggest the presence of cultural components that regulate offtake, such as that pregnant and juvenile animals are not targeted. In contrast, instances of pregnant females, young offspring, and entire groups of animals being killed have been documented in recent years (HEAL 2020; Fig. 7), further indicating that these hunting festivals are associated with indiscriminate killing of wildlife which raises additional conservation concerns. Unsustainable hunting and defaunation, for example, compromises ecosystem resilience and can lead to reduced forest regeneration with wider impacts on both the conservation and the carbon storage capacity of forests (Gardner et al. 2019).

### Study limitations

Wildlife trade surveys that touch upon illegal and/or unsustainable activities should be interpreted with care; not least because markets are complex systems that can vary over time (due to a variety of different factors) and those involved in the trade chains may distrust surveyors or have poor memory recall (D'Cruze et al. 2018a; Mayor et al. 2019; Newton et al. 2008). However, that 112 individuals were willing to talk to us, and that 99 of them openly admitted to their own involvement with illegal hunting, suggests that they were being truthful. Moreover, while there is a risk that our data may underestimate or overestimate the impact on wildlife in Jhargram, West Medinipur, and surrounding areas of West Bengal, it is important to clarify that our aim was not to assess the full extent of impact on wild populations or individual animal welfare.

Rather, our intention was to gain new insights into the diversity of wildlife being targeted (by hunters), to better understand what they might be used for (both by hunters personally and any subsequent consumers following onward sale), and to identify those wildlife species that might potentially be at risk. Likewise, a full inventory of wildlife being hunted in these two districts, including identifying species that are most targeted and the frequency of trade, was beyond the scope of this study. Specifically, we acknowledge that our use of local names to infer the species hunted and sold cannot be considered a complete taxonomic account, and that in some cases hunters may be referring to only one particular species that is not threatened by extinction or vice versa. Yet, despite the limitations of a “snapshot” survey-based approach, we believe that our findings represent valuable information that can be used to help provide information for future efforts to protect wildlife in West Bengal.

### Recommendations

Our study highlights the indiscriminate killing of a wide variety of species during illegal ritualistic hunts in West Bengal including potentially at least 25 that are listed on Schedule I of the Wildlife Protection Act. While recognizing that ritualistic hunting is a complex issue involving various stakeholders with diverse perspectives and goals (Sanctuary Nature Foundation 2021), our research provides valuable information that can inform future initiatives. Our recommendations are not exhaustive but include the following five key points: 1) Firstly, considering the potential for detrimental impacts on wildlife populations, it is imperative that wildlife crime be taken seriously, and this includes

increasing the risk of apprehension, prosecution, and punishment (Gomez et al. 2023); 2) While the decision by the Calcutta High Court to equate indiscriminate wildlife hunting with murder under Section 302 of the Indian Penal Code indicates that there is strong judicial will to end ritualistic hunts in West Bengal, effectively addressing the complex issue of illegal wildlife hunting and subsequent trade requires a multi-faceted strategy that extends beyond law enforcement alone. In particular, initiatives aimed at instigating positive changes in human behaviour, based on research into impactful messaging, including the animal welfare, conservation, public health, and legal risks associated with engagement in illegal wildlife trade activities, are also increasingly recognised as an essential component of an effective approach (Sanctuary Nature Foundation 2021; Moorhouse et al. 2024). Consequently, the Humane Committees proposed by the Calcutta High Court are likely to be more effective if they incorporate these types of human behaviour change initiatives into their future plans for delivering positive change for both wildlife and people; 3) Campaigns aimed at raising awareness locally about regulations governing the hunting of species and the negative impacts of utilisation and illegal trade in wildlife on local populations, conservation, animal welfare and public health may also be beneficial to motivate communities to stand against wildlife exploitation, particularly among younger generations (Bhattacharya et al. 2019); 4) In addition, further exploration of legal non-consumptive alternatives could also assist efforts to effectively curb these types of illegal hunting festivals in West Bengal, especially given that the majority of respondents in our survey expressed a willingness to embrace them if they were made available. In particular, to address the void left by the enjoyment derived from any ritualistic hunts, the development and promotion of “wildlife-friendly” tourism (as previously suggested by Dutta and Mondal 2020) such as bird watching could emerge as an important and viable solution (e.g., the “Catapults to Cameras” programme that promotes photography of wildlife instead of killing (Marik 2024)), or outdoor “festival” type non-consumptive but wildlife-focused activities that still bring elements of fun and celebration that are reportedly key motivations for people taking part in ritualistic hunting. Beyond personal enjoyment, these types of “wildlife friendly” initiatives could also help to reduce engagement in the hunting of wildlife for personal use or as a supplementary form of financial income by fostering a holistic, ethical, and sustainable approach to wildlife protection in the region. This type of community behaviour change is not unprecedented: the Angami tribe in Khonoma village in Nagaland, NE India stopped hunting in the 1990s driven by village leaders responding to the killing of endangered Blyth's tragopans (*Tragopan blythii*) and, although negotiations with villagers were reportedly on-going for several years, created the Khonoma Nature Conservation and Tragopan Sanctuary (KNCTS) in 1998 in the first community-led conservation project in India ([https://vikalpsangam.org/wp-content/uploads/migrate/Stories\\_PDFs/community\\_conservation\\_at\\_a\\_crossroads\\_in\\_khonoma.pdf](https://vikalpsangam.org/wp-content/uploads/migrate/Stories_PDFs/community_conservation_at_a_crossroads_in_khonoma.pdf)). There are also more examples of (self-imposed) hunting bans imposed by villages through the traditional village council in community lands in Arunachal Pradesh (Datta and Naniwadekar 2019). Many younger people are now engaged with ‘hunting’ and observing wildlife (including birds, butterflies and moths) with cameras and binoculars and taking pride in their natural heritage. Likewise, in the context of ritualistic hunting for wildlife derivatives as traditional medicine and

belief-based use (both for personal use and commercial profit), mechanisms to promote the substitution of sustainably sourced plant-based medicinal materials for wildlife-origin materials could also prove useful. Several studies have recently noted that this type of initiative could be effective in countries where most traditional medicine remedies are predominantly of botanical origin, and animal-origin remedies are known to have herbal alternatives.

## Additional information

### Conflict of interest

This study was funded by an animal welfare organisation; four of the authors are employed by the same organisation (NDC holds the position of Head of Research) and LAH received funding from the same organisation. HEAL is a wildlife conservation organisation. The results presented in this paper were in no way influenced by either the funding source, or our own personal views on animal welfare or conservation.

### Ethical statement

Ethical approval was obtained via Manchester Metropolitan University on 06/09/2022, EthOS Reference Number: 43711.

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### Author contributions

Conceptualization: Neil D'Cruze, Angie Elwin, Shubhobroto Ghosh, Alexander E. Asfaw; Investigation: Sangita Giri and Suvrajyoti Chatterjee; Data curation: Angie Elwin, Lauren Harrington, Emma Coulthard, John Norrey, Tiasa Adhya and Suvrajyoti Chatterjee; Writing—original draft preparation: Neil D'Cruze, Lauren Harrington, Angie Elwin; Writing—review and editing: Neil D'Cruze, Angie Elwin, Lauren Harrington, Shubhobroto Ghosh, Alexander E. Asfaw, Emma Coulthard, John Norrey, David Megson, Tiasa Adhya, Vasudha Mishra, Meghna Banerjee, Aditya Banerjee; Visualization: Angie Elwin, Lauren Harrington; Supervision: Neil D'Cruze, Shubhobroto Ghosh, David Megson; Project administration: Angie Elwin, Neil D'Cruze, Shubhobroto Ghosh; Funding acquisition: Neil D'Cruze, Shubhobroto Ghosh. All authors have read and agreed to the published version of the manuscript.

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### Data availability

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

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## Supplementary material 1

### Questionnaire survey questions

Authors: Neil D'Cruze, Angie Elwin, Shubhobroto Ghosh, Alexander E. Asfaw, Emma Coulthard, David Megson, John Norrey, Sangita Giri, Vasudha Mishra, Tiasa Adhya, Suvrajyoti Chatterjee, Meghna Banerjee, Aditya Banerjee

Data type: docx

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Link: <https://doi.org/10.3897/natureconservation.56.132178.suppl1>

## Supplementary material 2

### Species list

Authors: Neil D'Cruze, Angie Elwin, Shubhobroto Ghosh, Alexander E. Asfaw, Emma Coulthard, David Megson, John Norrey, Sangita Giri, Vasudha Mishra, Tiasa Adhya, Suvrajyoti Chatterjee, Meghna Banerjee, Aditya Banerjee

Data type: xlsx

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## Supplementary material 3

### Price data

Authors: Neil D'Cruze, Angie Elwin, Shubhobroto Ghosh, Alexander E. Asfaw, Emma Coulthard, David Megson, John Norrey, Sangita Giri, Vasudha Mishra, Tiasa Adhya, Suvrajyoti Chatterjee, Meghna Banerjee, Aditya Banerjee

Data type: xlsx





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## Research Article

# Modelling the present and future distribution of *Ambystoma altamirani* in the Transmexican Volcanic Belt, Mexico

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## Abstract

*Ambystoma altamirani* is a critically endangered, microendemic amphibian species inhabiting the high-altitude rivers and streams of the Trans-Mexican Volcanic Belt (TMVB), a region experiencing severe ecological disturbances. This study aims to assess the current and future distribution of *A. altamirani* under different climate and land-use change scenarios using ecological niche modelling (ENM). We also evaluate the connectivity of suitable habitats and the overlap with existing natural protected areas (NPAs). Using occurrence records and environmental variables, we modelled the species' potential distribution under two climate models (CN85 and MP85) for 2050. The results indicate a significant reduction in suitable habitat, particularly in areas such as the Sierra de las Cruces and the Chichinautzin Biological Corridor, with habitat losses projected to reach up to 13.95% by 2050 under the CN85 scenario. Forest cover loss between 2001 and 2023 further exacerbates this threat, especially in municipalities like Tlalpan and Ocuilán. Our analysis highlights the urgent need for targeted conservation efforts, including the preservation of mixed *Abies-Pinus* forests and the restoration of degraded ecosystems. The findings underscore the critical importance of integrated conservation strategies that address habitat degradation, climate resilience and ecological connectivity to ensure the long-term survival of *A. altamirani*.

**Key words:** Axolotls, Central Mexico, climate change, conservation strategies, Ecological niche modelling, habitat fragmentation, mole salamanders

## Introduction

*Ambystoma altamirani* is a critically endangered, microendemic amphibian species native to the high-altitude rivers and streams of the Transmexican Volcanic Belt (TMVB). This region, renowned for its rich biodiversity and high levels of endemism (Mastretta-Yanes et al. 2015; Sunny et al. 2017; Lemos-Espinal and Smith 2024) is one of Mexico's most ecologically disturbed areas, where habitat

degradation, invasive species and climate change are placing immense pressure on local ecosystems (García 2011; Parra-Olea et al. 2012; Figueroa et al. 2016). *Ambystoma altamirani* relies on the pristine conditions of oxygen-rich rivers and the integrity of temperate forests, particularly those dominated by *Abies* and *Pinus* species (Lemos-Espinal et al. 1999; Sunny et al. 2014; Lemos-Espinal et al. 2016; Woolrich-Piña et al. 2017). However, extensive land-use changes, including deforestation for agriculture, urban sprawl and both legal and illegal logging, have severely fragmented its habitat, further endangering the species (Lemos-Espinal and Smith 2015; Heredia-Bobadilla et al. 2017).

The TMVB, like many global biodiversity hotspots, is increasingly impacted by human activities that are driving significant biodiversity loss (CONABIO 2018). The Nevado de Toluca and Sierra de las Cruces, along with their protected natural areas, are amongst the key habitats where studies on *A. altamirani* have been conducted (Lemos-Espinal et al. 2016; Heredia-Bobadilla et al. 2017; Camacho et al. 2020; Guerrero-de la Paz et al. 2020; Ruiz-Reyes et al. 2024). These critical areas have experienced substantial forest loss, particularly of *Abies* and *Pinus* forest, due to legal and illegal logging as well as land conversion. Between 2011 and 2014, significant portions of the Bosque de Agua were cleared and this deforestation trend has worsened in recent years, exacerbated by increased illegal logging during the COVID-19 pandemic (López-García and Navarro-Cerrillo 2021). In the Nevado de Toluca, clear-cut deforestation occurred in 49.6 hectares in 2018 alone (González-Fernández et al. 2022). Given that 91.1% of Mexico's *Abies* forests are located within the TMVB (Sunny et al. 2017), their conservation is vital, not only for biodiversity, but also for maintaining essential ecosystem services such as water regulation, which benefits over 25 million people in central Mexico (Sunny et al. 2017). The limited dispersal ability of *A. altamirani*, combined with habitat fragmentation, makes the species especially vulnerable to environmental changes (Ruiz-Reyes et al. 2024). Ruiz-Reyes et al. (2024) showed that the species' potential distribution is concentrated in areas where *Abies* forests are interspersed with *Pinus* forests, while areas dominated solely by *Pinus* are less suitable. This emphasises the need to preserve mixed forest habitats to support viable populations of *A. altamirani*. In addition to habitat loss, *A. altamirani* faces significant threats from invasive species, particularly *Oncorhynchus mykiss* (rainbow trout), which has been introduced into many high-altitude rivers in Mexico (Zambrano et al. 2010; Estrella-Zamora et al. 2018; Guerrero-de La Paz et al. 2020).

The TMVB is one of Mexico's primary agricultural regions and the expansion of crops like corn, potatoes and oats has led to the conversion of forested land into farmland (Galicía and García-Romero 2007; Caro-Borrero et al. 2024). Traditional farming practices often result in soil erosion and habitat degradation (Galicía and García-Romero 2007; Aryal et al. 2018; Caro-Borrero et al. 2024), while the widespread use of agrochemicals contaminates the rivers and lakes where *A. altamirani* lives (Caro-Borrero et al. 2024). This reduces habitat quality and increases the species' susceptibility to diseases and environmental stressors (Valbuena et al. 2021). Pollution from agricultural runoff affects the health of amphibian populations and reduces their resilience to environmental changes, further heightening the risk of extinction (Egea-Serrano et al. 2012). Predictions suggest that 25% of global biodiversity could be lost within the next 50 years due to such unsustainable practices (Lanz et al. 2018).

While habitat destruction and invasive species are pressing concerns, climate change presents a long-term challenge to the survival of salamanders and therefore *A. altamirani* (Parra-Olea et al. 2012; Vargas-Jaimes et al. 2021). As climatic conditions shift, habitat suitability for the species may decline in currently occupied areas, potentially limiting *A. altamirani* to smaller, more isolated populations (Sunny et al. 2014). Therefore, we hypothesise that *A. altamirani*'s future distribution will be significantly reduced due to the combined effects of habitat degradation and climate change and that current natural protected areas (NPAs) may not sufficiently encompass suitable habitats for the species under future climate scenarios (Ochoa-Ochoa et al. 2012). The primary objective of this study is to model the current and future distribution of *A. altamirani* under different climate and land-use change scenarios using ecological niche modelling. Additionally, we aim to evaluate the connectivity of suitable habitats for *A. altamirani* using functional connectivity analysis, assess the overlap between highly suitable areas and existing NPAs and determine whether current conservation efforts are sufficient to protect the species. By identifying critical habitats and regions most vulnerable to environmental change, we seek to provide actionable insights for improving conservation strategies and ensuring the long-term survival of *A. altamirani*.

## Materials and methods

### Species occurrence and environmental variables

A total of 198 occurrence records for *Ambystoma altamirani*, including geographical coordinates, were collected from both fieldwork and several online databases, such as REMIB (<http://www.conabio.gob.mx/remib>), UNIBIO (<http://unibio.unam.mx/>), the Global Biodiversity Information Facility (GBIF; <https://www.gbif.org>), HERPNET (<http://www.herpnet.net/>), the Vertebrate Network (VerNet, <http://vertnet.org/index.html>), IREKANI (<http://unibio.unam.mx/irekani/>) and iNaturalist ([www.iNaturalist.com.mx](http://www.iNaturalist.com.mx)). To ensure consistency, only records from 2000 to 2023 were included, as significant land-use changes and climatic shifts in Mexico have primarily occurred over the last two decades. For example, between 2002 and 2019, Mexico lost 594 thousand hectares of humid primary forest, placing it amongst the top nine tropical countries in terms of primary forest loss (Hansen et al. 2013). The occurrence data were processed using the EcoNicheS package for R (Marmolejo et al. 2024) following these steps: (1) previsualisation of the data to remove erroneous or geographically implausible records, (2) elimination of duplicate entries and (3) subsampling to avoid pseudoreplication, ensuring that only records at least 1 km apart were retained. This method significantly reduces model overfitting (Segurado et al. 2006; Boria et al. 2014). After filtering, 73 records for *A. altamirani* remained for analysis (Fig. 2B). Climatic variables were sourced from WorldClim version 2.1 (Fick and Hijmans 2017) at a 1 km resolution. Topographic variables, including elevation, slope and aspect, were derived from elevation data using ArcMap 10.5 (Fick and Hijmans 2017). Land-cover variables, such as soil use and vegetation (series VI, scale 1:250000), were obtained from the National Institute of Statistics and Geography (INEGI 2017). The land-cover layer, based on satellite images from 2014 and last updated in 2017, was processed by



extracting different land-cover types, converting them into raster format and resampling them from categorical to continuous variables. This was achieved through a method that averages the eight surrounding pixel values and the previous pixel value ( $7 \times 7$  mean), as described by Hirzel et al. (2001). This procedure was performed using IDRISI TerrSet 18.21 software (Clark Labs 2020) through the Filter module (Gidey et al. 2017). All layers were handled in raster format with a 1 km resolution using the raster package (Hijmans 2023) in R (R Core Team 2022). A Pearson's correlation analysis, conducted with ENMTools (Warren et al. 2010) and implemented through the shinydashboard EcoNicheS for R package (Marmolejo et al. 2024), helped to discard highly correlated variables ( $r^2 > 0.7$ ; Dormann et al. (2013)). The selected variables deemed crucial for amphibian presence (Vargas-Jaimes et al. 2021; Ruiz-Reyes et al. 2024), included the percentages of grassland arid vegetation, *Pinus* forest, *Quercus* forest, *Abies* forest, agricultural land and cloud forest. Climatic factors, such as maximum temperature of the warmest month (BIO5), minimum temperature of the coldest month (BIO6), precipitation of the wettest month (BIO13) and precipitation of the driest month (BIO14), were also considered. The distance to urban areas was excluded to avoid bias, as these areas are more easily accessed by observers (Araujo and Guisan 2006). Instead, both current and future urban zones, derived from distribution maps, were assigned zero habitat suitability (González-Fernández et al. 2018; Sunny et al. 2019; Vargas-Jaimes et al. 2021; Rubio-Blanco et al. 2024).

### Environmental niche modelling

Environmental niche modelling for *Ambystoma altamirani* was performed using the Maxent algorithm (Phillips et al. 2006) through the ENMeval 2.0 package (Kass et al. 2021) in R to determine the most suitable model settings. Multiple models were generated, testing various combinations of regularisation values and feature classes, including linear (L), quadratic (Q), product (P), threshold (T) and hinge (H). A total of 100,000 background points were randomly generated and the block approach of ENMeval was used to spatially partition occurrences, as per the methodology of Radosavljevic and Anderson (2014). This method creates four non-overlapping geographic bins to ensure spatial independence between the training and testing datasets (Fourcade et al. 2018). Model performance was assessed using the area under the curve (AUC) from receiver operating characteristic (ROC) plots (Metz 1978; Phillips et al. 2006). Partial-receiver operating characteristic curves (Partial-ROC; Peterson et al. (2008)) were generated using the EcoNicheS package. To estimate potential distribution areas, we applied both the 10<sup>th</sup> percentile training presence threshold and the 60% percentile training presence threshold, allowing for robust predictions of *A. altamirani*'s present and future distributions in response to environmental changes.

### Land cover and climate change

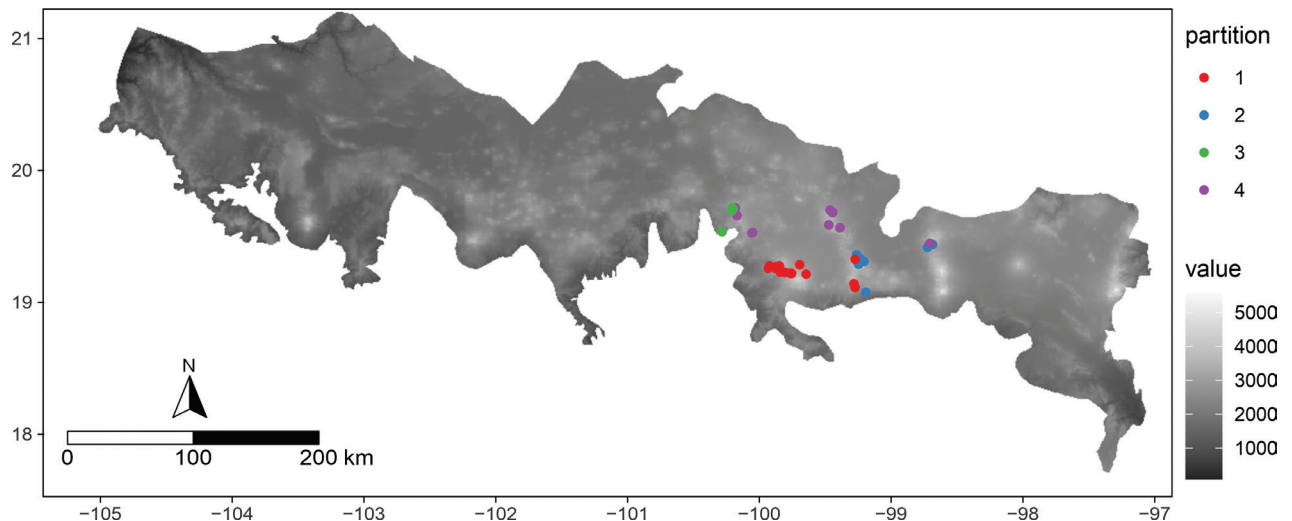
Land-use and vegetation projections for the year 2050 were generated using the Land Change Modeller for Ecological Sustainability (LCMES) module in IDRISI TerrSet, based on land-use and vegetation layers from 2011 (series V;

INEGI 2011) and 2014 (series VI; INEGI 2017). The model employed artificial neural networks, Markov chain matrices and transition suitability maps using multilayer perceptron or logistic regression (Mas et al. 2014; Ansari and Golabi 2019; Hasan et al. 2020). This approach predicts land-cover changes between different raster images from various time periods with the same number of classes in the same order (Mas et al. 2014). The analysis evaluates quantitative change by charting gains and losses across land-cover types (Mishra et al. 2014; Hasan et al. 2020) and estimates net change, persistence and specific transitions between land-cover classes (Gibson et al. 2018; Hasan et al. 2020). Finally, the algorithm applies a Markov chain model to forecast land-cover changes from time  $t = 1$  to time  $t+1$ , using transition probability and area matrices for each land-cover class (Hasan et al. 2020). Current and future urban areas were designated as areas of zero habitat suitability. Future potential distribution maps were generated using future bioclimatic variables from the CN-RM-CM5 (CN) and MPI-ESM-LR (MP) climate models for the year 2050, under the most extreme climate scenario (RCP 8.5). These climate models were selected from WorldClim's downscaled CMIP5 data ([https://worldclim.org/data/cmip5\\_2.5m.html](https://worldclim.org/data/cmip5_2.5m.html)) due to their proven effectiveness for Central America (Hidalgo and Alfaro 2015) and their high performance amongst the 40 available models (Kamworapan and Surussavadee 2019). The resulting continuous maps were reclassified into binary maps using both 90% and 10<sup>th</sup> percentile training presence thresholds. These thresholds exclude the lowest 10% of locality values, offering a more reliable prediction of habitat suitability (Radosavljevic and Anderson 2014). The binary maps were then used to evaluate changes in *A. altamirani*'s potential distribution under future climate scenarios. To assess the contribution of current natural protected areas (NPAs) in conserving *A. altamirani*, we calculated the percentage of highly-suitable areas that overlap with NPAs using both the 60% and 10<sup>th</sup> percentile training presence thresholds. This analysis was conducted using the EcoNicheS for R package. Finally, a comprehensive search was conducted using Global Forest Watch to assess the areas where the greatest loss of potential distribution for *Ambystoma altamirani* was identified. The objective was to determine whether these reductions in distribution are directly linked to the loss of vegetation cover. The analysis focused on correlating the predicted habitat loss from ecological niche models with observed deforestation trends, providing insight into the extent to which habitat degradation is contributing to the species' declining range.

## Results

### Ecological niche modelling

Based on the results of the ENMeval analysis, the L model with a regularisation multiplier (RM) of 1 was identified as the best-performing model for predicting the current distribution of *A. altamirani* in 2014, as well as its future distribution under the CN85 climate scenario in 2050. For the MP85 scenario in 2050, however, the optimal model was found to be the L model with a RM of 2 (Table 1). After applying filtering criteria to the occurrence data, a total of 73 records were retained for model calibration (Fig. 1). ENMeval models were used to estimate both current and future distributions under two global climate models (GCMs),



**Figure 1.** Occurrence records with the block method partition, including the *Ambystoma altamirani* accessible area (M) represented by grey-scale elevation layer.

**Table 1.** Predictive performance of present and future ENMeval distribution models for *Ambystoma altamirani* with two different global climate models and land-cover changes.

Year	RM	FC	10 percentile training presence	ROC	Partial-ROC
2014	1	L	0.3	0.98	1.968 ( $p < 0.001$ )
2050 cn85	1	L	0.3	0.98	1.968 ( $p < 0.001$ )
2050 mp85	2	L	0.3	0.98	1.968 ( $p < 0.001$ )

CN85 and MP85 and the models showed significantly better predictive performance than random chance (Table 1). The area under the curve (AUC) for all models was consistently high, with a value of 0.98, indicating strong predictive power ( $p < 0.001$ ). This was supported by partial ROC bootstrap tests, which revealed significant empirical AUC ratios of 1.968 across all models ( $p < 0.001$ ; Table 1), further confirming the robustness of these predictions (Table 1). The environmental variables that contributed the most to the species' distribution were the minimum temperature of the coldest month (Bio6), with a high permutation importance of 51.8%, followed by *Abies* forest cover, which accounted for 20.3% of the variation in habitat suitability. Other significant factors included slope (6.8%), altitude (6%) and arid vegetation cover (5.6%) (Fig. 2, Table 2). The positive correlation of Bio6 and *Abies* forest with the species' presence suggests that *A. altamirani* thrives in colder environments and forested areas, particularly in higher altitudes with dense *Abies* coverage (Fig. 2). Conversely, the presence of arid vegetation and *Quercus* forest negatively impacted the habitat suitability, highlighting the species' preference for moist, forested environments (Fig. 2, Table 2). In terms of future distribution under climate change scenarios, the models predicted a contraction in suitable habitat for *A. altamirani* by 2050. Using a 60% threshold, the models projected a habitat loss of 6.02% under the MP85 scenario and a larger loss of 13.95% under the CN85 scenario (Fig. 3, Table 3). This decline suggests that *A. altamirani* will face increasing challenges in maintaining its current range as climate conditions shift, particularly in the more sensitive areas of its distribution. When considering the 10 percentile training presence threshold, which accounts for potential future range loss,

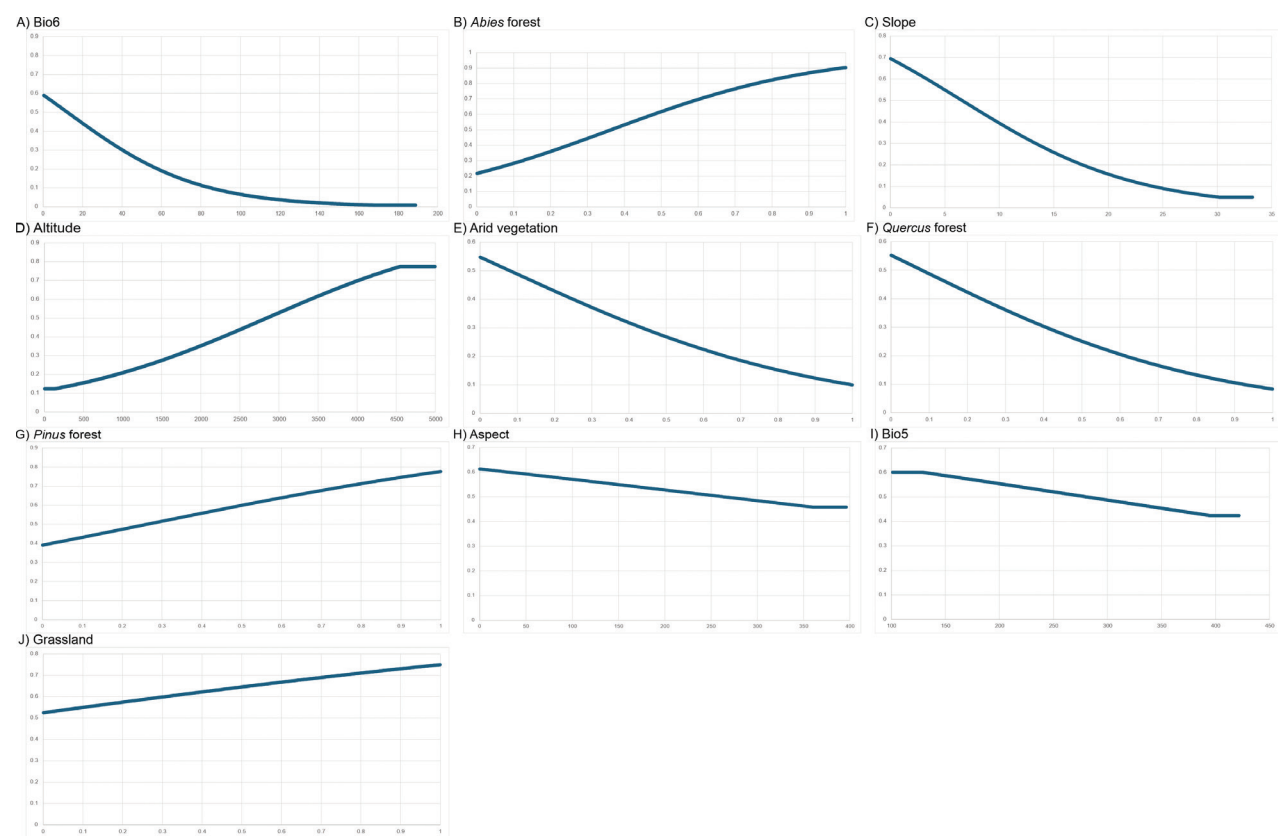


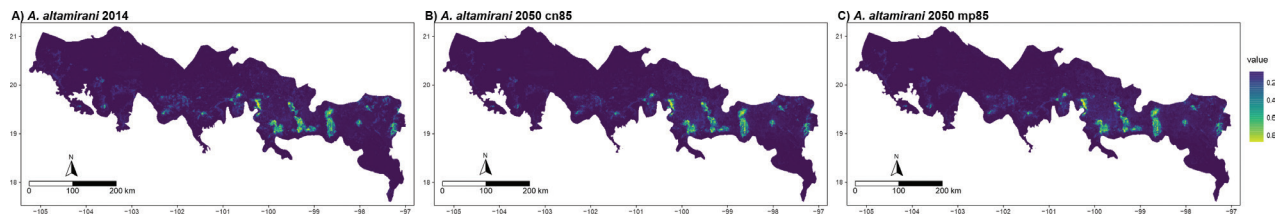
Figure 2. Response curves of the variables that influence the potential distribution of *Ambystoma altamirani*.

Table 2. The most important environmental variables associated with the presence of *Ambystoma altamirani*.

Variable	Permutation importance	Correlation
Bio6	51.8	Positive
Abies forest	20.3	Positive
Slope	6.8	Negative
Altitude	6	Positive
Arid vegetation	5.6	Negative
Quercus forest	4.5	Negative
Pinus forest	1.9	Positive
Aspect	1.6	Negative
Bio5	0.8	Negative
Grassland	0.7	Positive

the projected reductions were slightly lower, with losses between 4.73% (MP85 scenario) and 9.61% (CN85 scenario) (Fig. 3, Table 3). These habitat losses were most pronounced in critical areas such as the Sierra de las Cruces forest (Fig. 4), specifically within the south of the Sierra de las Cruces and Chichinautzin Biological Corridor (Fig. 4). These regions are known biodiversity hotspots and serve as key habitats for *A. altamirani*. The predicted contraction in these areas highlights the potential vulnerability of this species to habitat fragmentation and climate-induced shifts in environmental conditions (Fig. 4, Table 3).

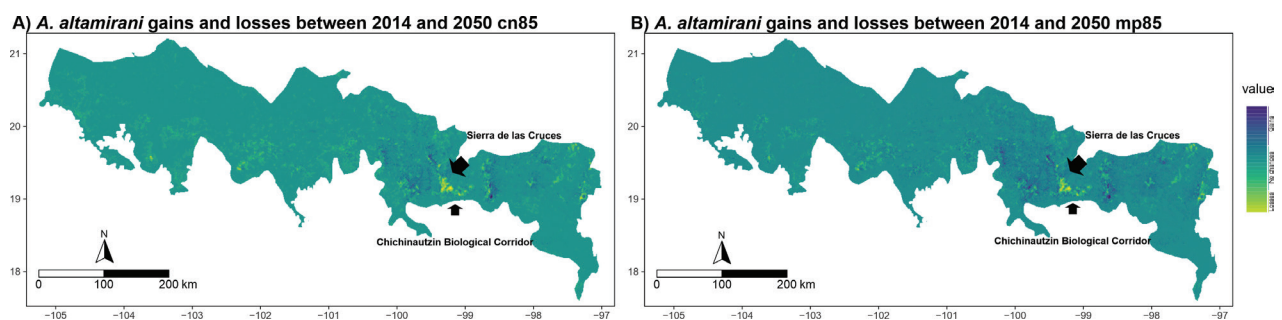
Overall, these findings suggest that *A. altamirani* is highly dependent on specific environmental conditions, particularly cold temperatures and forested



**Figure 3.** Present (2014) and future (2050) potential distribution maps for *Ambystoma altamirani*, generated for 2014 and under future climate scenarios CN85 and MP85 for 2050.

**Table 3.** Present and future suitability area (km<sup>2</sup>) and percentage of area loss of *Ambystoma altamirani* with two different global climate models and land-cover changes.

60%					10 percentile training presence				
2014 (km <sup>2</sup> )	2050 cn85 (km <sup>2</sup> )	Loss 2014–2050 (%)	2050 mp85 (km <sup>2</sup> )	Loss 2014–2050 (%)	2014 (km <sup>2</sup> )	2050 cn85 (km <sup>2</sup> )	Loss 2014–2050 (%)	2050 mp85 (km <sup>2</sup> )	Loss 2014–2050 (%)
1273.94	1096.22	13.95	1197.19	6.02	3410.1	3082.34	9.61	3248.65	4.73



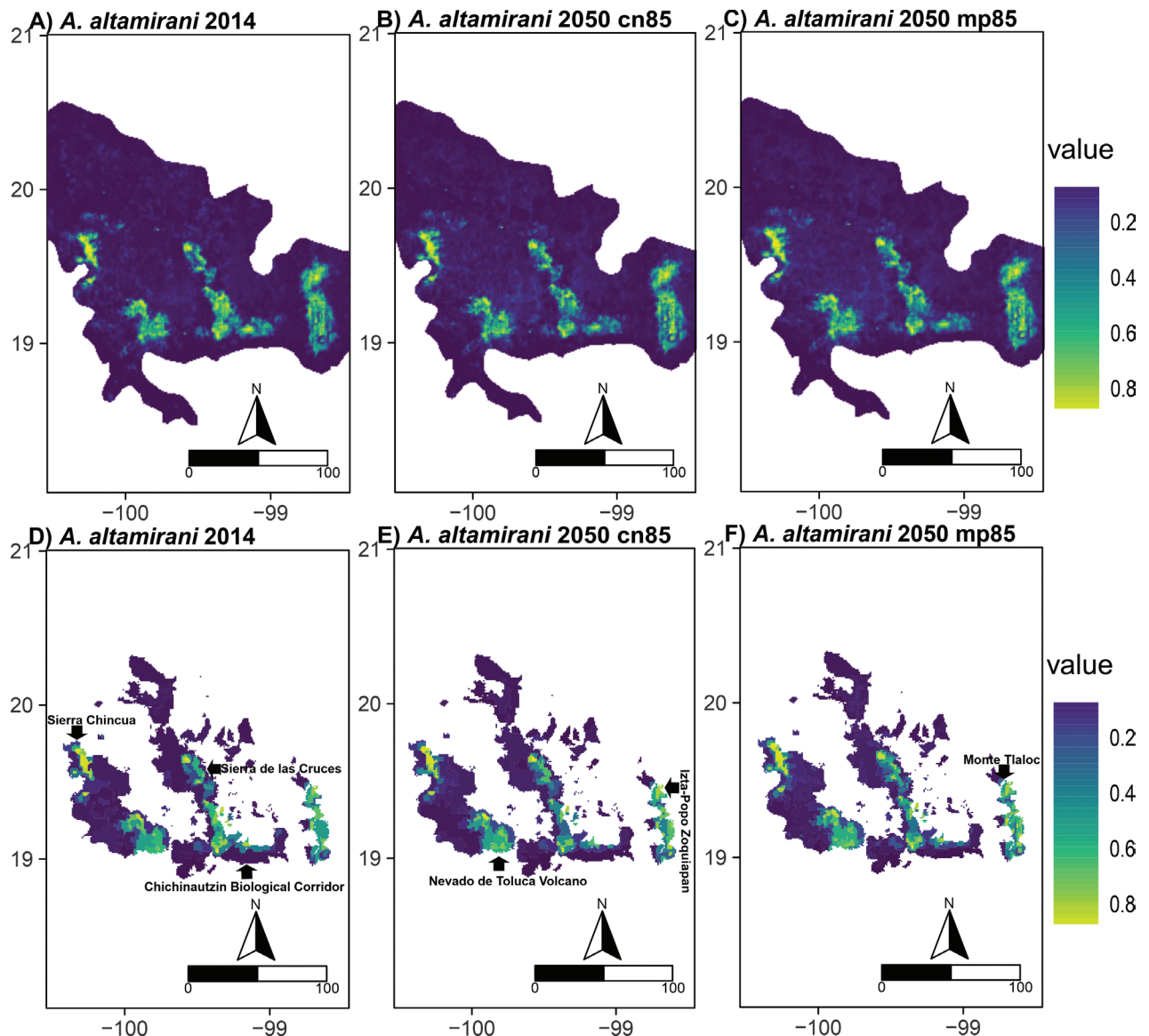
**Figure 4.** Gains and losses in the potential distribution of *Ambystoma altamirani* under two different global climate models (CN85 and MP85) and in response to land-use and vegetation cover changes.

habitats, which are projected to be significantly reduced in the coming decades. The models also underscore the importance of protecting key habitats, especially in the Sierra de las Cruces Forest, where the species faces the greatest risk of habitat loss. Conservation strategies should focus on mitigating the effects of climate change and preserving forested areas to ensure the survival of *A. altamirani* in the future.

A map was generated depicting the potential distribution of *A. altamirani* within areas where its occurrence is known (Fig. 5), as well as the species' potential distribution within federal and state-level protected natural areas (Fig. 6, Table 4). The results indicate that the regions with the highest potential distribution for *A. altamirani* include the Sierra de las Cruces, the Chichinautzin Biological Corridor (Fig. 6D) and the Izta-Popo Zoquiapan. However, current population records of *A. altamirani leorae* are limited to the Monte Tlaloc area (Fig. 6E. F), despite the broader potential distribution identified in the the Izta-Popo Zoquiapan area (Fig. 6E), the Nevado de Toluca Volcano (Fig. 6E) and the Sierra Chincua (Fig. 6D). These areas represent key conservation zones where suitable habitat conditions are present for the species, highlighting their importance in the conservation strategy for *A. altamirani* (Fig. 6).

The results indicate that, by 2050, both global climate models (CN85 and MP85) project a reduction in suitable habitat for *A. altamirani* within protected natural areas, using a 60% threshold. Under the CN85 scenario, the species is expected to



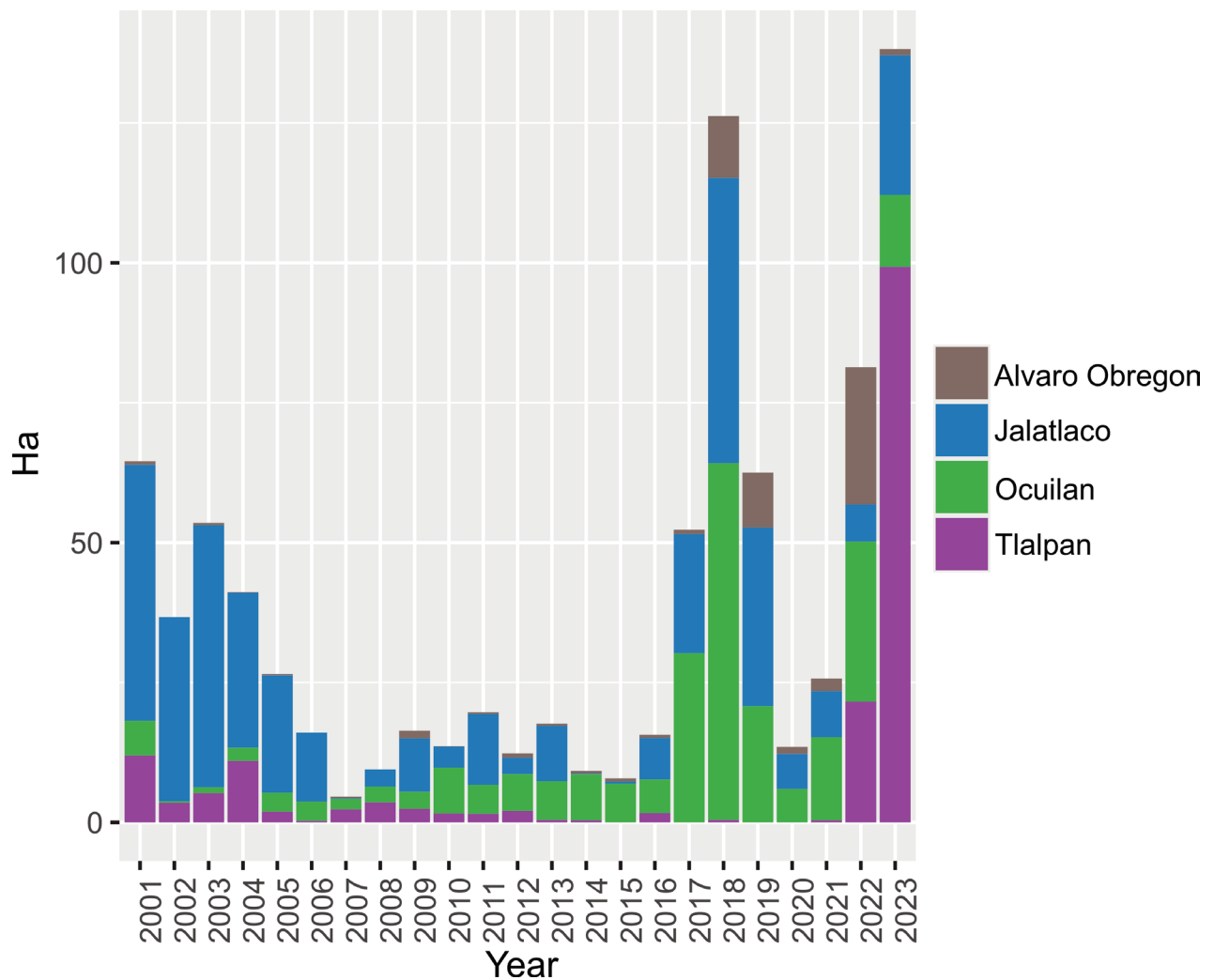


**Figure 5.** Present (2014) and future (2050) potential distribution maps for *Ambystoma altamirani*, considering known areas of occurrence and locations within protected natural areas, generated for the 2014 baseline and future climate scenarios CN85 and MP85 for 2050.

experience a significant 12.93% habitat loss, whereas the MP85 model predicts a more moderate reduction of 5.12%. Additionally, using the 10 percentile training presence threshold, the CN85 model shows an 8.11% decrease in suitable habitat, with a smaller reduction of 1.91% under the MP85 scenario. These findings emphasise the considerable impact of climate change on the future availability of suitable habitats for this critically endangered species (Table 4).

The results show varying levels of forest cover loss across four municipalities (Tlalpan, Álvaro Obregón, Jalatlaco and Ocuilan) between 2001 and 2023. Tlalpan experienced the most dramatic increase in forest loss in 2023, reaching 99.29 km<sup>2</sup>, with a smaller peak in 2022 (21.62 km<sup>2</sup>). In Álvaro Obregón, the highest deforestation occurred in 2018 (11.05 km<sup>2</sup>), followed by 2019 (9.82 km<sup>2</sup>). Jalatlaco had its most significant losses in 2018 (50.97 km<sup>2</sup>) and 2017 (21.26 km<sup>2</sup>). Ocuilan showed similarly high levels of deforestation in 2018 (63.75 km<sup>2</sup>) and 2017 (30.24 km<sup>2</sup>), with another spike in 2022 (28.56 km<sup>2</sup>).





**Figure 6.** Percentage of forest cover lost between 2001 and 2023 within the Sierra de las Cruces and the Chichinautzin Biological Corridor, as determined using data from Global Forest Watch, considering the municipalities in the region.

**Table 4.** Present and future suitability area (km<sup>2</sup>) and percentage of area loss of *Ambystoma altamirani* with two different global climate models and land-cover changes in the ANPS.

60%					10 percentile training presence				
2014 (km <sup>2</sup> )	2050 cn85 (km <sup>2</sup> )	Loss 2014–2050 (%)	2050 mp85 (km <sup>2</sup> )	Loss 2014–2050 (%)	2014 (km <sup>2</sup> )	2050 cn85 (km <sup>2</sup> )	Loss 2014–2050 (%)	2050 mp85 (km <sup>2</sup> )	Loss 2014–2050 (%)
930.88	810.50	12.93	883.25	5.12	2123.67	1951.48	8.11	2083.09	1.91

## Discussion

The findings of this study underscore the significant threats faced by *Ambystoma altamirani*, a critically endangered amphibian, whose survival depends on highly specific environmental conditions found in the TMVB (Ruiz-Reyes et al. 2024; Sunny et al. 2024). Habitat degradation, land-use changes, invasive species and climate change are all converging to increase the vulnerability of this microendemic species, reinforcing the need for urgent and targeted conservation actions. The results of the ecological niche models (ENMs) indicate that *Ambystoma altamirani* relies heavily on temperate forests dominated by *Abies* and *Pinus* species (Reyes-Olivares et al. 2024; Ruiz-Reyes et al. 2024; Sunny et

al. 2024), with the minimum temperature of the coldest month (Bio6) being the most important variable contributing to its distribution (51.8%). The fragmentation and degradation of these habitats, particularly in the Sierra de las Cruces region, are of particular concern (López-García and Navarro-Cerrillo 2021; Ruiz-Reyes et al. 2024). Both legal and illegal logging, along with agricultural expansion and urbanization, continue to erode the integrity of these forested areas, as confirmed by multiple studies (García 2011; López-García and Navarro-Cerrillo 2021; González-Fernández et al. 2022; Segarra et al. 2024).

It is also important to consider that these distribution models are predicting potential suitable areas for the species. However, even though some regions may appear to have the necessary environmental conditions, *A. altamirani* is not present in many of them. This suggests that the potential distributions predicted by the models might be overestimated. Consequently, *A. altamirani* could be facing even greater conservation pressures than what these models suggest, given that its actual range might be more restricted than the potential range indicates. This highlights the need for targeted conservation actions that focus on both habitat protection and understanding the species' real-world limitations within its predicted range. The loss of critical forest cover between 2011 and 2014 and continued deforestation since then have compounded the risk of extinction for *A. altamirani*, as these forests provide essential microhabitats that are becoming increasingly scarce (López-García and Navarro-Cerrillo 2021; González-Fernández et al. 2022). In particular, the high correlation between *Abies* forest cover and *A. altamirani*'s presence suggests that conservation efforts should prioritize mixed *Abies* forest along with aquatic habitats as streams (Ruiz-Reyes et al. 2024). Areas dominated exclusively by *Pinus* forests were found to be less suitable, underscoring the importance of maintaining diverse forest ecosystems to support viable populations (Jöks et al. 2023; Ruiz-Reyes et al. 2024). The trend of forest loss, particularly in the TMVB, jeopardises the future of this amphibian and highlights the urgent need for stricter enforcement of land-use regulations and sustainable forest management (García 2011; López-García and Navarro-Cerrillo 2021; Vargas-Jaimes et al. 2021; González-Fernández et al. 2022). While *A. altamirani* may have some capacity to adapt to changing environmental conditions (Sánchez-Sánchez et al. 2024), climate change poses a significant long-term challenge for the species. Our models predict a notable reduction in suitable habitat under future climate scenarios, with potential losses reaching up to 13.95% by 2050 (CN85 scenario). This habitat contraction will primarily affect areas that are already under pressure from human activities, such as the southern part of the Sierra de las Cruces and the Chichinautzin Biological Corridor (López-García and Navarro-Cerrillo 2021; Vargas-Jaimes et al. 2021). The observed patterns of forest cover loss in Tlalpan, Álvaro Obregón, Jalatlaco and Ocuilan between 2001 and 2023 are particularly concerning for *A. altamirani*, as our ecological niche models (ENMs) predicted significant reductions in the species' potential distribution in these areas. The most substantial deforestation events in 2018 and 2023 coincide with the predicted loss of suitable habitat, further exacerbating the conservation challenges for this critically endangered species. In particular, the deforestation in Tlalpan and Ocuilan, which reached 99.29 km<sup>2</sup> and 63.75 km<sup>2</sup>, respectively, in recent years, threatens critical forest habitats that *A. altamirani* relies on, especially

in regions where *Abies* and *Pinus* forests are essential for its survival. The increased land-use changes in these areas, coupled with climate change, are likely to fragment the already limited habitats further, isolating populations and increasing the risk of local extinctions.

These findings highlight the urgent need for targeted conservation actions to mitigate habitat loss and protect the remaining suitable environments for *A. altamirani*, especially in areas identified as high-risk through both deforestation trends and species distribution models. Likewise, these areas are known biodiversity hotspots and are critical for maintaining ecological connectivity, which is vital for the species' survival (Kirk et al. 2023; Qian et al. 2023; Zhou et al. 2023). The predicted loss of suitable habitat is particularly concerning given *A. altamirani*'s limited dispersal abilities (Ruiz-Reyes et al. 2024). The species is highly dependent on cold, oxygen-rich rivers and climate change is expected to reduce the availability of such aquatic habitats, further fragmenting populations. Despite the potential resilience of *A. altamirani* to rising aquatic temperatures observed under experimental conditions (Sánchez-Sánchez et al. 2024), as temperatures rise and precipitation patterns shift, the availability of cold-water streams could decline, leaving the species isolated in smaller, fragmented patches of suitable habitat (Johnson et al. 2024; Lamouille-Hébert et al. 2024). This isolation could hinder genetic exchange between populations, increasing the risk of local extinctions (Sunny et al. 2022). Mitigating the impacts of climate change will require a multifaceted approach that includes habitat restoration, the creation of ecological corridors to facilitate movement between suitable habitats (Sunny et al. 2022) and the protection of areas that are expected to remain suitable under future climate scenarios like the northern part of the Sierra de las Cruces, the Nevado the Toluca Volcano and the Sierra Chincua. The use of future bioclimatic variables in our models provided valuable insights into how the distribution of *A. altamirani* may shift, allowing conservationists to prioritize areas that will remain critical for the species' survival in the coming decades. In addition to habitat degradation and climate change, *A. altamirani* faces significant threats from invasive species, particularly *Oncorhynchus mykiss* (Estrella-Zamora et al. 2018; Zamora et al. 2018; Guerrero-de La Paz et al. 2020; Sunny et al. 2024). The expansion of trout farming, especially in the Sierra de las Cruces region, has further degraded critical habitats (Zambrano et al. 2010; Estrella-Zamora et al. 2018; Guerrero-de La Paz et al. 2020; Sunny et al. 2024). Furthermore, it is essential to investigate how *O. mykiss* may affect *A. altamirani* populations in other areas where the species is present. Additionally, the establishment of trout farms, particularly in municipalities like Isidro Fabela, has led to the degradation of riverine habitats, reducing the availability of suitable breeding sites for *A. altamirani*. Moreover, agricultural expansion in the TMVB exacerbates the pollution of aquatic ecosystems (Häder et al. 2020; Mushtaq et al. 2020). The widespread use of agrochemicals contaminates the rivers and streams where *A. altamirani* resides, further reducing habitat quality and increasing the species' susceptibility to diseases and environmental stressors (Suárez et al. 2021; Zambrano-Fernández et al. 2022; Paetow et al. 2023). Sustainable agricultural practices and stricter regulations on agrochemical use are essential to improving water quality and reducing the pressures on amphibian populations (Suárez et al. 2021; Zambrano-Fernández et al. 2022; Paetow et al. 2023).

## Conservation implications

Given the complex combination of threats facing *A. altamirani*, conservation strategies must adopt an integrated approach that addresses habitat loss, invasive species management and climate resilience. Protecting and restoring temperate forests, particularly those with a high proportion of *Abies* cover, is critical for maintaining viable populations (Sunny et al. 2018; González-Fernández et al. 2019). Special attention should be given to the Sierra de las Cruces and surrounding areas, which are home to the most vulnerable populations of *A. altamirani*. In addition, the regulation of trout farming and the removal of invasive *O. mykiss* from key habitats could alleviate some of the pressures on native amphibian populations. This, combined with habitat restoration and the implementation of ecological corridors, could help mitigate the effects of habitat fragmentation. Finally, addressing climate change through landscape-level conservation planning is essential for the long-term survival of *A. altamirani*. Identifying areas that are likely to remain suitable under future climate conditions and ensuring that these areas are adequately protected within natural protected areas (NPAs) will be critical for building climate resilience into conservation strategies. The overlap between suitable habitats and NPAs, as evaluated in this study, provides a roadmap for prioritizing areas for conservation and restoration efforts. Restoring degraded ecosystems are essential to mitigate the negative impacts on *A. altamirani*. Given the species' narrow habitat range and limited dispersal abilities, maintaining habitat connectivity and protecting the species' remaining habitats are critical for preventing its extinction.

## Conclusion

*Ambystoma altamirani* is at a critical juncture, facing a confluence of threats from habitat loss, invasive species, pollution and climate change. Immediate action is required to protect its remaining habitats and ensure the long-term survival of the species. Conservation efforts must focus on habitat protection, sustainable land-use practices, invasive species management and climate adaptation strategies. By taking a holistic approach, we can help safeguard this unique species and the valuable ecosystem services it provides for future generations.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

## Ethical statement

No ethical statement was reported.


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## Author contributions

All authors contributed to the study conception and design, material preparation, data collection and analysis. The first draft of the manuscript was written by Armando Sunny and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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## Data availability

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

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