

Reencounter with the past: occurrence of sei whale (Balaenoptera borealis) in an old hunting area in the south-eastern Pacific Ocean

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Abstract

The sei whale (*Balaenoptera borealis*) was intensively exploited throughout its range, with about 110.000 individuals hunted by pelagic fleets in Antarctic waters between 1960 and 1970. In addition, basic information on its distribution, migratory routes, and feeding grounds in the southeastern Pacific, has been poorly documented. In the case of Chile, recent information consists mainly of accidental records. This research presents the first sei whale photo-identification catalog for south-central Chile. From November 2019 to January 2020, 88 individuals were recorded from land-based and boat surveys at Caleta Chome. Of these, 12 individuals were photo-identified through scars or distinctive notches in the dorsal fins. The peak of sightings occurred during December 2019; two individuals were sighted on more than one occasion.

Keywords

Balaenoptera borealis, Caleta Chome, Chile, Sei whale, South-eastern Pacific

Introduction

The sei whale (*Balaenoptera borealis*) is an endangered mysticete (Cooke 2018) and the third largest whale after the blue whale (*Balaenoptera musculus*) and the fin whale (*Balaenoptera physalus*; Horwood 2018). This species presents a cosmopolitan distribution and pelagic with temperatures below 20 °C (Omura and Nemoto 1955; Gambell 1968, 1985). It migrates to the southern hemisphere during summer, from areas near the subtropical convergence where it reproduces, to areas near the Antarctic convergence (50°S–60°S) for feeding (Horwood 1987; Reeves et al. 1998; Rice 1998). Feeding zones are unpredictable, with a sudden influx into an area, followed by their disappearance and subsequent absence for years (Gambell 1985; Reeves et al. 2002; Jefferson et al. 2008). During the summer there are high concentrations of sei whales between 40° and 50°S with adult individuals reaching polar waters while juveniles or sub-adults stay north of the Antarctic convergence (Lockyer 1977; Acevedo et al. 2017). Six populations have been assumed for the southern hemisphere for management purposes; however, the scarce evidence has failed to identify separate populations within ocean basins (Kanda et al. 2006; Horwood 2018).

The International Whaling Commission estimated that by the 1940s the population declined from 191.000 to 37.000 individuals after the cessation of commercial catches in 1983 (Gambell 1985). Although the sei whale was not a target species for hunting until the early 1960s (Acevedo et al. 2017; Español-Jiménez et al. 2019) the decrease in the most profitable whales (B. musculus, B. physalus, Megaptera novaeangliae and Eubalaena australis) led to an increase in the hunting effort for this species. In South America, this species was heavily exploited throughout its range (Zerbini et al. 1997; Aguayo-Lobo et al. 1998a) where about 110.000 individuals were hunted by pelagic fleets in Antarctic waters between 1960 and 1970 (Horwood 2018). In Chile, it was the third most hunted whale species between 1929 and 1979 with at least 1,664 individuals captured (Aguayo 1974); however, due to the difficulty of differentiating from Bryde's whale (Balaenoptera edeni) during this whaling period, its hunting numbers are probably overestimated since many Bryde's whales would have been reported as sei whales (Valdivia et al. 1981; Gallardo et al. 1983; Aguayo-Lobo et al. 1998a). There were about 25 years (between 1974 and 1999) in which there was no research on sei whales, and the few studies that were conducted during this period did not consider the sei whale as an object of study (Gallardo and Pastene 1983; Gallardo et al. 1983; Guerra-Correa et al. 1987; Aguayo-Lobo et al. 1998b). Since the moratorium on whaling established in 1983, there has been a considerable reduction in sei whale research (Reeves et al. 2002); currently this species is one of the least known baleen whales in the world (Prieto et al. 2012; Acevedo et al. 2017; Horwood 2018).

Caleta Chome was founded by the Macaya Hnos. whaling industry on 1948 (Quiroz and Carreño 2019). By 1954, the sei whale was already within the productivity of the whaling plant in Caleta Chome and was the first documented record of sei whales for this region. The sei whale catch data for this area were for a long time under the name "S + B", since they considered the bryde (B) and sei (S) whales together due to their similarities, therefore there are no clear records of the number of individuals of sei whales caught in this area (Pastene 1982). In Caleta Chome, between 1951 and 1983, active whaling was carried out by the Trinidad Whaler owned by the Macaya Family (Quiroz and Carreño 2019). Given that the sei whale is endangered, studies of its populations are crucial to support its conservation. This study presents the first sei whale photo-identification catalog in south-central Chile and information on sightings.

Materials and methods

Study area

The sightings were in Caleta Chome in the Biobío region of Chile (36°40'S, 73°15'W; Fig. 1). Waters rich in nutrients from the Humboldt Current fertilize the coasts of this region in the spring and summer season when the winds are favorable to coastal upwelling (Sobarzo et al. 2007; Simpkins 2018), generating an increase in primary productivity and higher trophic levels (Thiel et al. 2007; Escribano et al. 2012; Anabalón et al. 2016). South of Caleta Chome, the freshwater discharge from the Biobío River provides nutrients, organic matter, and terrigenous particles to the adjacent coastal area, so positively influencing phytoplankton biomass and primary production (Masotti et al. 2018). Bathymetric accidents such as the Biobío canyon (Sobarzo et al. 2016) and an irregular coastline (Figueroa and Moffat 2000) are essential factors in the coastal dynamics of the area.

Sighting and data base

The sighting records were collected between November 2019 and January 2020 two days per week for a total of 25 days (41 hours and 15 min) of monitoring. The surveys (search of whales) were conducted during the morning between 08:00–12:00 AM (14 surveys) and afternoon between 06:00-09:00 PM (11 surveys). The sightings were made from a) land from a hill of 50–55 m of height using 10×42 binoculars and spotting scopes $15-45 \times 65$ (16 surveys); and b) aboard the boat El Felipe I (7.8 m in length) that periodically sails in Caleta Chome (9 surveys). The number of observers varied from 2 to 4. From the boat, the sightings were at a distance of no greater than 20 m and the identification of the species was carried out with photographic records using a Canon EOS77D camera with 100-400 mm zoom lens, and Canon SX530 semi-professional camera. The morphology of the dorsal fin, characterized by a prominent falcate fin that rises at a steep angle from the rear and central ridge along the head, allowed species identification (Acevedo et al. 2017). The individuals were individualized based on photographs by identifying the distinctive scars, notches, or holes in the dorsal fin (Würsig and Jefferson 1990). Only images of medium to high quality (> 4608 × 3456 pixels) that allowed to highlight their distinctive characteristics were used.

Daily Sea Surface Temperature (SST) between November 2019 and January 2020 data were obtained from Multi-Scale Ultra High Resolution (MUR, https://podaac.jpl.nasa. gov/dataset/MUR-JPL-L4-GLOB-v4.1) with a spatial resolution of 1 km². The SST for each sei whale sighting at sea was obtained from the near pixel to the sighting coordinate.



Figure 1. Study area **B** G.A: Arauco gulf, SMI: Santa Maria Island, Llv: Punta Lavapié, Bbc: Biobío canyon, Bbr: Biobío River and Ccp: Concepción **C** Localization of sightings in Caleta Chome. The red point shows the position of the sightings in the sea. The associated sampling number is indicated on each point (see Table 1). The black point shows the position of Caleta Chome, where sightings from land were made. The color palette in **A** and **C** panel shows the depth in meters. The bathymetric information for the area was obtained from the General Bathymetric Chart of the Ocean (GEBCO, GEBCO – The General Bathymetric Chart of the Oceans).

Data analysis

To have a better visualization of the results of the sightings, a detection index (Di) was established which was calculated based on the sum of the sightings made during intervals of 10 days (i.e., maximum interval between monitoring) and the effective sampling effort within that interval of days:

Di = sightings during 10 days / sampling effort for 10 days

Results

Between November 3 and January 18, we made 88 sei whale sightings. The number of sightings varied from November to January, registering a peak of sightings on December 11 and 20 (Fig. 2). We identified 17 groups of sei whale of 2 to 9 individuals (median = 4); most of these groups were registered during the month of December (10 groups). The SST range from 12.7 °C to 15.1 °C during the sightings, and the estimated depths of the sightings range from 16 to 137 m. On the other hand, sei whale sightings ware made at mean distance of 3.6 km from the coast. We highlight one event, where the distance was around 0.1 km. (Table 1).

Twelve individuals were photo-identified through distinctive scars or notches on the dorsal fins (Fig. 3), 83% of the individuals have some notch and one of them has a broken fin (# 002) and another two had lacerations (# 007 and # 009). Most individuals (see photographic sequence from # 009 to # 012 in Fig. 3) were photographed in December 2019. Two individuals (# 003 and # 009) were sighted more than one day in the area, individual # 003 was sighted 7 days after the first sighting and individual # 009 on the seventh and tenth day after the first sighting. The presence of a mother with a calf was registered for 5 different days.



Figure 2. Frequency in the detection index of the *Balaenoptera borealis* sighted during November and December 2019 and January 2020. The detection rate was calculated based on the effective sightings per day for 10 days standardized by the sampling effort. The numbers above and within each bar indicate the number of total sightings and the sampling effort in each date range, respectively.

Table 1. Summary of sightings of sei whales (*Balaenoptera borealis*) in Caleta Chome during the seasons from November 2019 to January 2020. Note: the number of individuals with * represents those sightings in which mothers with young were recorded.

No.	Date (d/m/y)	GPS Coordinates	Number of	Sighting place	Distance from	SST (°C)	Depth (m)
		(Latitude, Longitude)	individuals	shore (km)			
1	03-11-2019	_	1	Coast	-	-	_
2	06-11-2019	-	3*	Coast	-	_	_
3	09-11-2019	36°48'10.64"S, 73°13'45.58"W	1	Boat	4.4	12.9	113
4	17-11-2019	36°47'18.55"S, 73°14'54.26"W	4*	Boat	5.6	15.1	137
5	18-11-2019	_	5	Coast	-	-	_
6	28-11-2019	_	6	Coast	-	-	_
7	29-11-2019	-	4*	Coast	-	_	_
8	30-11-2019	_	4	Coast	-	-	_
9	01-12-2019	36°46'17.00"S, 73°13'40.75"W	6	Boat	1.6	13.5	122
10	02-12-2019	-	1	Coast	-	_	_
11	05-12-2019	_	3	Coast	_	-	_
12	06-12-2019	_	2	Coast	_	-	_
13	07-12-2019	36°48'35.88"S, 73°13'30.39"W	8	Boat	4.6	12.7	103
14	08-12-2019	36°49'17.70"S, 73°13'7.15"W	4	Boat	5.4	12.8	122
15	13-12-2019	36°46'44.59"S, 73°13'41.02"W	9*	Boat	1.1	13.3	105
16	14-12-2019	36°45'40.66"S, 73°13'18.85"W	8	Boat	2.4	12.9	116
17	22-12-2019	_	3	Coast	_	-	_
18	23-12-2019	_	1	Coast	_	-	_
19	30-12-2019	_	4*	Coast	_	-	_
20	31-12-2019	_	4	Coast	_	-	_
21	06-01-2020	_	1	Coast	_	-	_
22	08-01-2020	_	1	Coast	_	-	_
23	09-01-2020	_	2	Coast	_	-	_
24	10-01-2020	_	1	Coast	_	-	-
25	13-01-2020	_	1	Coast	_	-	-
26	18-01-2020	36°46'40.98"S, 73°12'54.39"W	1	Boat	0.1	12.7	16



Figure 3. Dorsal fin photographs of 12 identified sei whales (*Balaenoptera borealis*) at Caleta Chome, during the seasons from November 2019 to January 2020.

Discussion

With the moratorium established by the International Whaling Commission (IWC) in 1983, the cetacean records associated with hunting decreased and the only records of the sei whale for central Chile were only three reports and one scientific publication (Pastene 1982; Gallardo and Pastene 1983; Gallardo et al. 1983; Aguayo-Lobo et al. 1998a). Although there are records of sightings of this species in previous years (F. Silva obs. pers.), a high abundance of individuals was not recorded in this area and neither was the presence of mothers with young.

The sei whale is described as predominantly found in deep waters, occupying mainly pelagic habitats at distances greater than 110 km from the coast (Best and Lockyer 2002; Prieto et al. 2012); however, many of our sightings occurred near the coast at distances no greater than 6 km (see Table 1) consistent with what was observed for populations of sei whales in the South Atlantic (Weir et al. 2020). This greater abundance of sei whales near the coast and the feeding activity during the day, agree with the results obtained by Español-Jiménez et al. (2019) on the coast of the Gulf of Penas and Tres Montes. Another data to highlight is the sightings of the same individual on more than one occasion; this suggests a stay of at least 7–10 days. In addition, the presence of mothers with young during the observation season may be giving signs of safety and good environmental conditions for rearing. More than 50% of the identified individuals had scars on their fins. Among the possible natural threats that could have damaged its dorsal fin, is predation by killer whales (*Orcinus orca*) which are considered its only significant natural predator (Jefferson et al. 1991; Springer et al. 2006).

The occurrence of sei whales in the coastal upwelling system of Chile was reported by Gallardo and Pastene (1983), who associated high primary productivity with the concentration of sightings, proposing that the coasts of the Biobío region can be feeding areas for some cetaceans during their migrations. The continental shelf off Biobío is an important upwelling zone and has been described as one of the most productive areas within the Humboldt Current System (Montecino et al. 1998; Montero et al. 2007; Thiel et al. 2007; Daneri et al. 2012; Iriarte et al. 2012). Bathymetric features such as underwater canyons generate foraging areas for a variety of cetaceans, contributing to primary productivity and biodiversity (Moors-Murphy 2014). Croll et al. (2005) mention that bathymetric ruptures and the coastal upwelling process are important factors in the density of euphausiids, contributing to the formation of feeding areas. The high occurrence of individuals in Caleta Chome could be related to a migratory route close to the Biobío Canyon located to the south of the study area.

The oceanographic conditions in the distribution of this species are variable in relation to the migratory routes and the permanence in feeding and / or reproduction sites (Omura and Nemoto 1955; Gregr and Trites 2001; Sasaki et al. 2013; Murase et al. 2014). On the coasts of Chile, the presence of sei whales has been reported in areas with sea surface temperatures of 14.5 °C in spring (Clarke et al. 1978). In the Magellan Strait Acevedo et al. (2017) reported a thermal range between 5.7 and 10.9 °C. Our sightings are also within this temperature range, agreeing with the values reported for this species in other parts of the world (Sasaki et al. 2013).

Acevedo et al. (2017) reported sei whales in the Magellan Strait from November to May, with a peak of sightings (83.6%) occurring during December and January. They suggest that although there are records that support the hypothesis that whales forage in southern Chile, none of them provide information on systematic annual occurrences in these same areas. But this, in turn, can be supported by the fact that sei whales are known for their unpredictable presence in an area followed by their subsequent disappearance, as well as having a greater variation in distribution in their feeding grounds than most species of baleen whales (Tønnessen and Johnsen 1982; Horwood 1987). All these records that support the presence of the sei whale on Chilean coasts are at the same time of the year (January-May) within relatively small latitudes and, although they are not in consecutive years, there is a certain periodicity that could support the hypothesis that the sei whale forages in Chilean waters but their foraging areas change over the years (Pastene and Shimada 1999; Aguayo-Lobo et al. 2006; Guzmán 2006; Acevedo et al 2017; Español-Jiménez et al. 2019) This may be due to an underestimation of the presence of this species in Chile, since there is no systematic monitoring in all the areas that it has been recorded. In this work, the occurrence of the sei whale off the Chilean coast shows the existence of a passage zone and possible feeding area within the waters near the coast. Ecological knowledge about sei whales along the Chilean coast is scarce, therefore we highlight the importance of increasing sighting efforts around the Hualpén Peninsula Nature Sanctuary during upwelling events to understand if the presence of sei whales follows some seasonal pattern or corresponds to sporadic events.

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RESEARCH ARTICLE



Important plant areas (IPAs) in the Fergana Valley (Central Asia): The Bozbu-Too-Ungortepa massif

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Abstract

This paper discusses identifying Important Plant Areas (IPAs) in one of the most densely populated regions of Central Asia—the Fergana valley. The recognition of IPA sites is an attempt to introduce new ways of conserving local plant diversity with a high concentration of endemic species in Central Asia, where conservation methods of the former Soviet Union still prevail. The research revealed the current state and geography of many rare species and enriched the flora of Uzbekistan and Kyrgyzstan with several rare species. The second IPA is the transboundary territory of the Fergana valley, uniting the southern spurs of the Chatkal range and the Ungortepa-BozbuToo massif. We documented the distribution of 62 species in the IPAs under the sub-criteria of Plantlife International. Our study aimed at continuing studies on the IPAs in this region, addressing specific conservation challenges, such as conserving national endemics and endangered species that grow outside protected areas and GIS mapping of endemic species.

Keywords

Central Asia, endemic plants, Fergana Valley, important plant areas

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Introduction

Biodiversity hotspots represent regions with high species richness and centers of endemic species and are generally considered priority conservation regions. They provide a good natural laboratory for conducting studies testing the relationship between extinction risk and evolutionary history (Fu et al. 2022). As a rule, most biodiversity hotspots are covered by a network of protected areas. Globally, protected areas have been increasing significantly over the last few decades. However, the existing global network covers less than 20% of areas important for biodiversity and ecosystem services (Maxwell et al. 2022). It does not offer a sufficient contribution to the representativeness of these areas (Signorello et al. 2018). In many ways, representativeness is not achieved in poorly studied regions of the world.

The Mountains of Central Asia are an important global biodiversity hotspot (Myers et al. 2000) but a botanically poorly-known part of Asia (Manafzadeh et al. 2016; Li et al. 2020). The situation is even worse for the individual regions because dozens of botanical-geographic regions do not have flora lists pertaining to 50–60 years ago. Poor representation is a common problem for Central Asian species, with data often lacking (Wilson et al. 2021). However, many taxonomy, geobotany, and plant ecology studies were carried out (Tojibaev et al. 2020). One such region is the Fergana valley (FV)—the vast depression between the Tian-Shan and Pamir-Alay Mountain systems, mainly in eastern Uzbekistan and partly in Tajikistan and Kyrgyzstan.

In the previous stages of the study, we attempted to identify important plant areas (IPAs) in the FV, one of the most densely human-populated regions in Central Asia with a diverse array of endemic and endangered species. The badlands of the Chust-Pap adyrs (foothills) with a high concentration of endemic species of the western Tian-Shan were chosen as the first IPA of the FV. An overview of international references on IPA research in various countries was given in the first phase of IPA research (Tojibaev et al. 2022).

Identifying species diversity and distribution is key to the protection and prevention of biodiversity loss at both global and local scales, because robust data are paramount for well-informed decisions on policy, conservation, and species management (Dani Sanchez et al. 2021). IPAs consider species distribution and botanical richness and prioritize plants and habitats that are under threat, by identifying a network of key sites for their conservation (https://www.plantlife.org.uk/international). The guidelines developed by Plantlife for Europe (Anderson 2002) have been tested and implemented in many countries over the past two decades (https://www.plantlifeipa.org/ about). Based on this, each new IPA study should comply with accepted standards and subsequently be included in the Plantlife IPA database (www.plantlifeipa.org/home).

This work continues the identification of the IPA sites in FV, Central Asia. The second IPA site in the FV is located between Kyrgyzstan and Uzbekistan, in the southern part of the Chatkal Range. Two areas are clearly distinguished here. These are (a) the Bozbu-Too mountains (Kyrgyzstan) and (b) the lower reaches of the Mailisay river basin and the Ungortepa massif (Kyrgyzstan and Uzbekistan). Based on this, the second IPA site in FV has been named the Bozbu-Too-Ungortepa massif. According to the administrative zoning, the study area is located in the Chartak district of the Namangan region of Uzbekistan and the Aksy district of the Jalal-Abad region of Kyrgyzstan. Phytogeographically, the IPA site belongs to the South Chatkal district of the Fergana region, Mountainous Central Asian province (Tojibaev et al. 2016, 2017). The core flora of the area, mainly consisting of Mediterranean and Iran-Turanian elements, is heavily influenced by widely distributed species. The South Chatkal district is the southern habitat for some Tian-Shan and western Tian-Shan species, including *Tulipa bifloriformis* Vved., *Fritillaria sewerzowii* Regel, *Ferula tenuisecta* Korovin, and *Ferula prangifolia* Korovin.

The Bozbu-Too-Ungortepa massif and the adjacent Mailisay river basin of the Chatkal Range are poorly studied areas of the FV. The first detailed field studies were carried out in the middle of the last century (1949–1953) when the Institute of Botany of the Academy of Sciences of the Republic of Uzbekistan organized expeditions to study industrial reserves of rubber plants under the local name *tau-sagyz*. In 1949, during a route-geobotanical survey of the FV, large thickets of a rubber-bearing taxonomically unknown representative of the genus *Scorzonera* L. were found (Nabiev 1954). In 1950, this species was described as *Scorzonera uzbekistanica* Czevr. & Bondarenko (Chevrenidi and Bondarenko 1950).

A national approach to global conservation priorities

In Central Asia countries, the realization of IPAs programs, including the identification process, data accumulation, and GIS mapping of species across IPA criteria, is in dynamic development (Tojibaev et al. 2022). In many ways, all countries in Central Asia inherited the Soviet (in some places modernized) system of nature conservation practice. National biodiversity strategies and protected area management are very similar and the main approaches to the development of plants conservation strategies and their implementation remain. The first attempts to identify such IPA sites have been made (Dimeyeva and Vesselova 2015; Tojibaev et al. 2022). The results of the first phase of IPA research created a solid methodological basis. It is already becoming clear that the national IPA programs make it possible to use a pragmatic and proven approach to national plant conservation in local conservation practice. This program can complement other initiatives such as national biodiversity strategies and protected area management. Most importantly, regional data from Central Asia can be integrated into the global IPA network.

Materials and methods

Study area

The FV is an intermountain trough covering an area of about 22 000 km². Including the surrounding mountains, it covers up to 80 000 km² and is located

in Uzbekistan, Kyrgyzstan, and Tajikistan (Tojibaev et al. 2018a). The length of the valley is about 250–300 km. The width of the FV reaches 130 km and narrows to 5–10 km in the west. In the east, the valley is bordered by the Fergana Range (Kyrgyzstan), by the Alay (Kyrgyzstan and Uzbekistan) and Turkestan ranges (Kyrgyzstan, Tajikistan, and Uzbekistan) in the south, and the Chatkal (Kyrgyzstan and Uzbekistan) and Kurama ranges (Tajikistan and Uzbekistan) in the north. Quaternary deposits (pebble, crushed stone, sand, loam, sandy loam, and clay) compose the plains and fill intermontane, post-, and inter-adyr depressions. The surface of the FV, especially in its central part, is flat and represents the ancient terraces of the Syr Darya River (up to four terraces) and numerous alluvial cones (Naumenko and Zubov 2021).

The botanical significance of Central Asia in terms of global plant diversity and the use of this phrase concerning a specific geographical area were discussed in a previous publication (Tojibaev et al. 2022).

The study area is north of the Syr Darya River on the southern slopes of Chatkal Range. Tashkumyr town (Kyrgyzstan) is the eastern, and Kerben town (Kyrgyzstan) the western border. The southern border runs along the meridian of Yangikurgan and Uchkurgan towns (Uzbekistan), and further north along Avletim (Kyrgyzstan). The IPA site was located between 41°25'9.44"N, 71°41'31.76"E and 41°27'23.85"N, 72°10'53.88"E, with an altitude range from 550 to 2860 m above sea level. The site is 48–56 km long and 30–42 km wide (Fig. 1).



Figure 1. Study area. The Bozbu-Too-Ungortepa massif.

Climate and geomorphology

The climate of the IPA site and its surroundings is dry and warm. As no precise climatic data are available for this IPA site, extrapolations have been made based upon the average data for Namangan weather stations and using the environmental lapse rate (6 °C/1000 m). The annual rainfall in the lower zone (500–800 m) varies from 170–280 mm, and from 350–400 mm in the upper zone (1200–1500 m). The average temperature is 2–5 °C in January, and 27–28 °C and 7–10 °C in the upper part in July. The average annual temperature is 13.2 °C, and the lowest is 4 °C. The absolute minimum fluctuates around -20 °C. Rainfall occurs in spring and autumn-winter. There are two contrasting periods: spring (until the end of May) with green vegetation and summer when most of the vegetation burns out and the vegetation is a shade of yellow (Nabiev 1954).

The study area occupies two lower geomorphological stages—the foothills and the low mountains of South Chatkal. The variegated geological structure of the surface and the intense runoff characteristic of the region, causing erosion of slopes, have a significant effect on soil formation and the soil cover structure. For this reason, the predominant part of the site is devoid of a characteristic soil cover due to the vigorous erosion of the surface. This is one of the main factors affecting the formation of vegetation cover (Nabiev 1954).

Data collection

In the Uzbekistan part, the first field studies to identify the IPA were in 2013–2014 by Tojibaev's team but were stopped due to lack of funding. In Kyrgyzstan, the first field studies of the authors (G.A. Lazkov) began in the 1990s. However, targeted research under the IPA program was carried out only in 2020–2022. From the IPA surveys both in Kyrgyzstan and Uzbekistan, Lazkov's and Tojibaev's team field records, literature data from Nabiev (1954), Vernik and Rakhimova (1982), and herbarium collections data (mainly in TASH, FRU, LE, MW; Thiers 2022), a total of 1275 vascular plant species have been recorded in the IPA site.

Taxonomy

Taxonomic identification was based on Flora of Uzbekistan (1941–1963), Flora of Kyrgyzstan (1952–1965), Conspectus Florae Asiae Media (1969–1993), a taxonomic revision of some families of the flora of Kyrgyzstan (Pimenov and Kljuykov 2002; Lazkov 2006a), the newly published first four volumes of the new Flora of Uzbekistan (Sennikov 2016, 2017, 2019, 2022), the recently published data on Apiaceae (To-jibaev et al. 2020), *Tulipa* (Tojibaev and Beshko 2014), and the checklist of the Flora of Tian-Shan mountains (Tojibaev et al. 2020). The nomenclature of each taxon followed Plants of the World Online (POWO) http://www.plantsoftheworldonline.org), and International Plant Name Index (IPNI) www.ipni.org).

Site selection

Species and habitats were selected according to the proposed IPA criteria. The IPA site selection methodology fits the European IPA criteria set out in the IPA site selection manual and their modifications published in authoritative publications (Anderson 2002; Blasi et al. 2011; Darbyshire et al. 2017), the main of which are:

1. The IPA site satisfies either criterion A, B, C, or any combination of these.

2. Previously tested approaches in the conditions of mountainous Central Asia were applied, which makes it possible to ensure the creation of a regional network.

3. The IPA site contains those conditions necessary to ensure the long-term viability of the species or intraspecific taxa. Also, the selected IPA site contains multiple qualifying species to focus conservation action at the national and transboundary levels.

We used only selected vascular plants and habitats and existing data on their distribution on a national and regional scale. This is because, as in Kyrgyzstan, there is currently no classification of habitats, especially critical habitats, in Uzbekistan. Moreover, the studied area is typical for the south of mountainous Central Asia.

The boundaries of the Bozbu-Too-Ungortepa IPA site are broadly in line with the regional assessment of KBAs (Key Biodiversity Areas) in Central Asia (CEPF 2017). Biodiversity hotspots N°11 (Karatag Mountains), N°12 (Ungortepa), and N°05 of the contours (Aflatun-Padshaata) from the final report "Biodiversity hotspots in the mountainous region of Central Asia" are included in the Bozbu-Too-Ungortepa IPA.

Existing conservation threats were estimated based on field observations and analysis of the available literature data (Davletkeldiev 2006; Khassanov 2019; Eastwood et al. 2009).

Results

Criterion A (threatened species)

According to the generally accepted formulation, IPA sites with criterion A should hold significant populations of one or more species of global or regional conservation concern (Anderson 2002). After modifying IPA methodology as proposed by the authors in the previous stages of the IPA research in Central Asia (Tojibaev et al. 2022), 62 vascular plant species were recognized as belonging to criterion A. Following the national requirements, we also listed national and regional species of interest (Table 1). **Table 1.** The list of selected species including the criterion A of the Bozbu-Too-Ungortepa massif with IPA sub-criteria, IUCN Conservation status, general distribution and preserved herbaria.

No	Accepted species name	IPA	Conservati	servation status (IUCN)		Red Data	Preserved Herbaria	
		category	$\frac{1}{1000 \text{ km}^2} = \frac{1}{1000 \text{ km}^2}$		Category	Book		
1	Malus sieversii (Ledeb.) M.Roem.	Ai	Not evaluated		VU	KG	TASH, FRU, TAD,	
			The evaluated		A2acde		MW, AA	
2	Acanthophyllum pungens (Bunge) Boiss.	Aii	1,685,141.644	248	LC+EN	UZ	TASH, FRU, TAD,	
	19 1 5 . 0.						MW, AA	
3	Allium dodecadontum Vved.	Aii	19,569.468	92	VU+EN	KG	TASH, FRU	
4	Allium viridiflorum Pobed.	Aii	133.707	16	EN	UZ	TASH, FRU	
5	Anthochlamys tjanschanica Iljin ex Aellen	Aii	21,576.049	136	NT+EN	UZ	TASH, FRU, MW	
6	Crataegus knorringiana Pojark.	Aii	19,859.660	52	EN	KG	TASH, FRU	
7	Delphinium knorringianum B.Fedtsch.	Aii	23,231.587	24	NT+EN	KG, UZ	TASH, FRU	
8	Dorema microcarpum Korovin	Aii	21,800.193	46	NT+EN	KG, UZ	TASH, FRU, MW	
9	Eminium regelii Vved.	Aii	95,605.497	80	LC+EN	KG	TASH, FRU, TAD,	
							MW, AA	
10	Gamanthus ferganicus Iljin	Aii	15,513.182	136	VU+EN	UZ	TASH, FRU, MW	
11	Iris kolpakowskiana Regel	Aii	126,385.582	96	LC+EN	KG	TASH, FRU, MW, AA	
12	Hedlundia persica (Hedl.) Mezhenskyj	Aii	851,107.371	144	LC+EN	KG	TASH, FRU, TAD,	
							MW, AA	
13	Lamyropappus schakaptaricus (B. Fedtsch.)	Aii	4,166.438	108	EN	KG	TASH, FRU, MW	
	Knorring & Tamamsch.							
14	<i>Oreosalsola drobovii</i> (Botsch.) Akhani	Aii	34,390.040	80	NT+EN	TJ, UZ	TASH, FRU, TAD,	
							MW, AA,	
15	Seseli eryngioides (Korovin) Pimenov et	Aii	1,190.623	20	EN	KG	FRU, MW	
	V.N.Tikhom.							
16	<i>Tulipa ferganica</i> Vved	Aii	36,709.666	156	NT+EN	UZ	TASH, FRU, MW	
17	Allium tatyanae F.O.Khass. & F.Karimov	Aiii	13,360	8	CR	-	TASH	
18	Astragalus allotricholobus Nabiev	Aiii	316,899	24	EN	-	TASH, FRU, MW	
19	Corydalis bosbutooënsis Lazkov	Aiii	3,040	8	CR	-	TASH, FRU	
20	Gagea spelaea Levichev et Lazkov	Aiii	3,786	8	CR	-	FRU	
21	<i>Iris austrotschatkalica</i> Tojibaev, F. Karim.	Aiii	0	8	CR	-	TASH	
	et lurgunov			(0				
22	Acantholimon nabievii Lincz.	Aiii	2,039.723	48	EN	-	TASH, FRU, MW	
23	Allium arkitense R.M. Fritsch	Aiii	1 124,205	20	EN	-	TASH, FRU	
24	Allium gracillimum Vved	Aiii	9 463,085	28	VU+EN	-	TASH, FRU, TAD MW	
25	Allium haneltii F.O. Khass. & R.M. Fritsch	Am	865,233	36	EN	-	IASH	
26	Allium oreoscordum Vved.	Am	19 763,085	36	VU+EN	-	IASH, FRU, MW	
27	Allium pseudopskemense ined.	Aiii	2523	16	EN	-	FRU	
28	Astragalus bosbutooensis Nikitina et Sudn.	Am	356,940	20	EN	-	IASH, FRU	
29	Astragalus melanocomus Popov	Am	827,190	12	EN	-	LE, MW	
30	Astragalus pseudodianthus Nabiev	Aiii	1169,459	32	EN	-	TASH, FRU	
31	Astragalus spryginii Popov	Am	4442,536	20	EN	-	IASH, FRU, MW	
32	Cousinia knorringiae Bornmuller	Aiii	775,616	32	EN	-	TASH, FRU	
33	<i>Cousinia krauseana</i> Regel & Schmalh.	Aiii	37 072,599	36	NT+EN	-	TASH, FRU, MW	
34	Echinops knorringianus Iljin	Aiii	8,933.847	40	VU+EN	-	TASH, FRU, MW	
35	<i>Eremurus czatkalicus</i> Lazkov	Aiii	3,153,563	32	EN	-	TASH, FRU, MW	
36	Ferula incisoserrata Pimenov et J. Baranova	Aiii	749,070	16	EN	-	TASH, FRU, MW	
37	Fritillaria rugillosa Naumenko & Zubov	Aiii	4,885,728	32	EN	-	TASH, FRU	
38	Hedysarum gypsaceum Korotkova	Aiii	1,956.487	28	EN	-	TASH, FRU, MW	
39 ,	Hedysarum turkestanicum Regel et Schmalh.	Aiii	15 611,335	32	VU+EN	-	TASH, FRU, MW	
40	Hyalolaena intermedia Pimenov & Kljuykov	Aiii	2,969,10	24	EN	-	TASH, FRU	
41	Iris narynensis O. Fedtsch.	Aiii	2 735,900	28	EN	-	TASH, FRU, MW	
42	Limonium ferganense IIkonnGal.	Aiii	5 203,680	12	VU+EN	-	TASH, FRU, MW	
43	Oxytropis gymnogyne Bunge	Aiii	14,138.749	120	VU+EN	-	TASH, FRU, TAD MW	

No	Accepted species name	IPA	Conservation status (IUCN)			Red Data	Preserved Herbaria
		category	EOO km ²	AOO km ²	Category	Book	
44	Phlomoides adylovii Lazkov	Aiii	1,810.229	24	EN	-	FRU
45	Phlomoides kirghisorum Adylov, Kamelin	Aiii	2,605.687	40	EN	-	TASH, FRU
	& Makhm.						
46	Phlomoides alaica (Popov) Adylov,	Aiii	43,650.470	24	NT	-	TASH, FRU, MW
	Kamelin & Makhm.						
47	Phlomoides urodonta (Popov) Adylov et al.	Aiii	176.445	32	EN	-	TASH, FRU
48	Scutellaria xanthosiphon Juz.	Aiii	1,810,209	24	EN	-	FRU, MW
49	<i>Silene fetissovii</i> Lazkov	Aiii	2948,074	28	EN	-	FRU, MW
50	Seseli giganteum Lipsky	Aiii	893.356	20	EN	-	LE, MW, AA, TASH,
							FRU, TAD
51	Seseli unicaule (Korovin) Pimenov	Aiii	16 472,343	32	VU+EN	-	LE, TASH, FRU,
							WILR, MW
52	Tanacetopsis ferganensis (Kovalevsk.)	Aiii	5,310.020	24	VU+EN	-	FRU, MW
	Kovalevsk.						
53	Allium minutum Vved.	Aiv	10,420.306	20	VU+EN	TJ	LE, TASH, FRU, TAD
54	Astragalus syreitschikovii Pavlov	Aiv	95,139.963	68	LC+EN	-	MW, AA, TASH, FRU
55	Fritillaria sewerzowii Regel	Aiv	39,522.829	116	NT+EN	-	TASH, FRU, AA, MW
56	<i>Jurinea winkleri</i> Iljin	Aiv	6,661.322	108	VU+EN	-	TASH, MW
57	Leibnitzia knorringiana (B. Fedtsch.) Pobed.	Aiv	8,443.694	40	VU+EN	-	FRU, MW
58	Lagochilus knorringianus var. drobovii	Aiv	32,447.798	60	NT+EN	-	TASH, FRU, AA, MW
	(Kamelin & Tzukerv.) Lazkov						
59	Polycnemum perenne Litv.	Aiv	32,249.538	152	NT+EN	-	TASH, FRU, AA, MW
60	Pseudosedum ferganense Boriss.	Aiv	27,595.830	100	NT+EN	-	LE, MW, TASH, FRU
61	Tulipa bifloriformis Vved.	Aiv	15,621.720	164	VU+EN	TJ	TASH, FRU, MW,
							TAD
62	Takhtajaniantha tau-saghyz (Lipsch. &	Aiv	28,713.924	28	NT+EN	-	TASH, FRU, AA, MW
	G.G.Bosse) Zaika, Sukhor. & N.Kilian.						

Category Ai

This category includes globally threatened species. In the Bozbu-Too-Ungortepa IPA site, only one species was described as category *Ai*.

Malus sieversii (Ledeb.) M.Roem., Fam. Nat. Syn. Monogr. 3: 216 (1847)

= Malus kirghisorum Al.Fed. & Fed., Trudy Yuzhno-Kirgizsk. Eksped. 1: 220 (1949).

Note. *Malus sieversii* is grown and consumed worldwide, and there is increasing interest in the crop's evolutionary history and in variety improvement for traits such as fruit characteristics, disease resistance, and stress tolerance (Gross et al. 2012). The species is still found in the fragmented fruit and nut forests of Central Asia and is threatened by habitat degradation, mainly from agricultural development and overgrazing (Eastwood et al. 2009). In Central Asia, this species is considered vulnerable, meaning it is threatened with extinction in the wild according to the IUCN Red List categories and criteria (IUCN 2021). Threats include loss and degradation of habitat because of agricultural expansion and development, genetic erosion (grafting of commercial varieties and hybridization), and over-grazing. **Existing Conservation Threats.** Over-collection for firewood. **General distribution.** Afghanistan, Kazakhstan, Kyrgyzstan, Pakistan, Tajikistan, Uzbekistan, Xinjiang.

Distribution in FV. All mountain ranges of the FV.

Category Aii

Fifteen species were described in category Aii.

All species listed in sub-criterion *Aii* are included as threatened species in the Red Data Books of Kyrgyzstan (Davletkeldiev 2006) and Uzbekistan (Khassanov 2019). Our definition of sub-criterion *Aii* agrees with the formulation by Darbyshire et al. (2017), according to which the site must consist of species of IUCN Regional Red List or another regionally approved peer-reviewed threat list.

Acanthophyllum pungens (Bunge) Boiss., Fl. Orient. 1: 561 (1867)

Figs 2A, 3A

= Acanthophyllum albidum Schischk., V.L. Komarov (ed.), Fl. URSS 6: 893 (1936).

Note. This polymorphic species is widespread in Central Asia (Lazkov 2006a). Several species were described based on variations of this species, which were later recognized as synonyms of *A. pungens* (Bondarenko 1972; Lazkov 2006a; Lazkov and Sultanova 2014). These "small species" which were later recognized as synonyms, including *A. albidum* Schischk., are of conservation interest. The last taxa were described by Schischkin (1936) from Central FV (Margelan, Kosch-Karchi). These plants are distinguished by white tepals and small inflorescences (up to 15 mm) and, as a rare endemic of FV, were listed in the Red Data Book of Uzbekistan (Khassanov 2019).

Existing Conservation Threats. Habitat fragmentation.

General distribution. Kazakhstan, Kyrgyzstan, Mongolia, North Caucasus, Tajikistan, Transcaucasus, Uzbekistan, Xinjiang.

Distribution in FV. Foothills and lower mountains of FV.

Allium dodecadontum Vved. in Opred. Rast. Sred. Azii 2: 316 (1971) Figs 2B, 3B

Note. The species is a member of a small cluster (Alay-Fergana Geographical clade sect. *Acmopetala* sensu Fritsch et al. 2010) and comprises five species occurring in western Tian-Shan: partly in Kyrgyzstan, partly in Uzbekistan (Fritsch et al. 2010; Fritsch and Khassanov 2008). Together with *A. schachimardanicum* Vved. (endemic of the middle and upper belts of the Alay Range), *A. dodecadontum* is rec-



Figure 2. The wild habitat of some plants in FV A Acanthophyllum pungens B Allium dodecadontum
C Allium viridiflorum D Lamyropappus schakaptaricus E Delphinium knorringianum F Iris kolpakowskiana
G Seseli eryngioides H Hedlundia persica I Tulipa ferganica J Eminium regelii.

ognized as the most basal species of the cluster and is considered strictly endemic to Chatkal Range.

Existing Conservation Threats. Habitat fragmentation. **General distribution.** Kyrgyzstan, Uzbekistan. **Distribution in FV.** Mid-mountain belt of the Chatkal Range.



Figure 3. Distribution map of the species of sub-criterion *Aii*, based on GeoCAT **A** *Acanthophyllum pungens* **B** *Allium dodecadontum* **C** *Allium viridiflorum* **D** *Anthochlamys tjanschanica* **E** *Crataegus knorringiana* **F** *Dorema microcarpum* **G** *Delphinium knorringianum* **H** *Eminium regelii.*

Allium viridiflorum Pobed. in Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 11: 64 (1949)

Figs 2C, 3C

Note. This species is a representative of the bi-type *Allium* ser. *Verticillata* Tzag., which have whorled leaves (Fritsch 2016). *A. viridiflorum* differs from *A. verticillatum* Regel, the type species of the section, by the greenish color of tepals that are nine times longer than the pedicels, short stamens, ecological preference, and geographical distribution (Tojibaev et al. 2014b). *A. viridiflorum* was previously considered a local endemic of Arkit, Chatkal range, Kyrgyzstan (Lazkov and Sultanova 2014). A new species population was found in the Uzbekistan part of this IPA site (Tojibaev et al. 2014b).

Existing Conservation Threats. Habitat fragmentation, overgrazing.

General distribution. Kyrgyzstan, Uzbekistan.

Distribution in FV. Low and mid-mountain belt of the Chatkal Range.

Anthochlamys tjanschanica Iljin ex Aellen in Verh. Naturf. Ges. Basel 61: 170 (1950) Fig. 3D

Note. The genus *Anthochlamys* comprises five species (POWO, 2022) and is distributed in the Iran-Turanian floristic region (Sukhorukov and Konstantinova 2012). Except for *A. tjanschanica*, almost all the taxa can be found in Iran and western Afghanistan, which differs from other species by its distribution in the Tian-Shan Mountains (Sidorenko et al. 1968; Pratov 1972a).

The species belongs to the rare endemic species of FV with a very narrow distribution range. It inhabits gravelly and loessal slopes of foothills and does not grow in protected areas (Tojibaev et al. 2022).

Existing Conservation Threats. Habitat fragmentation, dry-farming, overgrazing. **General distribution.** Kyrgyzstan, Tajikistan, Uzbekistan. **Distribution in FV.** Foothills of FV.

Crataegus knorringiana Pojark. in Ref. Nauchno-Issl. Rabot, Otdelenie Biol. Nauk 1945: 7 (1947) Fig. 3F

Fig. 3E

Note. FV, the Chatkal Range, and the surrounding area, in particular, are important areas in the conservation of wild species of hawthorns (Pachomova 1976). Species known to be widely distributed throughout Central Asia, including *C. songorica* C.Koch., *C. turkestanica* Pojark., and *C. pontica* C.Koch, and some endemic species with narrow distribution both grow in these areas. The distribution of narrow-range species is bounded by the Chatkal river basin and the adjacent mountain ranges of the FV. This group includes *C. rematilobata* (Chatkal, Turkestan and Alay ranges), *C. tianschanica* Pojark. (Chatkal Range), *C. ferganensis* Pojark. (Chatkal, Fergana, Alay ranges), and *C. knorringiana* with distribution in the Chatkal and Alay ranges (Pachomova 1976). The last species were recorded in Bozbu-Too during the IPA surveys.

Existing Conservation Threats. Habitat fragmentation, human disturbance, over-grazing, and cutting threaten the species (Eastwood et al. 2009).

General distribution. Kyrgyzstan.

Distribution in FV. Mid-mountain belt of Chatkal and Alay ranges.

Delphinium knorringianum B.Fedtsch. in J. Roy. Hort. Soc. 61: 196 (1936) Figs 2E, 3G

Note. This rare ornamental plant is a relic species with a disjunctive area and is endemic to FV, where it was found on the northern slopes of the Atoynok Mountain Range and in the northern spurs of Turkestan and Alay ranges.

Existing Conservation Threats. Habitat fragmentation. **General distribution.** Kyrgyzstan, Uzbekistan.

Distribution in FV. Mid-mountain belt of the Atoynok Mountain Range and in the northern spurs of the Turkestan and Alay ranges.

Dorema microcarpum Korovin in Bot. Mater. Gerb. Inst. Bot. Zool. Akad. Nauk Uzbeksk. S.S.R. 8: 6 (1947)

Fig. 3F

Note. As mentioned by M.G. Pimenov (1988), it is difficult not to use the epithet "marvelous" for the Iran-Turanian genus *Dorema* D.Don because, compared to other genera of the Umbeliferae family, it has simple umbel located along the axis of the common inflorescence. This is the most striking feature of the genus *Dorema*, truly unique within the Umbelliferae. The hotspot of the genus is in the Kopet Dag Range (Turkmenistan and Iran) and the surrounding lowlands and plains (Pimenov 1983a, 1988).

Dorema microcarpum is the type species of the sect. *Microcarpa* Pimenov, and is considered the most eastern species of the genus. The mountain surrounding FV limits the distributed area (Pimenov 1988). The species is on the verge of extinction, caused mainly by habitat fragmentation. It is included in the Red Data Books of Kyrgyzstan (Davletkeldiev 2006) and Uzbekistan (Khassanov 2019).

Existing Conservation Threats. Habitat fragmentation, dry-farming, overgrazing. **General distribution.** Fergana, South Chatkal, Kurama, and Alay ranges (Kyrgyzstan, Uzbekistan).

Distribution in FV. Foothills of FV.

Eminium regelii Vved. in Schreder (ed.), Fl. Uzbekistan. 1: 540 (1941) Figs 2J, 3H

Note. The species is listed as a rare endangered species in the Red Data Book of Kyrgyzstan (Davletkeldiev 2006) and is sporadically distributed in western Tian-Shan and northern Pamir-Alay (Pazij 1972). In the IPA site, the species occurs as single individuals in silty soil and loess slopes in piedmonts and low-montane zones.

Existing Conservation Threats. Habitat fragmentation, dry-farming, overgrazing in early spring.

General distribution. Kazakhstan, Kyrgyzstan, Tajikistan, Uzbekistan. **Distribution in FV.** Foothills of FV.

Gamanthus ferganicus Iljin in Trudy Bot. Inst. Akad. Nauk S.S.S.R., Ser. 1, Fl. Sist. Vyssh. Rast. 2: 131 (1936) Fig. 4A

≡ Halimocnemis ferganica (Iljin) Akhani, Int. J. Pl. Sci. 168: 948 (2007).

Note. The genus *Gamanthus* Bunge is predominantly a desert and foothill genus, growing on sands and variegated outcrops. The genus is represented by five species (POWO 2022), mainly in Central Asia, with a disjunction into the Caucasus and Iran and the environs (*Gamanthus pilosus* (Pall.) Bunge). Of these, two species, *G. ferganicus* Iljin and *G. leucophyllus* Botsch., are endemic to Mountainous Central Asia. The first species is bound to the FV, occurring in saline crushed stone slopes of the foothills (Pratov 1972b). It differs from other species of the genus by having opposite leaves covered throughout with crisp, closely appressed hairs.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan, Tajikistan, Uzbekistan.

Distribution in FV. Endemic; low mountains of Chatkal Range (Ungortepa).

Iris kolpakowskiana Regel in Trudy Imp. S.-Peterburgsk. Bot. Sada 5: 263 (1877) Figs 2F, 4B

= Iridodictyum kolpakowskianum (Regel) Rodion., Rod Iris: 202 (1961).

Note. This is an ornamental and early flowering species with a reducing area. The distribution area includes piedmont plains, foothills, and low mountains (up to 1500 m) in western and northern Tian-Shan. The species populates silty-soil, loess, and stony slopes. Such landscapes predominate in FV and are places of intensive human activity. For this reason, the number of individuals is limited and declining. The species is included in the Red Book of Kyrgyzstan (Davletkeldiev 2006).

Existing Conservation Threats. Habitat fragmentation, dry-farming, overgrazing in early spring.

General distribution. Western and northern Tian-Shan (Kazakhstan, Kyrgyzstan, Uzbekistan).

Distribution in FV. Foothills of FV.

Hedlundia persica (Hedl.) Mezhenskyj, NULESU Coll. Fruit Ornament. Pl.: 32 (2018)

Figs 2H, 4C

≡ Sorbus persica Hedl., Kongl. Svenska Vetensk. Acad. Handl., n.s., 35(1): 70 (1901).
≡ Pyrus persica (Hedl.) M.F.Fay & Christenh., Global Fl. 4: 115 (2018).

Note. Although the species is rare and red-listed in Kyrgyzstan (Davletkeldiev 2006) and has a restricted distribution in Central Asia, Iran, Caucasus and Afghanistan, there are no immediate threats. Most of the Central Asian populations are in protected areas (Chatkal, Besh-Aral, Sary-Chelek, and Aksu-Djabagli reserves).

Existing Conservation Threats. Over-collection for firewood.

General distribution. Iran, Kazakhstan, Kyrgyzstan, Tajikistan, Turkey, Turkmenistan, Uzbekistan

Distribution in FV. Mid-mountain belt of Chatkal, Fergana, Alay and Turkestan ranges.



Figure 4. Distribution map of the species of sub-criterion *Aii*, based on GeoCAT **A** *Gamanthus ferganicus* **B** *Iris kolpakowskiana* **C** *Hedlundia persica* **D** *Lamyropappus schakaptaricus* **E** *Oreosalsola drobovii* **F** *Seseli eryngioides* **G** *Tulipa ferganica.*

Lamyropappus schakaptaricus (B.Fedtsch.) Knorring & Tamamsch. in Bot. Zhurn. S.S.S.R. 38: 909 (1954)

≡ Cirsium schakaptaricum O.Fedtsch. & B.Fedtsch., Consp. Fl. Turkest. 4: 287 (1912).

Note. The monotypic genus *Lamyropappus* Knorring & Tamamsch. belongs to the relicts of the flora of Central Asia (Kamelin 1973; Tojibaev et al. 2019). The unique species of the genus has been described as a species of *Cirsium* L. (as *C. schakaptaricum* O.Fedtsch. & B.Fedtsch.) (Fedtschenko and Fedtschenko 1912). Based on the results of taxonomical revisions of the genus, this species was allocated as a monotypic genus *Lamyropappus* in 1954 (Neustrueva-Knorring and Tamamschian 1954). The species has been described in the vicinity of the village of Shekaftar (Central Asia, FV, Kyrgyzstan). Locus classicus is located in boundary areas with Uzbekistan. According to published data (Tamamschian 1963; Tulaganova 1993a), the species' distribution area includes the Pribalkhash District, with deserts and mountains in the middle section of the Naryn river basin (Chatkal and Fergana ranges).

The species has been included in the first three editions of the Red Data Book of Uzbekistan (Pratov 2009). In the 1960s, all cited habitats became farmlands or inhabited settlements. Accordingly, the species' local population in Uzbekistan has disappeared. Tojibaev (2002), within the inventory of flora of southern slopes of the Kurama range in FV, studied boundary areas of Uzbekistan and a Shakaftar village in detail. However, populations of the species have not been found. The northern foothills of FV, in particular the vicinity of the village Shekaftar, are used as farmland. The species has not been found in fragmented natural landscapes. Following the results of these studies, the species has been excluded from the last edition of the Red Data Book (Pratov 2009). However, during field surveys of 2018–2019, a fragmented population of the species was found in the vicinity of Arbagish village in Uzbekistan.

Existing Conservation Threats. Habitat fragmentation, overgrazing.

General distribution. Pribalkhash Deserts, western Tian-Shan (Kazakhstan, Kyrgyzstan, Uzbekistan).

Distribution in FV. Foothills and low mountains in Naryn River Valley, Chatkal, and Fergana ranges.

Oreosalsola drobovii (Botsch.) Akhani in Phytotaxa 249: 162 (2016) Fig. 4E

≡ Salsola drobovii Botsch. in Bot. Mater. Gerb. Bot. Inst. Uzbekistansk. Fil. Akad. Nauk S.S.S.R. 3: 3 (1941).

Note. The genus *Oreosalsola* Akhani, a non-C4 lineage, was described as a new genus segregated from *Salsola* s.l. Morphologically and phylogenetically, *Oreosalsola* is very similar to *Salsola arbusculiformis* Drobow, i.e., the "Collinosalsola" group. The main differences are the relatively long inflorescences, glaucous annual branches, and deep

bifid divergent stigmas in *Oreosalsola* in contrast to dark green annual branches, shorter inflorescences, and less divided stigmas in "Collinosalsola" (Akhani et al. 2016). The genus comprises nine species, mostly distributed in Central Asia and Iran (POWO 2022).

Oreosalsola drobovii (Botsch.) Akhani is distributed in the central and western Tian-Shan area. The FV is the southern border of the species distribution. It is found as solitary plants, inhabiting dry gravelly and stony slopes of the foothills and the lower belt of mountains. In the dry and waterless slopes of the Kurama ridge, the population is vulnerable, but the semi-shrub life form allows it to survive the "waterless" periods. But, this biomorph is the limiting factor in the context of various external impacts, such as cutting and fragmentation of habitats. This species is not protected in the Fergana part of the range.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan, Uzbekistan.

Distribution in FV. Foothills and low mountains of Kurama and Chatkal ranges.

Seseli eryngioides (Korovin) Pimenov & V.N.Tikhom., in S.K.Cherepanov, Sosud. Rast. SSSR: 30 (1981)

Figs 2G, 4F

Note. This is one of the subendemic species of the Bozbu-Too-Ungortep IPA site with a few numbers of individuals. The distribution area is limited by the Chatkal range (Pimenov 1983b). Part of a known population inhabits protected areas in the Besh-Aral Nature Reserve (Davletkeldiev 2006).

Existing Conservation Threats. Unstudied. **General distribution.** Western and northern Tian-Shan (Kyrgyzstan). **Distribution in FV.** Foothills of FV.

Tulipa ferganica Vved. in Byull. Sredne-Aziatsk. Gosud. Univ. 21: 148 (1935) Figs 2I, 4G

Note. This is a subendemic species of FV and also grows on the southern macroslope of the Susamyr Range. All populations are located in the eastern part of the valley within the Chatkal and Alay ranges. Despite its wide distribution in this part of FV, there is a decrease in habitats and individuals in populations, especially around large settlements. Populations in the vicinity of Yangikurgan, Andijan, and Namangan are most threatened, with observed declines of over 25–30% in the last 20–25 years. Therefore, the presence of this species in the protected areas of the FV is important. Currently, the FV presents 11 protected areas with four types (including natural monuments). In terms of preserving wild tulip populations, the most significant protected area is the Sary-Chelek Reserve and other reserves located in Kyrgyzstan (Dekhkonov et al. 2021). *T. ferganica* has been found in almost all protected areas within Kyrgyzstan. However, further efforts are needed to document the presence of species in many protected areas across the region. According to Wilson et al. (2021), climate-changing models using *T. ferganica* and

T. korolkowii underline the restricted representation of these taxa in protected areas. The species is threatened by agricultural expansion, habitat fragmentation, and over-grazing.

Existing Conservation Threats. Habitat fragmentation, overgrazing.

General distribution. Western Tian-Shan and northern Pamir-Alay (Kyrgyzstan, Tajikistan, Uzbekistan).

Distribution in FV. Foothills and low mountains of FV.

Category Aiii

Thirty six species were described in category Aiii.

In this study, following Darbyshire et al. (2017), sub-criteria *Aiii* and *Aiv* we defined differently from those used within Europe (Anderson 2002). As noted in Anderson (2002) and analogical publications (Blasi et al. 2011; Marignani and Blasi 2012), European IPA sub-criteria focus on threatened national endemics and national nearendemics, using political boundaries. For sub-criteria *Aiii* and *Aiv* we focused on FV and the Afghan-Turkestan province, areas that are larger than the political boundaries of some European countries.

Species of sub-criteria *Aiii* were divided into two groups: "Highly Restricted Endemics" (HRE), which consist of species with a total range of less than 100 km²; and "Range Restricted Endemic" (RRE) with a total range of no more than 5000 km², but more than 100 km² (Darbyshire et al. 2017).

Highly Restricted Endemics of FV

Allium tatyanae F.O.Khass. & F.Karimov in Stapfia 99: 211 (2013) Figs 6A, 8A

Note. This species was previously described at this IPA site (Khassanov et al. 2013). Since 2013, only five herbarium specimens have been collected, including two type specimens kept at TASH (TASH000469, TASH000470). Research in recent years, including within the framework of this study, shows that the species is extremely rare and is not found in the adjacent territories of Uzbekistan and Kyrgyzstan.

Existing Conservation Threats. Habitat fragmentation, overgrazing. **General distribution.** Uzbekistan.

Distribution in FV. Subendemic; foothills of FV.

Astragalus allotricholobus Nabiev in Bot. Mater. Gerb. Inst. Bot. Akad. Nauk Uzbeksk. S.S.R. 14: 17 (1954) Fig. 8B

Fig. 8B

Note. This is one of the lesser-known species of *Astragalus* sect. *Chaetodon* Bunge. It was described by M.M. Nabiev at the IPA site area during his inventory study of the

flora of Maylisay river basin (1954). The species in the herbaria of Central Asia (Tashkent, Bishkek) is represented by only a few specimens. A possible reason for this is the natural rarity of populations and the narrow range of distribution areas.

The species is well distinguished from other representatives of the sect. *Chaetodon*, primarily from *Astragalus rumpens* Meffert allians, by the pubescence of the calyx (long, spaced, and shortly appressed) and beans (short, appressed hairs). This feature was the reason for the specific epithet, which in Latin means "beans otherwise hairy".

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan, Uzbekistan.

Distribution in FV. Endemic (Chatkal, Kurama); foothills of southern Chatkal.

Corydalis bosbutooënsis Lazkov in Bot. Zhurn. (Moscow & Leningrad) 91: 954 (2006)

Figs 6B, 8C

Note. This interesting species (sect. *Strictae*, subsect. *Flabellatae* Fedde), with a narrow range, was previously described at this IPA site (Lazkov 2006b). The author of the species limited the distribution area to the Bozbu-Too Mount massif. The species was considered a narrow endemic of Bozbu-Too, Kyrgyzstan in all publications (Lazkov and Sultanova 2014). During IPA field research in 2019–2020, we found local populations of this species in Uzbekistan on the rocky outcrops of Ungortepa. The discovered population in Uzbekistan occupied approximately 400×500 m and was represented by single and grouped plants of 5–10 individuals.

Existing Conservation Threats. Habitat fragmentation, overgrazing.

General distribution. Kyrgyzstan, Uzbekistan.

Distribution in FV. Endemic; foothills and low mountains of FV.

Gagea spelaea Levichev & Lazkov in J. Asia-Pacific Biodivers. 12: 343 (2019) Figs 6C, 8D

Note. The genus *Gagea* Salisb. is one of the largest genera in Liliaceae. Numerous studies have estimated that the number of species in the genus ranges from about 100 (Dahlgren et al. 1985; Xinqi and Turland 2000) to more than 320 (Levichev 2013; Peterson et al. 2016), which is largely due to the incomplete taxonomic revision of *Gagea* (Levichev 1999). Several new species from Iran (Zarrei et al. 2010a, 2010b), Western Europe (Peruzzi et al. 2007), and Turkey, and surrounds (Kayikçi et al. 2014) were described in the last decade. Central Asia is considered a territory with many newly described species of *Gagea*. According to current data, 34 species of *Gagea* are known in the FV (Tojibaev et al. 2018a), and, more recently, 36 in Kyrgyzstan (Levichev et al. 2019). However, this number is incomplete, since several undescribed taxa are present in Central Asia, including the mountains surrounding of the FV.

Described from FV, *Gagea spelaea* belongs to the group of white-flowered *Gagea* species. Until recently, this species was only known from the Chatkal Range's Sary-Chelek Nature Reserve, Kyrgyzstan (Levichev et al. 2019). New field surveys under the IPA FV project found two localities of the species in the Bozbu-Too area.

Existing Conservation Threats. Habitat fragmentation, farmlands.

General distribution. Kyrgyzstan.

Distribution in FV. Endemic; Chatkal Range (Bozbu-Too, Sary-Chelek).

Iris austrotschatkalica Tojibaev, F.Karim. & Turgunov in Turczaninowia 17(4): 12 (2014)

Figs 6D, 8E

Note. The species also was previously described at this IPA site (southern Chatkal ridge, Ungortepa) in 2014 (Tojibaev et al. 2014c). *Iris austrotschatkalica*, with a narrow range of edaphic tolerance, i.e., a stenobiont, grows only on dry, well-warmed stony soils. The vertical interval is also very narrow, from 1200–1300 m only. Despite a careful study of adjacent territories, the species remains known only from the locus classicus in Uzbekistan.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Uzbekistan.

Distribution in FV. Endemic; low mountains of Chatkal Range (Ungortepa).

Range Restricted Endemics

Acantholimon nabievii Lincz. in Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 21: 495 (1961) Figs 6E, 8F

Note. This species has a limited distribution in the form of a narrow strip along the northern foothills of the FV (Tojibaev et al. 2022). The main part of the population grows close to a large settlement (Chust, Kosonsoy towns).

Existing Conservation Threats. Habitat fragmentation, overgrazing. **General distribution.** Kyrgyzstan, Uzbekistan. **Distribution in FV.** Endemic; northern foothills of FV.

Allium arkitense R.M.Fritsch in Stapfia 80: 385 (2002)

Figs 6F, 8G

Note. The species, one of the rare species of the FV, was described over the past two decades (Fritsch et al. 2002). Prior to our research, the species was known only from the locus classicus (Fritsch 2016). New populations of the species were found during field surveys in Kyrgyzstan (Lazkov) and Uzbekistan (Tojibaev, Karimov, Hoshimov). Thus, the species has a transboundary range.

Existing Conservation Threats. Habitat fragmentation, overgrazing. **General distribution.** Kyrgyzstan, Uzbekistan. **Distribution in FV.** Endemic; northern foothills of FV.

Allium gracillimum Vved. in Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 9: 243 (1946)

Figs 6G, 8H

Note. Originally, the species was described as a narrow endemic of the Mogoltau Mountains, Tajikistan (Vvedensky 1971). Later, it was found in the southern part of the Kurama range, Tajikistan (Tojibaev's oral communication), and the Chatkal range (Lazkov and Turdumatova 2010). According to current data, the species distribution area covers Mogoltau, the southern slopes of Kurama (both in Tajikistan), and the Atoynak and Chatkal ranges (Kyrgyzstan).

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan, Tajikistan.

Distribution in FV. Endemic; low mountains of Mogoltau, Atoynak, Chatkal and Kurama ranges.

Allium haneltii F.O. Khass. & R.M. Fritsch in Linzer Biol. Beitr. 30: 282 (1998) Figs 6I, 8I

Note. This is a morphologically and geographically interesting species of *Allium* sect. *Brevidentia* F.O.Khass. & Yengalycheva (Fritsch et al. 1998). The species is widely separated from its closest relatives, i.e., the Afghan species *A. miserabile* Wendelbo and *A. circumflexum* Wendelbo (Fritsch et al. 1998). All relatives are distinguished by simple inner filaments, coriaceous outer bulb tunics, and boat-shaped bulblets.

The distribution area is limited by the foothills of Kurama and Chatkal ranges (Tojibaev et al. 2020). The main part of the population grows close to a large settlement and is threatened by habitat fragmentation.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Uzbekistan.

Distribution in FV. Endemic; northern foothills of FV.

Allium oreoscordum Vved. in Bot. Mater. Gerb. Glavn. Bot. Sada R.S.F.S.R. 5: 95 (1924)

Figs 6H, 8J

Note. This is one of the primitive *Allium* species in the flora of Central Asia. Characterized by the peculiar structure of the bulb, i.e., bulbs are attached to an ascending rhizome, they are narrowly conical, and bulb tunics are reticulate. The species was

described from Ungortepa, i.e., in the IPA area. Currently, the area is limited to the Chatkal and Uzun-Akhmat ranges (FV) and the species is known in 10–12 locations; most of them are found in the Bozbu-Too-Ungortepa IPA.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan, Uzbekistan.

Distribution in FV. Endemic; northern foothills and mid-mountains of FV.

Allium pseudopskemense ined. (sect. Cepa)

Figs 6J, 8K

Note. Allium sect. Cepa (Mill.) Prokh. is a small group within the genus Allium that includes 12 wild and cultivated species. Two of them, Allium cepa L. (common or bulb onion) and A. fistulosum L. (bunching onion) are economically important (Yusupov et al. 2021). In «Flora of the Kirghiz SSR» (Nikitina, 1951), three wild (A. galanthum Kar. & Kir., A. oschaninii O.Fedtsch., A. pskemense B.Fedtsch.) and one cultivated species (A. cepa) representatives of sect. Cepa have been recorded. The same number of species of sect. Cepa (A. cepa, A. oschaninii, A. praemixtum Vved., A. pskemense) was recorded in Uzbekistan (Sennikov 2017).

During field research at the IPA site in 2012–2013, Lazkov collected specimens distinctive from *A. pskemense*. A subsequent revision of all herbarium specimens in FRU and TASH with similar morphological features shows that recently collected specimens differed from *A. pskemense* due to important characteristics such as filament structure and distribution areas. Prior to that point, all collectors in Kyrgyzstan wrongly identified it as the closely related *A. pskemense*. The difference between the newly collected plants and *A. pskemense* is justified morphologically, genetically, and geographically. The plant from this IPA site has been named *Allium pseudopskemense* ined.

Existing Conservation Threats. Habitat fragmentation. **General distribution.** Kyrgyzstan. **Distribution in FV.** Foothills of FV.

Astragalus bosbutooënsis Nikitina & Sudn. in Izv. Akad. Nauk Kirgizsk. S.S.R., Ser. Biol. Nauk 1: 68 (1974) Figs 6K, 8L

Note. The species was described in the Bozbu-Too Range and was considered endemic to Kyrgyzstan (Kamelin 1981; Lazkov and Sultanova 2014). It belongs to the sect. *Xiphidium* Bunge (subsect. *Microtropidia* Kamelin), and is a loose, non turf-forming plant with elongated pink-purple inflorescences. All known locations were in the Bozbu-Too. However, in 1950 and 1972, very similar plants were collected from Uzbekistan, between the Pap and Charkesar foothills and Chatkal reserve, re-

spectively. Because of the rarity of this species in the Uzbekistan part of the Chatkal Range, it remained overlooked by subsequent researchers (Krasovskaya and Levichev 1986; Tojibaev 2010). The new records significantly expanded the distribution area of this rare plant species (Tojibaev et al. 2014b). According to the latest data, the species is a narrow endemic of the FV, with distribution in the Atoynak and Chatkal ranges (Tojibaev et al. 2020).

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan, Uzbekistan.

Distribution in FV. Endemic; foothills and low mountains of FV.

Astragalus melanocomus Popov in Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 10: 24 (1947) Fig. 9A

= Astragalus isophysus Nabiev in Bot. Mater. Gerb. Inst. Bot. Akad. Nauk Uzbeksk. S.S.R. 14: 20 (1954).

Note. The species belongs to sect. *Chaetodon* Bunge (Puchkova 1967, 1981; Podlech and Zarre 2013). In Central Asia, the sect. *Chaetodon* is represented by a large number of species endemic to mountainous Central Asia, including rare and threatened ones (Tojibaev et al. 2015). *A. melanocomus* is endemic to the mountain ranges surrounding the FV (Puchkova 1967, 1981; Tojibaev et al. 2015). From the study area, it is known only from two herbarium collections from 1912 and 1933, kept in LE (Ungor-Tepa Mountains, O. Knorring, sn, 1912; Environs of the village of Kassansay, Drobow and Sakhobutdinov, sn, 1933). Subsequent studies in both Uzbekistan and Kyrgyzstan did not reveal the presence of *A. melanocomus* in this IPA site and surrounding areas.

Existing Conservation Threats. Unknown.

General distribution. Uzbekistan.

Distribution in FV. Endemic; middle mountain belt of FV.

Astragalus pseudodianthus Nabiev in Bot. Mater. Gerb. Inst. Bot. Akad. Nauk Uzbeksk. S.S.R. 14: 21 (1954)

Fig. 9B

Note. The species was described by Nabiev (1954) from the gypsum slopes of the northern foothills of the FV, bordering the IPA site. Other closely related species differ in the large size of the flower, calyx, and beans, and legumes 1–2 mm long. According to Vinogradova (1981) the species belongs to sect. *Aureophora* Kamelin, consisting of four species, and are narrow endemics to the foothills and low mountains of western Tian-Shan and western Pamir-Alay. Podlech and Zarre (2013) classified the species into sect. *Dissitiflori* with more than 1954 species worldwide.

Nabiev (1954) also described several *Astragalus* species, which are still considered narrow endemics of foothills and low mountains of southern Chatkal, bordering the Bozbu-Too-Ungortepa IPA site, namely *A. allotricholobus* (see above), *A. isophysus* (syn. of *A. melanocomus* Popov), both belong to the sect. *Chaetodon*, and *A. caudicosus* Galkina & Nabiev (sect. *Ornithopodium*, sensu Podlech and Zarre 2013: 1782–1797).

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan, Uzbekistan.

Distribution in FV. Endemic; foothills and low mountains of Kurama and Chatkal ranges.

Astragalus spryginii Popov in Dimo, Soil Invest. Exped. Rivers Syr-daria & Amudaria 1: 51 (1915)

Fig. 9C

Note. This is a representative of subsect. *Spryginiana* Kamelin with interesting morphological features. Within the sect. *Xiphidium* (more than 60 species in Central Asia) is distinguished by its life form (shrub) and is easily identified by its bare ovary and beans (Kamelin 1981). Recently, the species were noted as a new species for the flora of Uzbekistan from the Bozbu-Too-Ungortepa IPA site, and detailed information on geography and conservation issues was given by Tojibaev et al. (2019).

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan, Tajikistan, Uzbekistan.

Distribution in FV. Endemic; foothills and low mountain belts of FV.

Cousinia knorringiae Bornm. in Beih. Bot. Centralbl. 34(2): 201 (1916) Figs 6M, 9D

Note. This species is narrowly distributed in the western Tian-Shan and is endemic to Kyrgyzstan (Sennikov 2010; Lazkov and Umralina 2015). It inhabits bare rocks and screes at elevations of 1200–1500 m above sea level. This species is of conservation interest because of its small population size and limited distribution (Sennikov 2010, 2021). *C. knorringiae* was first reported in eastern Uzbekistan on the southern foothills of Ungur-Tepa Mt., north of Paromon Village, in Yangi-Kurgan District, Namangan Region (Usmonov et al. 2021). The conservation status of the species is assessed as Endangered (EN), based on criterion D (estimated population size of 200–250 mature individuals), according to the IUCN Red List Categories and Criteria (version 3.1).

Existing Conservation Threats. Habitat fragmentation.

General distribution. Kyrgyzstan, Uzbekistan.

Distribution in FV. Foothills and low-mountain belt of Chatkal Range. Endemic to the Bozbu-Too-Ungortepa IPA site.
Cousinia krauseana Regel & Schmalh. in Izv. Imp. Obshch. Lyubit. Estestv. Moskovsk. Univ. 34(2): 48 (1882) Figs 6N, 9E

Note. The species belong to *Cousinia* sect. *Jurineopsis* (Juz.) Tscherneva, which is endemic to the Central Asian Mountains (Tscherneva 1993). The section includes 11 species; most are distinguished by a comparatively small distribution area. Earlier, Tscherneva indicated *C. krauseana* in the flora of Uzbekistan (Tscherneva 1962) and Kirgiz SSR (Tscherneva 1963) with a small distribution area limited by the northern foothills of FV. During the field surveys in the IPA area, a small population of the species was found on the border of Uzbekistan and Kyrgyzstan.

A new observation also confirms that the species has a narrow distribution area and is consistent with the latest data by Tscherneva (1993). However, the species has few herbarium records.

Existing Conservation Threats. Habitat fragmentation, farmlands. **General distribution.** Kyrgyzstan, Uzbekistan. **Distribution in FV.** Foothills and low-mountain belts of FV.

Echinops knorringianus Iljin in Bot. Mater. Gerb. Glavn. Bot. Sada R.S.F.S.R. 3: 173 (1922)

Fig. 9F

Note. The genus *Echinops* L. in Central Asia represents approximately 45 species, and only 2 are annual plants (Li 1993). Among them, *E. knorringianus* can be easily distinguished by the absence of felt or cobweb pubescence on the upper surface of the leaves and stems with glandular pubescence. In contrast to *E. nanus* Bunge (the second annual species of the genus in Central Asia), *E. knorringianus* has a narrow distribution area (FV), bounded by the stony and fine-earth slopes of the foothills.

Existing Conservation Threats. Habitat fragmentation, farmlands. **General distribution.** Kyrgyzstan, Tajikistan, Uzbekistan. **Distribution in FV.** Foothills around FV.

Eremurus czatkalicus Lazkov in Turczaninowia 14(3): 12 (2011)

Figs 6P, 9G

Note. This is another new noteworthy record from the Uzbekistan part of the FV. This species was previously known from Kyrgyzstan, and the distribution area of this species was in the Aflatun River Basin in the southern Chatkal Range (Lazkov and Pashinina 2011). Most available data on the distribution of the species belongs to the vicinity of the locus classicus. However, in 2019, V. Yusupov collected specimens of the species from the northern foothills of the FV in Kasansay town (https://www.plantarium.ru).

This information intensified field surveys for the species elsewhere in the northern foothills of FV (Uzbekistan). Several specimens were found between Pap and Chust towns, in the Karatag massif (near Kasansay town), and Arbagish village. The last two locations form part of the IPA site.

Existing Conservation Threats. Habitat fragmentation, farmlands.

General distribution. Kyrgyzstan, Uzbekistan.

Distribution in FV. Foothills and low-mountain belts of Kurama and Chatkal ranges.

Ferula incisoserrata Pimenov & J.V.Baranova in Byull. Moskovsk. Obshch. Isp. Prir., Otd. Biol., n.s., 84(3): 87 (1979)

Figs 6L, 9H

Note. The species is endemic to eastern FV with distribution in the Chatkal and Fergana ranges (Pimenov and Baranova 1979). It was previously recorded in the flora of Kyrgyzstan as a national endemic (Lazkov and Pashinina 2011; Laskov and Sultanova 2014). It was collected in the Uzbek part of FV in 2014, but was fully identified in 2020. The Uzbekistan population grows only in the Ungortepa area. The nearest populations are located in the Karavan pass (around 10–15 km, Nabiev 1959, specimen stored in TASH) and Bozbu-Too (around 35–38 km, Lazkov's collection of 2021).

Existing Conservation Threats. Habitat fragmentation, farmlands.

General distribution. Kyrgyzstan, Uzbekistan.

Distribution in FV. Endemic; low and mid-mountains of Chatkal and Fergana ranges.

Regarding the diversity of geophyte bulbous species, FV is still an insufficiently studied area. This is evidenced by the fact that dozens of new species belonging to the genera *Allium, Eremurus, Iris,* and *Tulipa* have been described in the FV in the last two decades (Tojibaev et al. 2020; Naumenko and Zubov 2021). The genus *Fritillaria* is no exception, which is represented in the FV by at least six (Naumenko and Zubov 2021) out of eight species recorded in the Tian-Shan mountains. The last species was described recently (Naumenko and Zubov 2021), and the *locus classicus* is located in the IPA site. This species' information is provided below.

Fritillaria rugillosa Naumenko & Zubov in Int. Rock Gard. 140: 4–11 (2021) Figs 6O, 9I

Note. In all previous field studies conducted in this region, this species was identified as *Fritillaria stenanthera* (Regel) Regel, which is endemic to western Tian-Shan. However, this species of *Fritillaria* is widespread across all mountain ranges of the region. Only Naumenko and Zubov, the authors of *F. rugillosa*, paid attention to the differences between the two species and recognized the plants from eastern Fergana as a new species. According to the authors, *F. rugillosa* differs from *F. stenanthera* by an open/flat, wide-wheel-shaped perianth, sideways directed at flowering; perianth segments

without a pronounced pinch between the nectary horn and the bend of a segment; dark, violet, or purple filaments, pubescent by 2/3 at a base, white and glabrous in the upper part, or sometimes fully pubescent along the entire length (Naumenko and Zubov 2021). Naumenko and Zubov (2021) assumed that it is highly probable that it is distributed in the Uzbekistan part of the FV. Field surveys in 2021 confirmed the presence of the species in the Uzbekistan part of the FV. Populations of this species have been recorded in the vicinity of Arbagish.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan, Uzbekistan.

Distribution in FV. Foothills and low mountain belts of Chatkal Range.

Hedysarum gypsaceum Korotkova in Bot. Mater. Gerb. Inst. Bot. Akad. Nauk Uzbeksk. S.S.R. 14: 13 (1954)

Figs 6Q, 9J

Note. This species is known from the boundary area between Uzbekistan and Kyrgyzstan, where it grows on rocky slopes at 900–1500 m. The distribution area of the species almost coincides with the Bozbu-Too-Ungortepa IPA site. The majority of specimens stored in TASH were collected from the Kyrgyzstan part. The latest collection from the territory of Uzbekistan was in 1951 (Nabiev 1959). According to the field surveys of 2015, the species is rare in the Uzbekistan part of the Chatkal range (vicinity of Arbagish village). Field studies in subsequent years determined the distribution area in the Uzbekistan part of the FV and the current condition of local populations. No more than 150–200 individuals of this species were found in the research area. The population consists of individuals of different ages, and seed regeneration was observed (Tojibaev et al. 2019).

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan, Uzbekistan.

Distribution in FV. Endemic; foothills and low mountain belts of Kurama and Chatkal ranges within the FV.

Hedysarum turkestanicum Regel & Schmalh. in Izv. Imp. Obshch. Lyubit. Estestv. Moskovsk. Univ. 34(2): 21 (1882) Fig. 5, 9K

Note. The species is a narrow endemic to the southern slopes of the Chatkal Range. It was described in 1882 by Regel and Schmalhausen based on collections by Krause (Regel 1882). The locus classicus is in the vicinity of the village of Nanay, bordering Kyrgyzstan and Uzbekistan. For many years the plant was known only from type specimens. Another collection was carried out 69 years later, in 1951 by N.V. Galkina in the vicinity of Nanay. However, it is difficult to determine whether these specimens were collected in the Uzbekistan part (Tojibaev et al. 2014c). New infor-



Figure 5. Specimen of *Hedysarum turkestanicum* Regel & Schmalh. collected 70 years after the date of the type specimens collection.

mation on species distribution became available only in 2020 and 2021 when the staff of the Institute of Botany of the Academy of Sciences (Ortikov E. and Juramuradov I., respectively) collected two specimens of this species from the species' locus classicus. However, the scientific significance of these collections lies in the fact that they indicate the preservation of the species in such a narrow area, which is directly located near a large settlement.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan, Uzbekistan. **Distribution in FV.** Endemic; foothills of southern Chatkal Range.



Figure 6. The wild habitat of some plants in FV. A Allium tatyanae B Corydalis bosbutooënsis C Gagea spelaea D Iris austrotschatkalica E Acantholimon nabievii F Allium arkitense G Allium gracillimum H Allium oreoscordum I Allium haneltii J Allium pseudopskemense K Astragalus bosbutooënsis L Ferula incisoserrata M Cousinia knorringiae N Cousinia krauseana O Fritillaria rugillosa P Eremurus czatkalicus Q Hedysarum gypsaceum R Limonium ferganense S Seseli unicaule.

Hyalolaena intermedia Pimenov & Kljuykov in Bot. Zhurn. (Moscow & Leningrad) 67: 887 (1982) Fig. 9L

Note. The species was described from the environs of Kyzyl-Tokay (South Chatkal, Kyrgyzstan) based on herbarium specimen of the authors of the species (Pimenov and Kljuykov 1982). Until now, this species was considered nationally endemic to Kyrgyzstan, distributed only in the Chatkal Range (Pimenov and Kljuykov 2002; Lazkov and Umralina 2015). However, during an expedition to the hard-to-reach border areas north of Namangan, Tojibaev et al. collected *H. intermedia* Pimenov & Kljuykov, a new record for Uzbekistan (Lyskov et al. 2019). The species differs from all other *Hyalolaena* species in the branched cord-like roots and stubble-like concave ultimate segments of the basal leaves (Pimenov and Kljuykov 1982).

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan, Uzbekistan.

Distribution in FV. Endemic; foothills of Chatkal Range (Naryn and Kasansay River Basins).

Iris narynensis O.Fedtsch. in Izv. Imp. S.-Peterburgsk. Bot. Sada 5: 159 (1905) Fig. 10A

Note. *Iris narynensis* is endemic to the low mountain belts of the FV (both in Tian-Shan and Pamir-Alay parts). The flowers are vaguely reminiscent of *I. kuschakewiczii* and its related species (Ikinci et al. 2011). The species belong to the group of rare Juno Iris species of Central Asia. In the Tian-Shan part, it is known from Ungortepa (Kyrgyzstan), the surroundings of Arbagish (Uzbekistan), and Bozbu-Too (Kyrgyzstan).

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan, Uzbekistan.

Distribution in FV. Endemic; foothills and low mountains of FV.

Limonium ferganense Ikonn.-Gal. in Trudy Bot. Inst. Akad. Nauk S.S.S.R., Ser. 1, Fl. Sist. Vyssh. Rast. 2: 262 (1936) Figs 6R, 10B

Note. *Limonium ferganense* is one of the few species of the genus endemic to the FV. The majority of populations of the species grow in the foothills of the Chatkal and Fergana ranges within the Naryn River Basin (Linchevsky 1993). The closest species are *L. narynense* Lincz., another species endemic to the mountains of the middle section of the Naryn River. *L. ferganense* is distinguished by the lack of fruitless branches in the inflorescence.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan, Uzbekistan. **Distribution in FV.** Foothills of Chatkal and Fergana ranges.

Oxytropis gymnogyne Bunge in Mém. Acad. Imp. Sci. Saint Pétersbourg, Sér. 7, 22(1): 25 (1874)

Fig. 10C

Note. The genus *Oxytropis* DC. in Central Asia is mainly represented by species common in the mid and high-mountain regions (Filimonova 1983). *O. gymnogyne* belongs to a group of species endemic to Central Asia, distributed from foothills (450–500 m) up to the mid-mountain belt (1800–2000 m). In the Tajikistan part of western Tian-Shan (Mogoltau), the species are found at 450–1000 m altitude (Abdusalamova 1978). In northern Fergana, including the IPA site, the species is distributed on stony and gravelly slopes of foothills between 500–900 m.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan, Uzbekistan.

Distribution in FV. Foothills and mid-mountain belt of Chatkal, Kurama, and Mogoltau ridges.

As one of the largest genera in Lamiaceae, *Phlomoids* Moench. is well known for its high local microendemism in Central Asia (Salmaki et al. 2012).

The tribe *Phlomideae* Mathiesen originally comprised six genera (Scheen et al. 2010), including the mostly Central Asian *Phlomoides* (L.) Moench, and *Pseuderemostachys* Popov. According to the World Checklist (Govaerts et al. 2021) and the above circumscription, *Phlomideae* contains 200 records, whereas Kamelin and Makhmedov (1990) recognized about 250 species within the genera. The species are distributed from Europe to Mongolia, China, and India, with the highest number of species found in Central Asia, Afghanistan, Iran-Turanian, and Himalayan regions (Salmaki et al. 2012).

One of the centers of local endemism of *Phlomoides* is in the study area. At least 26 species of the genus grow in the FV; seven of them are endemic to the FV (Gulomov 2021).

Phlomoides adylovii Lazkov in Novosti Sist. Vyssh. Rast. 36: 32 (2004) Figs 7A, 10D

Note. The species is considered one of the narrow endemic species of *Phlomoides* Moench. in the FV (Lazkov 2011, 2016). It was described by G.A. Lazkov in the Kurpsay gorge (Atoynak ridge), bordering the eastern part of the IPA site (Lazkov 2004). Currently, it is known from four localities, two of which are located at Bozbu-Too, Ungortepa of this IPA site (Lazkov 2004, 2011).

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan. **Distribution in FV.** Endemic; foothills of Chatkal and Fergana ranges.



Figure 7. The wild habitat of some plants in FV **A** *Phlomoides adylovii* **B** *Phlomoides kirghisorum* **C** *Phlomoides urodonta* **D** *Seseli giganteum* **E** *Scutellaria xanthosiphon* **F** *Phlomoides alaica* **G** *Silene fetissovii* **H** *Tanacetopsis ferganensis.*



Figure 8. Distribution map of the species of sub-criterion *Aiii*, based on GeoCAT **A** *Allium tatyanae* **B** *Astragalus allotricholobus* **C** *Corydalis bosbutooënsis* **D** *Gagea spelaea* **E** *Iris austrotschatkalica* **F** *Acantholimon nabievii* **G** *Allium arkitense* **H** *Allium gracillimum* **I** *Allium haneltii* **J** *Allium oreoscordum* **K** *Allium pseudopskemense* **L** *Astragalus bosbutooënsis*.



Figure 9. Distribution map of the species of sub-criterion Aiii, based on GeoCAT A Astragalus melanocomus B Astragalus pseudodianthus C Astragalus spryginii D Cousinia knorringiae E Cousinia krauseana F Echinops knorringianus G Eremurus czatkalicus H Ferula incisoserrata I Fritillaria rugillosa J Hedysarum turkestanicum L Hyalolaena intermedia.



Figure 10. Distribution map of the species of sub-criterion Aiii, based on GeoCAT A Iris narynensis
B Limonium ferganense C Oxytropis gymnogyne D Phlomoides adylovii E Phlomoides kirghisorum
F Phlomoides alaica G Phlomoides urodonta H Scutellaria xanthosiphon I Silene fetissovii J Seseli giganteum
K Seseli unicaule L Tanacetopsis ferganensis.

Phlomoides kirghisorum Adylov, Kamelin & Makhm. in Opred. Rast. Sred. Azii 9: 99 (1987)

Figs 7B, 10E

Note. Originally, the species was described as *Eremostachys ferganensis* Ubuk. based on specimens collected from the Bozbu-Too Mountains (Ubukeeva 1960). Adylov and Makhmedov (1987) reported this species in Kyrgyzstan in the foothills and low mountains of Alay, Chatkal, and Fergana ranges. Current knowledge of the distribution area of this species shows that it is limited by FV (Lazkov and Sultanova 2014). Most of the population is located on the border between Kyrgyzstan and Uzbekistan. During field surveys in 2020–2021, several plants belonging to this species were collected in the Uzbekistan part of the IPA site (Arbagish and surrounds).

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing.

General distribution. Kyrgyzstan, Uzbekistan. **Distribution in FV.** Foothills of Chatkal Range.

Phlomoides alaica (Knorring) Adylov, Kamelin & Makhm. in Opred. Rast. Sred. Azii 9: 107 (1987)

Figs 7F, 10F

- ≡ Phlomis alaica Knorring in Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 15: 338 (1953).
- = Phlomis knorringiana Popov in V.L.Komarov, Fl. URSS 21: 648 (1954).
- ≡ Phlomoides knorringiana (Popov) Adylov, Kamelin & Makhm. in Opred. Rast. Sred. Azii 9: 107 (1987).

Note. The type specimens described as *Phlomis knorringiana* Popov differ from *Phlomis alaica* Knorring (currently *Phlomoides alaica*) in having short stellate (not stellate with longer middle rays) hairs on the bracts and the calyx (Lazkov 2011). Because plants with similar morphological characteristics do not have separate geographical distribution areas and plants with intermediate forms were observed, Lazkov (2011, 2016) combined the two species while maintaining the priority of the epithet "*alaica*".

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing.

General distribution. Kyrgyzstan, Uzbekistan.

Distribution in FV. Foothills of Alay and Fergana ranges, Ak-Shyyrak, At-Oynok, Bozbu-Too, Chaar-Tash, and Moldo-Too Mts.

Phlomoides urodonta (Popov) Adylov, Kamelin & Makhm. in Opred. Rast. Sred. Azii 9: 106 (1987)

Figs 7C, 10G

Note. According to Lazkov, *P. urodonta* is the type species of the monotypic series *Urodontae* Lazkov, characterized by white or whitish corolla and narrowly triangular calyx teeth on the sides without denticles (Lazkov 2011). The conservation value of the species is increasing due to the small distribution area. The species is endemic to southern Chatkal in Kyrgyzstan. Previously, Lazkov (2011) reported four herbarium specimens (three of them were collected on the territory of the Sary-Chelek reserve). Later, another population was recorded by him in the study area.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan.

Distribution in FV. Low mountains of Chatkal Range.

Scutellaria xanthosiphon Juz. in Bot. Mater. Gerb. Bot. Inst. Komarova Akad. Nauk S.S.S.R. 14: 367 (1951)

Figs 7E, 10H

Note. Described from the Kugart river basin in the Fergana range, Kyrgyzstan. Alay and Fergana ranges in Kyrgyzstan cover the current distribution area of this species (Abdullaeva 1987). These ranges surround the FV from the northeast and south (south-east). Lazkov and Sultanova (2014) support these data and in the "Cadastre" for this species indicate near Fergana regions of Kyrgyzstan, which includes the southern slopes of the Chatkal and Fergana ranges and the northern slopes of the Alay and Turkestan ranges. Field studies in 2020–2021 showed the presence of populations of the species located in the areas between the Fergana and Alay ranges, which fall in the Bozbu-Too Mountains.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan.

Distribution in FV. Middle mountain and subalpine belts of Chatkal, Fergana, and Alay ridges.

Silene fetissovii Lazkov in Novosti Sist. Vyssh. Rast. 29: 66 (1993) Figs 7G, 10I

Note. Thus far, the species is known from only a few localities in stony-rocky outcrops of the belt of forests and bushes of eastern Fergana. It was described by Lazkov (1993) in the territories adjacent to the IPA site (Chatkal Range, Padsha-Ata River Basin). Some paratype specimens were collected by Aydarova and Ubukeev from Bozbu-Too

in 1977 (kept in FRU). Based on current literature data and field observations from 2010–2020, the species is endemic to the Chatkal and Fergana ranges within the FV.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan.

Distribution in FV. Middle mountain belt of Chatkal and Fergana ranges.

The Flora of Kyrgyzstan is recognized as rich in species of the genus *Seseli* L. (Pimenov and Kljuykov 2002; Lazkov and Sultanova 2014). Out of 46 species known throughout Central Asia (Pimenov 1983b), 21 (Pimenov and Kljuykov 2002) or 23 (Lazkov and Sultanova 2014) species of the genus were recorded in the flora of Kyrgyzstan, and eight species are endemics (Pimenov and Kljuykov 2002). Most endemic *Seseli* species are distinguished by a small distribution area or are known from the type locality (*S. luteolum* Pimenov, *S. kashgaricum* Pimenov, Kljuykov). Some of the national endemics are distributed in eastern Fergana bordering Uzbekistan. Field surveys from 2010–2021 in Uzbekistan identified two *Seseli* species before being registered as national endemics of Kyrgyzstan (Lazkov and Umralina 2015).

Seseli giganteum Lipsky in Trudy Imp. S.-Peterburgsk. Bot. Sada 23: 140 (1904) Figs 7D, 10J

Note. The species belongs to the bi-type sect. *Microseseli* Pimenov (Pimenov 1978). The second species is *S. turbinatum* Korovin, which differs in life form (polycarpic), morphology (root system, fruit, corolla color, and branching type), and geography (Pamir-Alay, Nurata).

S. giganteum is rare and endemic to the southern Chatkal. It was described from the valley of the Khoja-Ata river basin (Pimenov 1974). The species is known from a few localities outside the locus classicus, but none goes beyond the southern Chatkal (basins of the Padsha-Ata and Kasan-say rivers). According to all published data, the species is considered a strict endemic to Kyrgyzstan (Pimenov 1974, 1978; Lazkov and Sultanova 2014; Lazkov and Umralina 2015).

The species was recorded near the vicinity of Nanay village in the Uzbekistan part of FV. This is close to the Ungortepa massive of the IPA site (by Tojibaev K.Sh., Turginov O.T.). The species grows on the loessal slopes of open shrublands.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan.

Distribution in FV. Middle mountain belt of Chatkal Range.

Seseli unicaule (Korovin) Pimenov in Novosti Sist. Vyssh. Rast. 11: 245 (1975) Figs 6S, 10K

Note. In contrast to the previous species, *S. unicaule* (sect. *Sclerrhiza* Popov & Sdobnina, subsect. *Lehmanniana* Pimenov) has a wider distribution area and, accordingly, is represented by a large number of herbarium specimens kept at TASH, LE, FRU, WILR, and MW. The geography of the species was revised in detail by M.G. Pimenov (Pimenov 1974, 1978, 1983b). The distribution area is limited to the drier foothills of eastern Fergana. The main habitats are in the Tian-Shan part of the valley, and a small disjunction is located in the eastern part of the Alay range (Pimenov 1983b).

During the recent field investigation of plant diversity in the Uzbekistan part of the IPA site specimens of *S. unicaule* were collected (https://www.plantarium.ru) from the neighborhood of Airbagsh village. Accurate investigations of all the surrounding areas did not give positive results. Based on field studies in 2019–2021, it can be concluded that the Uzbekistan populations are represented only in the foothills of Arbagish.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan.

Distribution in FV. Middle mountain belt of Chatkal Range.

Tanacetopsis ferganensis (Kovalevsk.) Kovalevsk. in Novosti Sist. Vyssh. Rast. 9: 270 (1972)

Figs 7H, 10L

Note. This species is endemic to eastern Fergana and is known from several localities in the eastern part of the Chatkal, Atoynak, and Fergana ranges (Kamelin and Kovalevskaya 1993). It grows between rocks on the stony slopes of the middle and upper mountains. Only one locality is known in the study area; the gorge of the Archaly-Sai river, which is part of the Bozbu-Too.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan.

Distribution in FV. Middle and high mountain belts of Chatkal and Fergana ranges.

Category Aiv

Ten species were included in category Aiv.

As noted by Darbyshire et al. (2017) for the large majority of plant species belonging to IPA sub-criteria *Aiv* there are no formal global conservation status assessments in Central Asia. This is also an actuality because many cases focus on country-level distributions. Poor representation is a common problem for Central Asian species (Wilson et al. 2021), and international evaluation is rare due to the lack of close cooperation between neighboring countries.

Allium minutum Vved. in Byull. Sredne-Aziatsk. Gosud. Univ. 19: 124 (1934) Figs 11A, 12A

Note. During field observations in the vicinity of the Arbagish village in 2014 and later in 2021, a white-flowered *Allium* species was collected for the first time from the foothills of the Chatkal range in Uzbekistan. The species grew on the Artemisia steppe between 1100 and 1200 m. Detailed studies revealed that they did not match any *Allium*



Figure 11. The wild habitat of some plants in FV A Allium minutum B Astragalus syreitschikovii
C Fritillaria sewerzowii D Jurinea winkleri E Lagochilus knorringianus var. drobovii F Polycnemum perenne
G Pseudosedum ferganense H Tulipa bifloriformis I Leibnitzia knorringiana J Takhtajaniantha tau-saghyz.

species reported from the foothills or low mountain belt of the Chatkal range. After examining all *Allium* species from FV, the morphological characters of these specimens were identical to those of *Allium minutum* Vved., which was previously recorded only in the Alay and Trans-Alay ranges, and in Kyrgyzstan and Tajikistan (Vvedenskyi 1971; Lazkov and Sultanova 2014). Therefore, *A. minutum* is here reported as a new record for the flora of Uzbekistan and the Chatkal range in particular.

According to Vvedenskyi (1971) and Khassanov and Esankulov (2015), *A. minutum* is growing on gravelly and variegated outcrops of the upper belt of mountains. Examination of all published data (Vvedensky 1971; Khassanov 2000; Khassanov and Esankulov 2015) and herbarium specimens kept in Tashkent (TASH) showed the absence of data outside the Alay and Trans-Alay ranges. There is a high probability that the plants from southern Chatkal belong to a new species of the *Allium* genus. However, the plants from the highlands of the Alay Range and the low mountains of Chatkal do not differ morphologically.

Therefore, the lack of data, especially regarding the distribution in the intermediate regions between Chatkal and Alay ranges, necessitated us to accept southern Chatkal plants as *A. minutum*.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan, Tajikistan, Uzbekistan.

Distribution in FV. Middle and high mountain belts of Chatkal and Fergana ridges.

Astragalus syreitschikovii Pavlov in Byull. Moskovsk. Obshch. Isp. Prir., Otd. Biol., n.s., 44: 34 (1935)

Figs 11B, 12B

Note. The species belong to sect. *Myobroma* (Stev.) Bunge in sensu Kovalevskaya (1981) or sect. *Caprini* in sensu Podlech and Zarre (2013: 702). Initially, the species was described by N. Pavlov as endemic to the Syr Darya Karatau, Kazakhstan (Pavlov 1935). The author classified the species as an autochthonous Turanian element and assumed they would hardly be found in other regions of Central Asia. According to current data, *A. syreitschikovii* is endemic to limestone in the foothills and low mountains of western Tian-Shan (Kovalevskaya 1981) and is distributed to the north of the Chu-Ili mountains, Syr Darya Karatau, Santalash, and Chatkal range. The southern border of the species distribution falls to the northern side of the Akhangaran river basin.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kazakhstan, Kyrgyzstan, Uzbekistan. **Distribution in FV.** Foothills and low mountains of Chatkal Range.

Distribution in P.V. Poolinns and low mountains of Chatkai Range.

Fritillaria sewerzowii Regel in Bull. Soc. Imp. Naturalistes Moscou 41(I): 443 (1868) Figs 11C, 12C

≡ Korolkowia sewerzowii (Regel) Regel, Gartenflora 22: 163 (1873).

= Fritillaria discolor Mottet, Dict. Prat. Hort. 2: 431 (1893–1894).

= Korolkowia discolor Regel, Gartenflora 35: 349 (1886).

Note. Endemic to western Tian-Shan and one of the most ornamental species in the flora of Central Asia. Widespread in almost all mountain ranges of western Tian-Shan.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kazakhstan, Kyrgyzstan, Uzbekistan. **Distribution in FV.** Foothills and low mountains of Alay, Chatkal, and Kurama ranges.

Jurinea winkleri Iljin in V.L. Komarov (ed.), Fl. URSS, 27: 722, 616 (1962) Figs 11D, 12D

Note. The species are mostly distributed in FV. A disjunction is located in the Zirabulak low mountains (western Pamir-Alay). Current field surveys show that populations of the species survived only in the foothill zone. In the northern part of the valley, several isolated and fragmented populations are found in the Chust-Pap foothills (Tojibaev et al. 2022). This population stretches in fragments in a northeastern direction via the north of Namangan city, and relatively good micro populations are recorded in the variegated slopes of the vicinity of Arbagish and adjacent territories of Kyrgyzstan.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan, Uzbekistan. **Distribution in FV.** Foothills of FV.

Leibnitzia knorringiana (B.Fedtsch.) Pobed. in Fl. URSS 28: 594 (1963) Figs 11I, 12E

Note. The genus *Leibnitzia* Cass. are a unique example of the Asian–North American biogeographic disjunction pattern (Hansen 1988; Baird 2009). Two species of the genus, i.e., *L. lyrata* (Sch.Bip.) G.L.Nesom and *L. occimadrensis* G.L.Nesom, are of biogeographic interest because they are two American members of a predominately Asian genus (Nesom 1983). The other four Asian members of *Leibnitzia* occupy mostly high-altitude (upper limits of 3100–5000 m above sea level vary by species), semiarid habitats in the Himalayan region, China, Japan, Korea, Bhutan, Mongolia, Siberia (Hansen 1988), and Kyrgyzstan (Nesom 1983; Hansen 1988; Lazkov and Sultanova 2014). Among them, *L. knorringiana* is distinguished by the smallest distribution area, limited to the Naryn river valley, Talas, and the Chatkal and Fergana Ranges within Kyrgyzstan (Tulaganova 1993b). Only one population was found at the Bozbu-Too-Ungortepa IPA site, growing in the tract of Taldi-Bulak (Mountain Bosbu-Too).

Existing Conservation Threats. Farmlands, overgrazing.

General distribution. Kyrgyzstan.

Distribution in FV. Middle mountain belt and highlands of Naryn River Valley, Talas, Chatkal, and Fergana ranges.



Figure 12. Distribution map of the species of sub-criterion Aiv based on GeoCAT. A Allium minutum
B Astragalus syreitschikovii C Fritillaria sewerzowii D Jurinea winkleri E Leibnitzia knorringiana
F Lagochilus knorringianus var. drobovii G Polycnemum perenne H Pseudosedum ferganense I Tulipa bifloriformis J Takhtajaniantha tau-saghyz.

Lagochilus knorringianus var. *drobovii* (Kamelin & Tzukerv.) Lazkov, KH Bot. Monogr. Rev. Ser. 1: 230 (2016) Figs 11E, 12F

≡ Lagochilus drobovii Kamelin & Tzukerv. in Novosti Sist. Vyssh. Rast. 20: 166 (1983).

Note. Originally, the species was described as *Lagochilus drobovii* Kamelin & Tzukerv. and was limited to a narrow range in the villages of Shakaftar and Sumsar in southern Chatkal, Kyrgyzstan (Kamelin and Tschukervanik 1983; Tschukervanik 1987). Later, Lazkov (2016: 230) considered these plants as a variety of *Lagochilus knorringianus*, because the type specimen of *L. knorringianus* and the type specimen of *L. drobovii* were collected together. According to Lazkov (2016) *L. knorringianus* var. *drobovii* differs from *L. knorringianus* var. *knorringianus* by calyx, and sometimes the entire plant is more or less pubescent with short and long multicellular setaceous hairs. Here we report *L. knorringianus* var. *drobovii* as a new record for the Flora of Uzbekistan based on specimens collected in Arbagish village, Uzbekistan.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan, Uzbekistan.

Distribution in FV. Low mountains of Chatkal Range.

Polycnemum perenne Litv. in Trudy Bot. Muz. Imp. Akad. Nauk 7: 81 (1910) Figs 11F, 12G

Note. The genus *Polycnemum* L. is represented by 7–8 species distributed in North Africa, the Mediterranean, Europe, Asia Minor, Siberia, and Central Asia (Sidorenko 1968; corrected by POWO 2022). Three species grow in Central Asia (Pratov 1972c). Among them, *P. perenne* is distinguished by a life form (semi-shrub, the other two are annuals) and narrow distribution (endemic to western Tian-Shan and Alay). The main part of the range is located in the western Tian-Shan (from the Syr Darya Karatau in the north to the Kurama Range in the south), where it is confined to rocks, stony and gravelly slopes of foothills, and a low mountain belt (800–1600 m). The species is widespread in the study area.

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kazakhstan, Kyrgyzstan, Tajikistan, and Uzbekistan.

Distribution in FV. Foothills and low mountains of Alay, Chatkal, and Kurama ridges.

Pseudosedum ferganense Boriss. in Trudy Bot. Inst. Akad. Nauk S.S.S.R., Ser. 1, Fl. Sist. Vyssh. Rast. 1: 112 (1933)

Figs 11G, 12H

Note. The genus *Pseudosedum* (Boiss.) A.Berger falls into the category of medium size genus within Crassulaceae DC. and comprises about 14 registered taxa (Tojibaev et al. 2018b). Ten species grow in Central Asia (Pratov 1974), two species are noted for northern China (Kanjun 2001), and three isolated species belong to the "Flora Iranica" area (Jansson and Rechinger 1970).

Previously, *P. ferganense* was recorded as a new species for the flora of Uzbekistan (Tojibaev et al. 2018b). It occupies an isolated position within the genus and belongs to the monotype sect. *Tuberaria* Boriss. (Borissova 1939), with the roots forming tu-

bers. In the Uzbekistan part of the FV, the species grows in cracks in the rocks in the northern foothills and low mountains, and populations extend from the Chap tract to the Ungortepa massif and lower reaches of the Mailisay river basin (Arbagish).

Existing Conservation Threats. Habitat fragmentation, farmlands, overgrazing. **General distribution.** Kyrgyzstan, Uzbekistan.

Distribution in FV. Foothills and low mountains of Alay, Chatkal, and Kurama ranges.

Tulipa bifloriformis Vved. in Opred. Rast. Sred. Azii 2: 320 (1971)

Figs 11H, 12I

Note. The species is one of the most widespread tulip species, endemic to western Tian-Shan (Tojibaev et al. 2020; Dekhkonov et al. 2022). However, no threat of risk of reduction or extinction of the species has been identified in the study area and adjacent territories of western Tian-Shan. Moreover, this species showed increased seed germination and can easily colonize disturbed habitats (Tojibaev and Kadirov 2010). Nonetheless, climate modeling for Central Asian tulips suggests that climate change will have a significantly negative impact on the range size of all species, including those currently widespread like *T. bifloriformis* (Wilson et al. 2021).

General distribution. Western Tian-Shan.

Distribution in FV. Northern foothills and low mountains of FV.

Takhtajaniantha tau-saghyz (Lipsch. & G.G.Bosse) Zaika, Sukhor. & N.Kilian in PhytoKeys 137: 72 (2020)

Figs 11J, 12J

≡ Scorzonera uzbekistanica Czevr. & Bondarenko in Fl. Uzbekistan. 6: 436, 516 (1962).

≡ Scorzonera tau-saghyz subsp. usbekistanica, in Bot. Zhurn. (Moscow & Leningrad) 71: 1672 (1986).

Note. This species belongs to, perhaps, the most famous and interesting group of plants studied from an economic point of view. The discovery of a new rubber plant species (*Scorzonera uzbekistanica* Czevr. & Bondarenko) back in 1949 in eastern Fergana led to the explorations of this interesting territory. According to the authors of the new species, the discovered plants belonged to *Scorzonera* L. genus and differed from the already known *S. tau-saghyz* Lipsch. & Bosse (endemic to the Karatau mountains, western Tian-Shan, Kazakhstan) in a number of morphological features, first of all by "yellow latex, bare and on the edge of the jagged outer leaves of the wrapper and achenes, pubescent only along hardly noticeable veins". These distinctive features made it possible to recognize the Maylisay plants as new (Chevrenidi and Bondarenko 1950). However, the protologue was given diagnostic signs only in Russian. A valid description was given in the 6th volume of the Flora of Uzbekistan (Kovalevskaya 1962)...

Later, Kamelin and Tagaev (1986), *S. uzbekistanica* identified it as a subspecies of *S. tau-saghyz* (*Scorzonera tau-saghyz* subsp. *usbekistanica* (Czevr. & Bondarenko) Tagaev. Nevertheless, subsequent research on the genus *Scorzonera* did not properly investigate the eastern Fergana plants. Even in the Conspectus Florae Asiae Media (Tagaev 1993), this species is referred to as a synonym of *S. tau-saghyz*. Currently, all available modern sources, including Zaika et al. (2020), POWO (2022), accept *S. uzbekistanica* as a synonym of *Takhtajaniantha tau-saghyz*.

Existing Conservation Threats. Habitat fragmentation, farmlands. **General distribution.** Kazakhstan, Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan **Distribution in FV.** Foothills of southern Chatkal.

Socio-economic issues

The FV is one of the most densely populated regions of Central Asia and one of the major areas of concern regarding the conservation of natural landscapes. For centuries, the valley ecosystems have been under the pressure of anthropogenic impacts (Tojibaev et al 2018a). Since the mid-1970s, habitats of many endemic and rare plant species of FV have been impacted by humans. However, populations of some species were not found during field surveys in the 1970s (Vernik and Rakhimova 1982).

Human impacts on the environment by uncontrolled development and climatic changes have exacerbated the threatened status of numerous plant species and habitats in FV (Tojibaev et al. 2022). In densely populated regions with limited natural ecosystems, like FV, effective conservation actions need to consider the social, cultural, and economic interests of local humans. This postulate applies to IPA programs as well (Blasi et al. 2011). Since IPAs inevitably fit into a wider context and are not designed to exist in isolation, they should be considered when designing ecological networks (Blasi et al. 2011; Jongman and Pungetti 2004) and ecological corridors, planning the zonation of existing protected areas and defining key biodiversity areas (Eken et al. 2004). In the Uzbekistan part of the FV, such programs are still relevant despite the inability to create new protected areas.

Discussion

IPA Assessment

Within the FV Bozbu-Too-Ungortepa, the IPA site is important for wild plant conservation. Using the IPA criteria for the FV (Tojibaev et al. 2022), Bozbu-Too-Ungortepa massif qualifies as an IPA under criteria A.

Endemism and a new record for the national floras

Due to research within the framework of the Bozbu-Too-Ungortepa IPA, the massif has become one of the most studied areas of the FV, where many endemic and threat-

ened species of local flora are concentrated. Our research has shown that at least five species (*Allium tatyanae*, *Astragalus bosbutooënsis*, *Corydalis bosbutooënsis*, *Hedysarum turkestanicum*, and *Iris austrotschatkalica*) are endemic to this relatively small area, which is one of the highest rates in mountainous Central Asia. Of these, four species have been found and described by some of this paper's authors (G.A. Lazkov, K.Sh. Tojibaev, and F.A. Karimov). It is also noteworthy that *Iris austrotschatkalica* was described as the result of the identification of the IPA site (Tojibaev et al. 2014a).

Our inventory of the flora within the study area has significantly enriched the known flora of Uzbekistan with species previously noted only in countries adjacent to Uzbekistan. Some of these new records have been published prior to this work, including those on *Astragalus spryginii* Popov, and *Pseudosedum ferganense* Boriss. (Tojibaev et al. 2018b). Here we report the presence of more species newly discovered among the flora of Uzbekistan, including *Hyalolaena intermedia* Pimenov and Kljuykov (Lyskov et al. 2019), *Allium arkitense, A. minutum, Phlomoides alaica, Lagochilus knorringianus* var. *drobovii*, and *Corydalis bosbutooensis*. Additionally, modern research within the framework of the IPA has found some species here that were previously considered extinct in this area, including *Lamyropappus schakaptaricus*, and *Hedysarum gypsaceum* (Tojibaev et al. 2019).

Contribution of the present research

The second IPA site in FV occupies a transboundary position between Uzbekistan and Kyrgyzstan. The site was selected based on species endemism at national (Uzbekistan and Kyrgyzstan), regional (FV) and botanical province (Afghan-Turkestan province) levels. The research results of the current stage of IPA identification in the FV was completed in line with the core aims of the IPAs Programme (Anderson 2002; Darbyshire et al. 2017, 2019). The best available data in Central Asia was used to highlight key sites with high concentrations of endemic and threatened plant species, growing outside of protected areas. We also considered the criteria proposed by Shaltout and Eid (2016), which allow for species vulnerability, irreplaceability, and richness of vascular plant species in the IPA site.

The first studies on the identification of IPA sites were characterized by determining IPAs within different countries, and compiling lists of rare and threatened species (Al-Abbasi et al. 2010; Hall et al. 2011; Llewellyn et al. 2011; Artemov 2012; Olonova et al. 2013; Dimeyeva and Vesselova 2015; and etc.). Data conversion into maps was associated with a number of works (Blasi et al. 2009, 2011), methodical improvement of IPA criteria for a global approach to plant conservation (Darbyshire et al. 2017), and just a few were devoted to the transboundary IPAs (Shuka and Malo 2010; Shuka et al. 2014). The IPA research remains relevant due to the global loss of biodiversity and critical habitats around the world. Such critical habitats include the following regions: the tropics, with its high species diversity (https://www.kew.org/science/our-science/ projects/tropical-important-plant-areas; see also Darbyshire et al. 2019; Dani Sanchez et al. 2021; andect.); Europe, with its high level of floristic data (Sparrius et al. 2019); and Central Asia (Tojibaev et al. 2022), botanically poorly-known part of Asia. Due to this, the IPA study carried out in the FV has some advantages. In particular, among the wide range of IPA studies, ours is one of the few that applied geospatial analysis of rare and threatened species for Red List assessment (Bachman et al. 2011). In addition, we provide detailed information on the botanical geography and taxonomy of species. Together, the information provided will contribute to the rapid dissemination of recent, easily accessible, data concerning understudied species and habitats, as well as the definition of practical boundaries (Blasi et al. 2011) in botanically poorly studied areas.

Further research

Although the current data on the plant diversity of Bozbu-Too-Ungortepa IPA is satisfactory, the next stage requires extensive botanical research in the following areas: (1) comprehensive surveys to build up a basic inventory of the plants in the area based on grid mapping; (2) vegetation studies to determine and map the major vegetation types; and (3) targeted monitoring programs taking into account threats to rare habitats and species in the future.

Lastly, any serious conservation planning within the Bozbu-Too and surrounding transboundary area should implement proper socio-economic research programs to examine ways to engage local stakeholders (Margules and Pressey 2000).

Conclusion

The second phase of IPA research in the FV shows the effectiveness of the basic principles of IPA in the conditions of mountainous Central Asia. In contrast to the previous stage, here we explored a transboundary territory by combining the efforts of scientists in two neighboring countries (Uzbekistan and Kyrgyzstan). This is an important step in the creation of a regional database of rare and endangered species, as well as in that of a regional Red list, which can serve to focus conservation action at the transboundary level. Continuing studies, such as these in transboundary areas, contribute to increasing the level of floristic knowledge in poorly studied areas. Furthermore, the studies would attract the attention of government environmental organizations as well as international organizations at the global scale, to the conservation of threatened species and critical habitats. Capacity building requires the standardization of approaches, extensive field research on both potentially threatened species and critical habitats, as well as new research into the causes of plant habitat degradation.

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REVIEW ARTICLE



Numerous uncertainties in the multifaceted global trade in frogs' legs with the EU as the major consumer

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Abstract

The commercial trade in frogs and their body parts is global, dynamic and occurs in extremely large volumes (in the thousands of tonnes/yr or billions of frogs/yr). The European Union (EU) remains the single largest importer of frogs' legs, with most frogs still caught from the wild. Amongst the many drivers of species extinction or population decline (e.g. due to habitat loss, climate change, disease etc.), overexploitation is becoming increasingly more prominent. Due to global declines and extinctions, new attention is being focused on these markets, in part to try to ensure sustainability. While the trade is plagued by daunting realities of data deficiency and uncertainty and the conflicts of commercial interests associated with these data, it is clear is that EU countries are most responsible for the largest portion of the international trade in frogs' legs of wild species. Over decades of exploitation, the EU imports have contributed to a decline in wild frog populations in an increasing number of supplying countries, such as India and Bangladesh, as well as Indonesia, Turkey and Albania more recently. However, there have been no concerted attempts by the EU and present export countries to ensure sustainability of this trade. Further work is needed to validate species identities, secure data on wild frog populations, establish reasonable monitored harvest/export quotas and disease surveillance and ensure data integrity, quality and security standards for frog farms. Herein, we call upon those countries and their representative governments to assume responsibility for the sustainability of the trade. The EU should take immediate action to channel all imports through a single centralised database and list sensitive species in the Annexes of the EU Wildlife

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Trade Regulation. Further, listing in CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora) can enforce international trade restrictions. More joint efforts are needed to improve regional monitoring schemes before the commercial trade causes irreversible extinctions of populations and species of frogs.

Keywords

amphibians, biodiversity, CITES, disease, over-exploitation, sustainability, taxonomic status, wildlife trade

Introduction

Three decades ago, initial signs of global declines in amphibian populations were reported (Blaustein and Wake 1990; Pechmann and Wilbur 1994). Thirteen years ago, Stuart et al. (2008) edited their compendium "Threatened World of Amphibians" as a result of the Global Amphibian Assessment and synthesised knowledge on the science and threats detrimentally impacting amphibian species on a global scale. Threats such as habitat destruction (Cox et al. 2006), pollution (Blaustein and Johnson 2003), domestic use and trade (Mohneke 2011; Turvey et al. 2021), international trade (Andreone et al. 2006; Carpenter et al. 2014; Auliya et al. 2016) and climate change (Blaustein et al. 2010) have been well studied in many areas, but amphibians are also particularly vulnerable to pathogens, such as ranaviruses (Cunningham et al. 1996; Daszak et al. 1999; Miller et al. 2011; Bayley et al. 2013), mycotic diseases (Daszak et al. 1999; Fitzpatrick et al. 2018) and parasites (Kim et al. 2016). Similarly, a recent study revealed that frogs act as intermediate hosts of the trematode Alaria alata and human consumption of frogs' legs containing larvae of the parasite can promote alariosis, a potentially deadly infection (Korpysa-Dzirba et al. 2021). However, it has also been emphasised that these threats can causally and synergistically interact (Ficetola et al. 2007; Sodhi et al. 2008; Hayes et al. 2010; Ford et al. 2020). As early as 1993, amphibian mortalities were attributed to the chytrid fungus, Batrachochytrium dendrobatidis (Bd) (Berger et al. 1998), with several possible extinctions and its spread across central America up to the late 1980s (Cheng et al. 2011). In the years that followed, the scale of this panzootic disease (chytridiomycosis), became apparent and scientific papers highlighted the fungal disease with more than 500 amphibian species around the world affected by Bd (Scheele et al. 2019). In addition, a new fungus specifically affecting salamanders, Batrachochytrium salamandrivorans (Bsal) was also identified (Martel et al. 2013). Notably, during the human pandemic COVID-19, commercial trade is both the principal source and the most viable means of spreading emerging zoonotic diseases (see Vora et al. 2022).

The international trade of live amphibians infected with either *Bd* or *Bsal* has since been highlighted (e.g. Fisher and Garner (2007); Kriger and Hero (2009), Catenazzi et al. (2010); Yuan et al. (2018); Fitzpatrick et al. (2018); Hughes et al. (2021); Thumsová et al. (2021)) and its detrimental impact threatens naïve populations with extinction (Martel et al. 2014; Stegen et al. 2017). To date, considerable research has contributed
to an increased understanding of regional, national and global declines of amphibians and understanding of the spread and pathogenicity of diseases. However, the impact of wildlife trade and associated diseases on local populations remains poorly understood.

While the international amphibian pet trade includes a broader range of species with many frogs still coming from the wild (Auliya et al. 2016; Hughes et al. 2021), species harvested for consumption as food (e.g. frogs' legs trade), represent only a small number of species. However, annual exports for the food trade are in the thousands of tonnes or hundreds of millions of individuals (Kusrini and Alford 2006; Gratwicke et al. 2010). Notwithstanding the considerable implications on species survivorship, we know less about the impacts of trade than most other threats in terms of effect on local biotic communities and their ecosystems, the spread of diseases and issues resulting from the interaction of wild-caught and farmed species (Lutz and Avery 1999; Dökenel and Özer 2019; Ribeiro et al. 2019). While the history of frog farming is marked by many setbacks, it has steadily increased in scale in recent years (FAO 2020; Dodd and Jennings 2021). Despite this growth, the potential ecological impact of frog farms is often neglected (see below) and over-exploitation of wild-caught frogs is ongoing (IUCN SSC Amphibian Specialist Group 2020e; Çiçek et al. 2021; Hughes et al. 2021). In addition, the taxonomic status of taxa exploited for consumption is not unequivocally clarified [e.g. the Fejervarya cancrivora complex at least three species (Kotaki et al. 2010; Kurniawan et al. 2011; Yodthong et al. 2019), taxonomic challenges in Pelophylax spp., i.e. P. lessonae and P. ridibundus (Holsbeek et al. 2008; Holsbeek and Jooris 2010; Hauswaldt et al. 2012) and the Limnonectes kuhlii complex (e.g. McLeod et al. 2011; Dehling and Dehling 2017; Stuart et al. 2020; Suwannapoom et al. 2021)]. Likewise, it is necessary to create an accurate and up-to-date database of the role the major consuming countries take in terms of numbers of wild caught/farmed animals, supplying countries, harvest locations, farms involved (cf. with data records of the Law Enforcement Management Information System (USFWS-LEMIS Database 2023), mortality figures etc., with a focus on the European Union (EU) (Veith et al. 2000; Potočnik 2012; Cicek et al. 2021) and Switzerland (see Dubey et al. (2014); Dufresnes et al. (2018)). For example, TRACES is an online platform of the EU established to certify imports of animals and their products according to sanitary standards (https://ec.europa.eu/food/animals/traces_en, see Suppl. material 3), but lacks speciesspecific data, missing an important opportunity to monitor species in trade.

Enforcement of laws, regulations and quotas or harvest limits is particularly challenging for the transport and trade of frogs' legs. Many species are very similar in their morphology and as products are skinned, processed and frozen, gross mislabelling is likely and hard to verify (Veith et al. 2000; Dittrich et al. 2017; Ohler and Nicolas 2017). In fact, it is impossible for enforcement authorities to assign frogs' legs to a species without genetic methods; hence, authorities can only check documents enclosed in a consignment and assume that they are true.

Herein, we provide an overview on the EU's central role as the primary ultimate destination for the global trade in frogs' legs and its corresponding responsibility for resulting ecological risks and impacts. Furthermore, our review summarises knowledge on the current status of international trade in both live frogs and parts for human consumption. We primarily outline certainties (e.g. loss of biodiversity, destabilisation of ecological communities in their ecosystems, flawed farming operations, genetic pollution) against the manifold uncertainties underlying this trade (lack of documentation to assess sustainability of trade; species identification of individual frozen frogs, skinned frog bodies or parts thereof; and international regulation of species not listed in the appendices of CITES). Clear identification of these deficiencies should oblige policy-makers from responsible consuming countries to follow revised and newly-implemented legislation and, where appropriate, apply the precautionary principle as a crucial safeguard for the survival of many amphibian species. Understanding the dimensions of the frogs' legs trade is challenging (since much of the global data is not available after 2009, as monitoring stopped), even when we had better data (Suppl. material 1: fig. S1). Initially, Asia dominated the export trade (especially India, Indonesia and China, but China ceased in 2007), followed by Europe (until 2006) and the US (a small proportion, almost entirely gone by 2008) (Atlas of Economic Complexity 2023; see Suppl. material 1, Fig. 1). However, these trends have not remained consistent and many complexities have revealed themselves more recently. Thus, understanding and updating our knowledge of global trade is paramount to effective interventions if we want to ensure a sustainable trade. We offer these suggestions to enable long-term sustainability of the trade, as well as the amphibian populations it is dependent upon and the humans whose livelihoods are intricately intertwined.

Methods

Apart from information retrieved from previous studies (Altherr et al. 2011; Auliya et al. 2016), this review is mainly based on a systematic literature survey from conscientiously extracted relevant published information related to the international trade in frogs' legs (e.g. taxonomy, ecology, disease, threats and conservation). For the identification of relevant publications, we used a number of English [e.g. x-country, x-species (e.g. Fejervarya) frog, trade, frogleg / frogs' legs, frog meat, commercial, culture, farming, threats (that could specifically be "pollution" or "climate change") and Indonesian [katak/kodok (for "frog"), Jawa, x-jenis (scientific name of a given species), dagang (trade), ancaman (threat), kaki (leg), pada (thigh)] search terms in Google Scholar searches. These terms were used because they would be in publications that feature amphibian trade in either English or Bahasa Indonesia. Number and order of terms entered per language was changed during searches. Searches in Bahasa Indonesia were implemented because Indonesia is recognised as the current major supplier of frogs' legs to European markets (e.g. Warkentin et al. (2009); Altherr et al. (2011); Potočnik (2012); EUROSTAT (2020)). Additionally, publications from the International System for Agricultural Science and Technology (AGRIS) of the FAO were scanned for "frog legs" (https://agris.fao.org/, see Suppl. material 3).

Taxonomy largely followed Frost (2021) and relevant papers that outline cryptic, look-a-like species or where taxonomic status remains uncertain (e.g. Holsbeek et al. (2008); Hasan et al. (2012); Yodthong et al. (2019)). With reference to the North American bullfrog, Rana catesbeiana listed in the genus Lithobates (Dubois 2006), most recent studies now list the genus as Aquarana (Dubois et al. 2021), while the trade data still refer to Lithobates. In order to avoid confusion, in this study, we use Lithobates. In addition, AmphibiaWeb (https://amphibiaweb.org/, see Suppl. material 3) was surveyed to filter information relevant to species involved in the commercial food and pet trade. Databases documenting species and volumes imported into the EU include EUROSTAT (https://ec.europa.eu/eurostat/web/main/data/database, see Suppl. material 3) and were filtered from the sub-database "EU trade since 1988 by HS2,4,6 and CN8" (categories 02082000 and 02089070 are frogs' legs fresh, chilled or frozen) selected for the period 2010 to 2019. Remarkably, imports of live frogs are not specifically documented by EUROSTAT, but assigned to an unspecific customs tariff number, generally describing "animals, other, live". Additionally, there is distinction between import of "wild" versus "cultured/farmed" specimens. We also extracted import data from the United States Fish and Wildlife Service (USFWS) and LEMIS databases for the period 2015-2020 (USFWS-LEMIS Database 2023), focusing on species that are traded either in kg or in large numbers and known to be relevant for human consumption (e.g. Hoplobatrachus rugulosus and Lithobates catesbeianus).

A study was simultaneously conducted for a current snapshot/analysis of the French market (the EU's major consuming nation of frogs' legs). Data were retrieved from the French Customs statistics for the period 2010–21 (LeKiosque.finances.gouv. fr; accessed 16 April 2019 and 26 April 2022). Additionally, in December 2021, an online survey of the French market was carried out. Websites used for this included major supermarkets, frozen food brands, Asian food supermarkets (i.e. Auchan, Cora, Monoprix, Picard, Tang Frères etc.). Another market survey of e-mail alerts was conducted between 23November 2021 and 9 February 2022. The survey was conducted using Google Alert with the keywords "frog legs" in French and in singular and plural forms, asking to receive all new content regardless of the source (News, Blogs, Web). The commercial offers were sorted and analysed.

An advanced search on "The IUCN Red List" (conducted August 2022), based on the following filters; (a) Taxonomy > Amphibia, (b) Threats > Biological Resource use > Intentional use and (c) Use and Trade > Food (Human), was also completed. The resulting species were assigned to their native regions/countries and tabulated with information on current IUCN Red List status ([IUCN 2021], amending updated assessments in January 2023 according to [IUCN 2022]), CITES appendix listing and information indicating a regional overharvest or overexploitation in general (see Table 3, Suppl. materials 2, 4). Subsequently, all CITES-listed amphibian species were filtered in SPECIES+ (https://www.speciesplus.net, see Suppl. material 3), a website developed by CITES and UNEP-WCMC that includes all species in appendices/annexes of CITES (n.b., only 2.5% of amphibian species are CITES listed), the EU Wildlife Trade Regulations and the Conservation of Migratory Species (CMS). CITES Appendix listings were checked with the species filtered in the IUCN Red List where international trade for consumption (food) was indicated. Those species were entered in the CITES trade database (https://trade.cites.org/, see Suppl. material 3) to record information on trade (e.g. years, volumes, countries of export and import and sources of trade) and to check if specific population trends are emerging. Indonesian harvest and export quotas were surveyed in the period 2015 to 2022, according to the annual published quota lists (e.g. Indonesian Ministry of Environment and Forestry (2022)).

Once we had a list of species potentially traded for food, we were able to pair that list with the IUCN data mapping species distributions. First, we downloaded amphibian ranges from the IUCN website (https://www.iucnredlist.org/). We then uploaded these into ArcMap 10.8 and selected all species in trade using the "joins and relates" function, before extracting these species. Species ranges were then dissolved so that each species was represented by a single polygon (though this could be a multipart polygon). This was then split into groups of 30 species before overlaps were counted using the "count overlapping polygons" toolbox for each subset. This was purely for processing and all species were included in total. These were then all converted to a raster with a 10 km resolution and each stack was summed using the "mosaic to new raster" function to sum values and map the number of species being consumed in each geographic area.

In addition, we used "union" to combine species' ranges with a map of the world (from thematic mapper), the species range country combinations dissolved to list each species once for each country it was in and the summary statistics tool was used to calculate the number of species being traded for consumption for each country. This table was then related to the original country map to show the number of species being traded for consumption per country. This was then repeated for just those species being traded internationally for consumption.

Results

After describing current import volumes of frogs' legs into the EU and the main supply regions, we highlight the species that make up the international frogs' legs trade, describe national consumption trends and finally provide information on threats impacting species/populations, indicate amphibian population trends and broader ecological impacts of the frogs' legs trade.

The role of the European Union and its member States

In the study period 2010 to 2019, total imports of frog's legs into the EU numbered 40.7 million kg. This total weight can be converted, when 1 kg equals 20–50 individual frogs (Veith et al. 2000), to at least 814 million and up to roughly 2 billion frogs.

The EU's role of responsibility should also imply sustainable harvest and trade of species in supplier countries. The following example makes it clear what abuses accompany this trade; (1) according to Indonesia's annual harvest/export quotas for *E. cancrivora* (there is no information on how Indonesia derives its annual quotas, so there is no data basis for sustainable trade), in the period 2016–2020, ca. 295.3 million kg were exported (1 kg equates to 15–22 individual specimens [cf. with Veith et al. (2000) above]; Indonesian Ministry of Environment and Forestry 2016–2020) resulting in ca. 5.4 million individual *F. cancrivora*; (2) to date there is no information on mortality prior to export. As early as 1986, an estimated pre-export mortality rate of 10–20% was reported, but mortality during the export process may be highly variable (Niekisch 1986). Herein, we assume that every export also includes an estimated number of dead animals for which the importer is also responsible; here we refer to the EU.

Regarding the export of live animals, wholesalers have been found to have mortality rates of around 45% for amphibians, meaning live trade levels may need to be in higher volumes to satisfy demand when many frogs die in transit, with many coming from the wild (Ashley et al. 2014).

In the study period 2010–19 (EUROSTAT 2020), Belgium leads EU countries in imported quantities of frogs' legs, with a total of 28,430 tonnes (69.8%), ahead of France with 6790 tonnes (16.6%), followed by the Netherlands (2620 tonnes; 6.4%), Italy (1790 tonnes; 4.3%) and Spain (923.4 tonnes; 2.2%) (Table 1). Smaller quantities were imported by the United Kingdom (68.8 tonnes), Croatia (28.5 tonnes), the Czech Republic (27.8 tonnes), Poland (12.5 tonnes), Romania (2.8 tonnes) and Germany (1.8 tonnes). Within the EU, Belgium re-exports a large part of its imports to other EU countries. For example, Belgium re-exported 20,920 tonnes to France (> 73% of all its imports in the study period) and 1410 tonnes to the Netherlands (ca. 5% of all its imports in the study period) and, accordingly, Belgium consumed 21% of its total imports.

	Major EU importers	Major suppliers of fr	ogs' legs into the EU
Belgium	28,429	Indonesia	30,019.4
France	6794.4	Vietnam	8439.4
Netherlands	2621.5	Turkey	1593.7
Italy	1787.2	Albania	586.5
Spain	923.4		

Table 1. Main EU importers/consumers and suppliers of frogs' legs (in tonnes) for the period 2010-2019. Source: EUROSTAT (2020).

France and the frogs' legs trade

Due to the introduction of advanced technologies of freezing methods in the 1970s, storage constraints were reduced and transport routes of frogs' legs became possible. This transformed the traditional frogs' leg trade in France, bringing some local frog populations to the brink of extinction (Ohler and Nicolas 2017 and references therein). Since at least the 1980s, France has historically been considered the main consumer of frogs' legs. According to Le Serrec (1988), France imported a total of 4522 tonnes of frogs' legs in 1983. Based on this fact, France initiated studies to gain clarity on species

composition as well as potential ecological damage from intense commercialised trade (MNHN 2012; Ohler and Nicolas 2017).

From 2010-19, France imported 30,015 tonnes of fresh, refrigerated or frozen frogs' legs (ca. 600-1500 million frogs; Veith et al. (2000)), according to French customs statistics (https://leKiosque.finances.gouv.fr/). France's main suppliers are Indonesia (24,102 tonnes or 80.3%), Vietnam (3941 tonnes or 13.1%), Turkey (1017 tonnes or 3.4%), Belgium (226 tonnes or 0.8%) and Albania (219.6 tonnes, 0.7%). For the same period, the quantities imported from Belgium to France differ widely depending on whether the data source is Eurostat or French customs due to two different statistical concepts. France separately lists the country of direct export origin and country of original export when the country of origin is not an EU country. Original origin prevails in the French statistical data. As a result, some frogs' legs are considered by the French methodology as imported from Indonesia and not from Belgium, even if they have transited through Belgium. Annual imports did not fluctuate significantly between 2017 and 2020, with an average of 2669 tonnes/year. A drop to 1826 tonnes is prominent in 2021, still a relatively high figure despite the paralysis of international trade due to COVID-19. Similarly, France also is a hub for re-exportation of frogs' legs. From 2017–20, France shipped 385 tonnes of frogs' legs, mainly destined for markets in Belgium (292 tonnes; 75.8% of total tonnage shipped), Luxembourg (24.4 tonnes; 6.4%) and Germany (16.6 tonnes; 4.3%). In 2021, it is notable that France also re-exported 13.9 tonnes (3.6%) to Vietnam.

Results of the online market survey in December 2021 indicate 20 frogs' legs food products readily available. Of these 20 products, 11 originated from Indonesia, three from Vietnam, one from France and one from the "EEC (Turkey, Albania etc.)". This last indication is confusing because the European Economic Community (EEC) was dissolved in 1993 excluding Turkey and Albania and both are not EU member States. With regard to the indication of France as a source country, these products are precooked frogs' legs that do not originate from France and the species indicated is "wild *Limnonectes* [*Rana*] *macrodon*" endemic to western Indonesia (cf. Table 2). Four sources do not provide information on the country of origin within the product description or packaging. Regarding species name, six sources indicate *Rana macrodon*, three *Fejervarya cancrivora*, another three *Hoplobatrachus rugulosus*, one "*Rana macrodon* or *Fejervarya cancrivora*" (here we assume the sourcing from different suppliers, resulting in insufficient traceability for species identification) and one *Rana esculenta*.

For six sources, both product description and packaging do not indicate a species name. With regard to EU legislation, lack of information (species or country of origin) is a violation of EU rules [Commission Regulation (EC) No 2065/2001 of 22 October 2001 detailing rules for the application of Council Regulation (EC) No 104/2000 as regards informing consumers about fishery and aquaculture products; https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32001R2065&from=FR). In eight sources, origin is highlighted as "wild", three refer to "fishing" (e.g. fresh water, rice fields) and, in one, "collected" is indicated as the source. Not a single product, however, indicates a captive bred or farmed source. Besides raw or cooked frogs' legs,

"frairine" is also offered for sale, a mixture of pork and frogs' legs seasoned with white wine. For this mixed product, there is no information on the origin or species involved.

An additional market survey through Google Alert for more than 10 weeks (see Methods) identified 38 commercial offers for frogs' legs (20 from Belgium and 18 from France). Regarding the offers from France, trends from the December 2021 study are largely confirmed, with only one offer indicating an origin "Vietnam and/or Indonesia captive bred".

In addition to imports, the French market is also supplied with wild-caught native species. Short marketing circuits, such as local restaurants, are supplied with Rana temporaria, a nationally protected species in France (https://www.legifrance.gouv.fr/ loda/id/JORFTEXT000017876248/, accessed April 2022, see Suppl. material 3). Despite the legal framework for harvest, numerous exemptions are granted. For example, > 2 million *R. temporaria* are legally caught each year in the Franche-Comté region (https://www.bourgogne-franche-comte.developpement-durable.gouv.fr/ranaculturebourgogne-franche-comte-dossiers-de-a6583.html, accessed June 2022, see Suppl. material 3). An exemption may exist if an offtake of < 1500 frogs is requested, as this is considered "familial". Poaching offences are also recorded and a distinction is made between captures without a permit, those exceeding quotas or if the captures are outside authorised time periods. In October 2018, a couple were fined €2500 for the capture of 4000 R. temporaria, even though they possessed a permit for the capture of 1000 specimens (https://robindesbois.org/en/a-la-trace-n23-le-bulletin-de-la-defaunation/ RobindesBois, "On the Trail" No. 23, 2019). In the same year, during eight inspections and three searches conducted under a judicial warrant, a total of 171 traps were seized, enabling the release of 17,950 grass frogs (R. temporaria) and 10 m³ of eggs into the natural environment (Office national de la chasse et de la faune sauvage; ONCFS, 9 May 2018).

Major suppliers of species for the frogs' legs industry in the EU

There is no doubt that the trade in frogs' legs for consumption is a global issue, with most countries involved in the trade as exporter, importer or some combination (Grat-wicke et al. 2010; Suppl. material 1: figs 2, 3). In recent decades, there have been four major source regions exporting edible frogs or body parts (wild and/or farmed) into the EU: (1) East Asia, i.e. China and Taiwan (Warkentin et al. 2009; Altherr et al. 2011; Shreshta 2019), (2) Southeast Asia, i.e. Indonesia and Vietnam (Niekisch 1986; Kusrini and Alford 2006; Warkentin et al. 2009; Gratwicke et al. 2010; Ohler and Nicolas 2017; Shreshta 2019), (3) South Asia, i.e. India and Bangladesh (Niekisch 1986; Le Serrec 1988; Warkentin et al. 2009) and (4) eastern Europe i.e. Turkey and Albania (Warkentin et al. 2009; Şereflişan and Alkaya 2016; Çiçek et al. 2021). The United States, another major importing country for frogs and their body parts, is supplied from Asia and South America (Warkentin et al. 2009; US USFWS-LEMIS Database 2023). Based on LEMIS data, main suppliers for the US market for *L. catesbeianus* were Mexico (labelled as wc, "wild capture"), Ecuador (farmed) and China (farmed).

Hoplobatrachus rugulosus was imported from Thailand (farmed) and Vietnam (wc) and *L. forreri* only from Mexico.

For most recent trade routes from source countries to importers and consumers into the EU, see Fig. 1.

Within the study period 2010–19, Indonesia clearly represents the leading supplier for the European Union's frogs' legs with 30,019.4 tonnes (74%), followed by Vietnam (8439.4 tonnes; 21%), Turkey (1593.7 tonnes; 4%) and Albania (586.5 tonnes; 1%) (Table 1, Fig. 1).

Comparatively smaller amounts were supplied by China (37.7 tonnes), India (15 tonnes), Thailand (9.2 tonnes), Malaysia (7.6 tonnes) and South Korea (0.3 tonnes), resulting in less than 1% of the EU's total imports (EUROSTAT 2020).

Indonesia

Europe has been the major importer of frogs' legs for many decades, with exports from Indonesia contributing to 83% of all European imports (Kusrini and Alford 2006). Already in 1969, Indonesia exported frog's legs (as fishery products; Mikrimah (2009)) to Europe and, in the 1970s, Indonesia was considered the third largest exporting country of frogs' legs after India and Bangladesh (Susanto 1994; Warkentin et al. 2009). While EU imports of frog's legs exported from Indonesia amounted to > 3 tonnes of frog's legs in 1987, exports in 1993 increased to 4.7 tonnes, corresponding to 94–235 million individual frogs (cf. Veith et al. 2000).



Figure 1. The EU as the major consuming region of frog's legs in the period 2010-2019, with major supplying countries in SE-Asia (Indonesia, Vietnam) and eastern Europe (Turkey, Albania) and major importing countries (Belgium, France, Netherlands, Italy and Spain). Sources: TRAFFIC (2018); EU-ROSTAT (2020).

Species involved in the international food trade are mainly represented by members of the family Dicroglossidae (Fejervarya and Limnonectes) (Kusrini 2005). However, at least 14 anuran species are exploited for the food trade and just four 'species' dominate the trade (Fejervarya cancrivora, F. limnocharis, L. macrodon and Lithobates catesbeianus). Of these, only the latter species, the non-native to Indonesia, L. catesbeianus, is cultured from farms (Altherr et al. 2011) (Table 3). According to Kusrini (2005), the export of 28-142 million frogs annually is approximately only one seventh of the animals harvested for the domestic market across Indonesia, with many smaller species consumed in Indonesia (local species are favoured), while larger ones of at least 100 mm snout-vent length (only about one eighth of the frogs caught) are destined for exports (Kusrini 2005; Kusrini and Alford 2006). Interestingly, Ohler and Nicolas (2017) provide size estimates of Fejervarya cancrivora in the French trade and ascertain that many individuals are, indeed, smaller than 100 mm. While major harvest regions in Indonesia include Sumatra and Java (Kusrini and Alford 2006), exploitation of anurans for food in Kalimantan appears to be less common, but frog's legs are traded "from Sulawesi to big exporting cities, such as Makassar or Jakarta before leaving the country" (Iskandar 2014).

Export quotas within Indonesia list species, but on reaching the EU, species level information is not recorded (see Table 2). DNA analysis showed that *Fejervarya cancrivora* was clearly the most dominant species imported into the EU and imports declaring other species i.e. *Limnonectes macrodon*, *Fejervarya limnocharis* and *Lithobates catesbeianus*, had been mislabelled (Ohler and Nicolas 2017).

Annual export quotas

Annually, Indonesian authorities publish harvest and export quotas of CITES and non-CITES species native to the Indonesia (but possibly not the actual export values). For species listed in Table 2, harvest/export quotas issued for the period 2015–2022 were determined (Indonesian Ministry of Environment and Forestry 2015–2022).

Amongst quotas established for edible frog species, trade for the purpose of "consumption" is indicated for both *Fejervarya cancrivora* and *F. limnocharis*. However, only in 2015, for *F. limnocharis*, a specific number of individuals was designated for consumption (Table 2). While export quotas for *F. cancrivora* in 2015 were only considered for pets (according to the recorded details), in 2016, a 37,155fold increased quota was set for consumption purposes. From then onwards, quota figures declined steadily, stagnating in the last two years with the collapse in 2019 remaining unexplained. It also remains unclear what reasons the number of individuals per kilo were reduced as of 2018 (Table 2). In 2015–16, export quotas for skins of *Limnonectes macrodon* were established and, thereafter, no quotas were allocated to the species. There is no information on the whereabouts or use of the skinned bodies and the fact why no quotas have been established for the species since 2017 (Table 2).

	2015	2016	2017	2018	2019	2020	2021	2022
Fejervarya	2250 (pet)	83,599,250	78,498,000	72,086,805	4,100,850	56,985,845	56,985,845	56,985,845
cancrivora		(cons.) [1kg	(cons.) [SVL	(cons.) [SVL				
		= 22 indiv.]	= 22 indiv.]	= 15 indiv.]	= 15 indiv.]	= 15 indiv.]	≥ 9 cm]	≥ 9 cm]
Fejervarya	12,150;	3600 (pet)	11,270	630 (pet)	1080 (pet)	1235 (pet)	1235 (pet	1235 (pet)
limnocharis	(10,000		(pet)					
	for cons.)							
Limnonectes	540 (pet)	540 (pet)	588 (pet)	0	90 (pet)	95 (pet)	95 (pet)	95 (pet)
kuhlii								
Limnonectes	10,350;	10,350;	0	0	0	0	0	0
macrodon	10,000	9000 (skin);						
	(skin)	1350 (pet)						

Table 2. Indonesian export quotas of species known to be consumed nationally and internationally; cons = consumption; indiv. = individuals; SVL = snout-vent length. Sources: Indonesian Ministry of Environment and Forestry (2015–2022).

Farming operations in Indonesia

In 1982, commercial frog farming was established in Indonesia only involving non-native species (Kusrini and Alford 2006). In 1983, *Lithobates catesbeianus* was introduced to Indonesia for the purpose of commercial farming (Susanto 1994) and, despite Susanto's comprehensive booklet on frog cultivation, 20 years later, there was no evidence that commercial breeding of this species has shown successful trends (Kusrini 2005). Despite government support programmes for the commercial breeding of frogs, the initiative remained less promising mainly because costs of harvesting wild-caught native species are lower (Kusrini 2005). Not only are high costs of breeding bullfrogs leading many farms to stop breeding *L. catesbeianus*, the susceptibility of the species to disease is also a factor (Kusrini and Alford 2006). More recent information on frog farms in Indonesia is not available, but examination of stable isotopes of frogs' legs in the trade from Indonesia indicate that commercial frog farms are still not established and that wild-sourced populations are being harvested, not farmed species (Dittrich et al. 2017).

Vietnam

Indonesia and Vietnam represented the largest exporters of frogs' legs in the period 2003–2007 (Altherr et al. 2011). In 2006 alone, Vietnam exported 573 tonnes of frog's legs (UN Commodity Trade Statistics Database 2010, in Altherr et al. (2011)), while, in the period 2010–2019, Vietnam supplied the EU with > 8400 tonnes frog's legs, representing the second largest supplier of frogs' legs into the EU (EUROSTAT 2020).

It is challenging to determine sources of current frogs' legs from Vietnam, whether they are farmed or wild-caught. According to Nguyen (2014), the governmental regulation of frog farming operations in Vietnam was meagre. Exports of frog's legs from Vietnam to Canada are based on permits documenting captive-reared *H. rugulosus* (Gerson 2012). Quoc (2012) also states that the harvest of wild-sourced individuals is unstable and very difficult to estimate, thus quantities for neither wild-caught nor farmed frogs can be indicated in a "value chain framework of the frog industry". Nevertheless, forensic research could confirm frog's legs of *H. rugulosus* that have been sourced from farms (Dittrich et al. 2017). Collection of wild individuals is intended to replenish frog farms, still a prospect considered challenging with *H. rugulosus* (Borzée et al. 2021).

Farming operations in Vietnam

According to Nguyen (2000), households in the Provinces of Hanoi, Ha Tay and Hai Duong have established breeding frog farms, but do not keep up with national demand and the majority of frogs for national consumption are sourced from wild populations.

The many risks associated in frog farming in southern Vietnam, Tien Giang Province and Ho Chi Minh City, have been highlighted by Nguyen (2014). In particular, private established farms raise concerns about quality standards and risk management. Interviews with representatives of various interest groups revealed that efforts to produce frogs commercially often lack the necessary husbandry for successful breeding, starting with choice of location for such a project, selection of suitable stock and species composition, as well as knowledge of breeding, diseases, hygiene for animals and humans, environmental pollution etc. (Nguyen 2014). In recent years, frog farming operations in Vietnam experienced an upswing and the country is considered the second largest producer of farm0raised frogs (U.S. Soybean Export Council 2019). Specially trained staff who are familiar with diseases inherent in frog farming, as well as the correct application of drugs/chemicals for treatment and prophylaxis, are needed to assure required/standardised biosecurity measures (see Thinh and Phu (2021)).

India

India, formerly considered the country with the largest frogs' legs exports (Abdulali 1985), is discussed here only in passing. In 1985, India and Bangladesh listed their main edible frog species i.e. Euphlyctis hexadactylus and Hoplobatrachus tigerinus in CITES Appendix II, as a result of dramatic population declines (Oza 1990), with exports completely stopped in 1987 and 1989, respectively. In place of India, Indonesia stepped in and became increasingly the main supplier for frogs' legs (see Warkentin et al. (2009)) in the late 1980s. However, it is astonishing that, in 2018, India apparently exported 5 tonnes frogs' legs to the Netherlands, despite its export ban of 1987. In this case, a confusion of the country codes (ID/IN) in the EUROSTAT database cannot be ruled out, but, alternatively, the export ban in India could have been circumvented. Independent of this, Humraskar and Velho (2007) indicate that the trade ban on frogs' legs did not have the desired effect in India. Trade data in the period 2010-2019 indicate that India contributed exports of 15 tonnes into the EU (equal to 0.05% of total imports into the EU [EU imports from Indonesia in the same period amounted to 74%]). According to export data provided by "Seair Exim Solution", frogs' legs (without naming species utilised or how they were sourced) originating from India were shipped to Poland via Thailand (https://www.seair.co.in/frog-legs-export-data/hscode-73023000.aspx, accessed March 2022, see Suppl. material 3).

Farming operations in India

In response to the export ban of frogs' legs for the international market imposed in 1987, initial establishment of frog farms was reported one year later. At that time, the frogs' legs trade was organised under the Seafood Exporters Association, who proposed that the Indian government set up frog breeding centres (Vijayakumaran 1988).

However, it seems that a nationwide establishment of commercially operating frog farms is still in its infancy in India, compared to some SE-Asian countries. In a more recently published study, possibilities for establishment of commercial frog farming in Goa were explored, based on the known issues of the frog trade (e.g. wild harvest); thus, to commercially produce frogs would in turn "minimise illegal poaching" (see D'Silva (2015)).

Turkey

In 2017, Turkey exported 547 tonnes of frogs for the food trade (Turkey Statistical Institute 2017, in Alkaya et al. (2019)) and, according to EUROSTAT (2020), in the same year, > 107 tonnes were imported from Turkey by France, Italy and Spain. Between 2010 and 2019, Turkey supplied EU-countries with > 1593 tonnes of frog's legs (EUROSTAT 2020). Şereflişan and Alkaya (2016) note that, at the national level, harvest and trade of frog's legs in Turkey appears negligible. The focus is essentially on international trade activities involving five companies exporting frogs' legs as the commodities "frozen frogs' legs", "chilled frogs' legs" and "processed form as live frog" to the EU and Switzerland. The authors reiterate the need for commercial frog farming because the wild harvests signal overexploitation. Species of economic value include four *Rana* spp. (*R. dalmatina, R. macrocnemis, R. camerani, R. holtzi*) and two *Pelophylax* spp. (*P. bedriagae, P. ridibundus*) (Şereflişan and Alkaya 2016). Wild *P. ridibundus* collected for export also include live specimens and frozen legs, 1000 tonnes of which are exported annually (see Alkaya et al. (2018) and references therein).

Farming operations in Turkey

According to Dökenel and Özer (2019), *P. ridibundus* is the primary species for EU imports and, in recent years, it has been involved in farms of the private and public sectors. However, the occurrence of zoonotic pathogens in frog farms highlights the need for the development of sustainable frog husbandry to protect animal and human health.

Albania

Between 2010 and 2019, Albania's share of the EU market was 1% (= 590 tonnes) and, according to Jablonski (2011), populations of *Pelophylax epeiroticus* and *P. shqipericus* were utilised both nationally and traded internationally for food. So far, however, there is no conservation management plan in place for the threatened *P. shqipericus* (Eco Albania 2019) and the species is of particular concern as offtake levels for trade purposes are considered unsustainable (Gratwicke et al. 2010).

Farming operations in Albania

To the best of our knowledge and research, we were unable to uncover any evidence of established farms for the commercial breeding of *Pelophylax* spp. for export and little documentation exists of export levels. In 1996, a French businessman invested in a frog farm, motivated in part by the fact that in the mid-1990s frogs' legs in France became rare (cf. above). Mainly due to a socio-economic and political crisis, this farming project failed (https://www.discover-cee.com/roadtrip-cee-albania-how-a-french-guy-discovered-tirana-as-best-place-to-start-his-fintech/, accessed May 2022, see Suppl. material 3). Therefore, we conclude that current export figures all refer to wild-sourced individuals.

Trends in EU frogs' legs imports

Import data for the period 2010–19 were compared with data of the previous decade (see Altherr et al. (2011)) and three trends stand out: (1) a decrease of roughly 12.3% in EU imports of frogs' legs (now 40,700 tonnes instead of 46,400 tonnes) with marked fluctuations underscoring this decline (Fig. 2), (2) the role of Belgium as the highest importing country with 70% of imports in the period under review (in contrast, France's import volumes decreased from 23% to 17% and those of the Netherlands' from 17% to 7%) and (3) the significant increase in the role of Vietnam in exporting frogs, from 8% to 21% of total imports, with China simultaneously dropping from 3% to less than 1%.

Forensic studies have shown that the species composition and labelling in Indonesia's trade has changed over recent decades (Ohler and Nicolas 2017). *Fejervarya limnocharis* and *Limnonectes macrodon* were amongst the most common documented species exported (Kusrini 2005), but *F. cancrivora* represents the major species in trade.



Figure 2. EU's frogs' legs imports (tonnes) during the period 2000-2019. Source: EUROSTAT (2020).

United States

While this study focuses on the EU, the current role of the United States is briefly highlighted, as the US also represents a major consumer of frogs' legs (cf. Warkentin et al. (2009); Gratwicke et al. (2010); Altherr et al. (2011)). In the period 2015–2020, at least four anuran species were imported by the US for consumption, Lithobates catesbeianus, L. forreri, L. grylio and Hoplobatrachus rugulosus (USFWS-LEMIS Database 2023). Lithobates catesbeianus (either alive, dead or legs only) represented the major species by a large margin, predominantly supplied by Mexico (mainly wild), Ecuador, and China (farmed) (Fig. 3). This species, the American Bullfrog, Lithobates catesbeianus, has also been widely introduced into Latin America and Europe for commercial breeding purposes (Carraro 2008). In 2018, imports of H. rugulosus emerged and were declared as exports from Thailand either as captive-bred or ranched, while exports from Vietnam also included wild individuals. Mexico exclusively supplied the United States with wild sourced *L. forreri*, shipped as meat or legs. In 2015–16, the US imported more than 90 tonnes of meat of L. grylio, all noted as captive bred (USFWS-LEMIS Database 2023), but this species is native to the United States (Fig. 3). It is noteworthy that the large quantities of frogs' legs of species harvested in Indonesia and eastern Europe have no sales in the USA.



Figure 3. Anuran species imported for the purpose of consumption into the US in the period 2015-20, in which weight (left) is compared to the number of individuals (right) to illustrate how unequally these variables are aligned with each other. Source: USFWS-LEMIS Database (2023).

National/domestic use

As can be seen in the individual IUCN Red List assessments on exploited amphibian species (Suppl. materials 1, 4, Fig. 2), many species are harvested at local/national levels for consumption, medicinal and/or spiritual purposes (e.g. Nepal 1990). Although this issue is not the focus of this paper, some light can be shed on aspects of local use of frogs for consumption from a conservation perspective. International trade activities can only claim to be sustainable if offtakes for national needs are also managed sustainably. This implies that monitoring of harvest levels for both local/national and international consumption need to be in place (Leader-Williams 2002). There are numerous published examples that describe the domestic trade of amphibians and the impact it may have on local frog populations. Species harvested for consumption within national borders and across range States, are reported for Greece (Hatziioannou et al. 2022), West and Central Africa (Mohneke et al. 2009, 2010; Akinyemi and Ogaga 2015; Efenakpo et al. 2015), Burundi of eastern Africa (Verbanis et al. 1993), India (Pandian and Marian 1986; Ahmed 2012; Talukdar and Sengupta 2020), Nepal (Shresta and Gurung 2019), PDR China (Zhang et al. 2008; Chan et al. 2014; Turvey et al. 2021), Malaysia (Hardouin 1997), Vietnam (Nguyen 2000), Mexico (Barragán-Ramírez et al. 2021) and the USA (Ugarte 2004; Ugarte et al. 2005), as exemplars of some countries/regions. The proportion of national vs. international trade is of particular interest when some countries document high annual exports for the international frogs' legs industry on a regular basis, while ignoring the fact that some species have been consumed locally for decades/centuries (Angulo 2008; Onadeko et al. 2011; Ahmed 2012). It would not be problematic if species are traditionally consumed at the local/national level and this use was deemed sustainable. However, harvest for international exports (above local/traditional harvest) often means overexploitation of local populations (Oza 1990 and cf. species compiled in Suppl. material 4). In addition, for Indonesia, it has been estimated that offtakes of edible frogs on a national level are up to 142 million frogs or seven times as many as that of annual international exports (see Kusrini (2005)), with no documentation of the impact on wild populations and highlighting the need for better monitoring of base populations and trade.

Species diversity consumed and evaluated in the IUCN Red List

The conservation of species in trade only makes sense if the species or species complexes are known. The trade in animals with unclear taxonomic status ignores a fundamental condition, namely the lack of any data basis for taxa to, for example, conduct a non-detriment finding for the species to evaluate threat (see below). In order to obtain an overview of the species involved in the food trade (whether at local, national or international level) and their respective origins, the IUCN Red List was filtered (Fig. 4; Suppl. material 4). Regions where most species are harvested for consumption are Southeast and East Asia and it is also these regions that supply the EU market with most of their frogs' legs. Furthermore, many species are consumed in Central America and (northern) South America, all of which are traded either locally, nationally or exported to the USA (predominantly *L. catesbeianus* from breeding farms; https://www.fao.org/fishery/en/culturedspecies/rana_catesbeiana/en, accessed March 2022, see Suppl. material 3). Interestingly, the EU is not a consumer of species from these regions. Likewise, all species consumed in Africa, with the West African region forming a species focus, are consumed in Africa and the EU is not a consumer of African species (Suppl. material 1, Fig. 4).

At least 187 species of anurans and salamanders/newts are collected locally/nationally for food and for the international frogs' legs industry (Suppl. material 4). According to information of Red List assessments, the local/national use of 13 species (filtered by the search criteria given above) was not explicitly stated, was more generally indicated (i.e. "species in the genus are also commonly used for food") or the use has been not necessarily considered a threat (e.g. Leptobrachium hainanense, IUCN SSC Amphibian Specialist Group (2020a); Suppl. material 4). Of the remaining 174 species, all but two are consumed on a local/national scale. For Lithobates pipiens, only national trade for research purposes is indicated (IUCN SSC Amphibian Specialist Group 2022i); however, according to Herrel and van der Meijden (2014), L. pipiens is also involved in the international trade, without details on the purpose of exports. Of all species of amphibians for which we found data, at least 20 species are potentially involved in international food trade activities. In some species (for example, Limnonectes shompenorum, IUCN SSC Amphibian Specialist Group (2022g)), cross-border trade was assumed, but not substantiated. In other species, the Red List assessment notes the presence of trade, i.e. Rana amurensis (IUCN SSC Amphibian Specialist Group 2020f). Therefore, in Red List assessed species that indicate international trade or questionable cross-border trade, uncertainty is involved in individual assessments (Table 3, Suppl. material 2).

Threat status, population trends and sustainability

Amongst the 30 species compiled in Table 3 and Suppl. material 2 that are consumed and traded locally, nationally and/or internationally (relevant for the European frogs' legs trade), uncertainties persist in several species regarding the level of exploitation.

When we submitted the first version of this work in August 2022, several Red List assessments for these species were outdated with 16 species last assessed ≥ 15 years ago, i.e. 11 species assessed in 2004 and five species assessed in 2008, while in 2022/23, several of those species had been re-assessed, leaving six species with outdated assessments (see Table 3, Suppl. material 2).

Current Red List assessments of aforementioned 30 amphibian species, now refer to 24 that have been evaluated "Least Concern", three "Near Threatened (NT)", one "Vulnerable (VU)", one "Endangered (EN)" and one "Critically Endangered (CR) (Table 3, Suppl. material 2).

Population trends of the 30 species indicate 20 species "decreasing", five species "stable", two species "increasing" and three species with an "unknown" population trend; it is worth mentioning here in addition that 14 species assessed "Least Concern" have a decreasing population trend (Table 3, Suppl. material 2).



Figure 4. Number of species per country in trade for consumption, see Suppl. material 1: figs S2, S3 for more detailed range data and for species in international trade. Notably African species are largely consumed domestically rather than exported (Suppl. material 1: figs 2, 4). Source: IUCN (2022).

Notable is the fact, that the two large-legged species, i.e. *Limnonectes blythii* and *L. malesianus* had been assessed "Near Threatened" in 2004 with decreasing populations in both species (van Dijk and Iskandar 2004b; van Dijk et al. 2004c); however, both were re-evaluated as "Least Concern" in 2021, despite decreasing population trends (IUCN SSC Amphibian Specialist Group 2022d, f).

Outdated assessments are further exacerbated by the fact that the species are regionally overharvested for consumption as well as being involved in the international trade at uncertain levels. However, of all 30 species known to be consumed, 16 species have special mention of harvest that might influence their conservation status. Of these, 13 species (*Leptodactylus fallax, Limnonectes blythii, L. kuhlii, L. leporinus, L. macrodon, L. malesianus, Lithobates pipiens, Pelophylax caralitanus, P. kurtmuelleri, P. ridibundus, P. shqipericus, Rana amurensis* and *R. chensinensis*), have either "regional overexploitation-collection" or "harvest leading to declines" explicitly stated in their IUCN Red List assessments. Another three species (*Fejervarya cancrivora, Hoplobatrachus rugulosus, Limnonectes microtympanum*), have these same parameters as 'presumed' within their Red List assessments (Table 3, Suppl. material 2). A detrimental harvest impact is indicated for *Rana dybowskii* for the medicinal trade (Kuzmin et al. 2004) and in *Limnonectes grunniens* and *Pelophylax bedriagae*, harvest for the food trade is considered a significant threat. In *Limnonectes ibanorum* and *L. ingeri*, harvest is considered detrimental due to the species' unfavourable life history traits (Table 3, Suppl. material 2).

Of the 187 species filtered from the IUCN Red List that are collected for either local, national or international consumption (Suppl. material 4), assessments of population trends since 2004 to 2021 reflect an increase in population declines, while the number of species in threat categories also seems to increase over the course of the Red List assessments; however, the species' to be evaluated as well as the geographical distribution play decisive roles; thus, a threat to species evaluated as "Data Deficient" cannot be ruled out and geographically widespread species assessed as "Least Concern" may be threatened at the national level (cf. Figs 5, 6).

Table 3. Anuran species in the European frogs' legs trade where overexploitation and/or taxonomy is/are important limiting factor(s) for sustainable commercial trade. Distribution: Information here is based on IUCN Red List assessments and more recent literature. Country codes follow acronyms provided in the CITES Trade Database, https://trade.cites.org/cites_trade_guidelines/en-CITES_Trade_Database_Guide.pdf; "?" next to country denotes uncertainty; RLA: Red List Assessment and year when the species was most recently assessed, with 'outdated' used to designate RLAs > 10 years old; LC: Least Concern, DD: Data Deficient, NT: near threatened, VU: vulnerable; Pop. trend: population trend (↑: increasing; →: stable; ↓: decreasing; ?: unknown); CITES: listed in either appendices I-III or in the annexes of the European Union Wildlife Trade Regulations (EU-WTR) A-D; Information: *): Assessment involving uncertainty. Sources: IUCN (2021) and therein published Red List assessments of the species concerned; Indonesian quotas – Indonesian Ministry of Environment and Forestry (2022); Frost (2021) for adjusting English names, taxonomy and distribution.

Species	Distribution	RLA (year)	Pop.	CITES /	Information on taxonomy, threat, trade,
			Trend	EU WTR	farming operations & exploitation levels
Fejervarya cancrivora Crab- eating grass frog	ID, MY, TL	LC (2020) last assessment of 2004!	Ļ	_	 assumed overharvest*(IUCN SSC Amphibian Specialist Group 2022a) utilised locally, nationally and internationally export quota sharply increased in 2016 to more than 83 million animals for consumption and since then strong fluctuations. 2022 harvest/export quota Indonesia: 59,985,100 / 56,985,845 specimens Imported to the EU by millions as frogs' legs taxonomy remains uncertain in some
					populations
Fejervarya limnocharis Common Asian grass frog	BD, BN, KH, CN, HK, IN, ID, JP, LA, MO, MY,	LC (2004, outdated)	\rightarrow	_	 harvested for human consumption, found in local and national trade (Nguyen 2000; van Dijk et al. 2004a) probably also in international trade
	MM, NP, PK, PH, SG, TW, TH, VN				 2021 harvest/export quota Indonesia: 1300 /1235 specimens for the pet trade, in 2015, also harvested for consumption (cf. Table 2) cryptic species complex
<i>Fejervarya</i> moodiei Northern crab- eating grass frog	BD, IN, MM, PH, TH, KH?	LC (2020) last assessment of 2004!	?	_	 originally thought to be known only from the type locality Manila (Luzon Island, Philippines, with unclear taxonomic validity identified by DNA barcoding in French frogs' legs imports (Ohler and Nicolas 2017) locally consumed in the Philippines (IUCN SSC Amphibian Specialist Group 2022b)
Hoplobatrachus rugulosus Asian rugose bullfrog	KH, CN, HK, LA, MM, TW, TH, VN	LC (2020) last assessment of 2004!	Ţ	_	 large individuals may be overharvested locally IUCN SSC Amphibian Specialist Group (2022c) wet rice agroecosystems appear to balance the impact of exploitation locally, nationally and internationally traded for food harvest of large numbers of wild individuals is ongoing, either directly to be marketed or to restock farms, for example, in Vietnam large numbers of frogs' legs imported into the EU meat is considered a delicacy in restaurants in Vietnam (Naruar 2000)

Species	Distribution	RLA (year)	Pop.	CITES /		Information on taxonomy, threat, trade,
			Trend	EU WTR		farming operations & exploitation levels
Hoplobatrachus	AF, BD, BT?,	LC (2008,	\rightarrow	II / B	٠	introduced to Madagascar
<i>tigerinus</i> Asian	CN?, IN,	outdated)			•	intense harvest before the 1990s has
bullfrog	MM, NP, PK					detrimentally impacted populations (India,
						Bangladesh)
					•	legal export banned in India and Bangladesh
						since the late1980s
					•	utilised locally, nationally, internationally (frogs'
						legs industry)
					•	taxonomic confusion with <i>H. rugulosus</i> *
					•	species is farmed (e.g. in Vietnam or Thailand),
						occasionally hybridisation with <i>H. rugulosus</i> to
						increase production
Limnonectes	ID, LA?, MY,	LC (2021)	Ļ	_	•	major threat is consumption (locally /
<i>blythii</i> Blyth's	MM, SG, TH	last				nationally / internationally)
giant frog		assessment			•	population decline > regional overharvest
		of 2004!			•	taxonomic uncertainty > <i>blythii</i>
						complex*(IUCN SSC Amphibian Specialist
						Group 2022d)
					•	relatively large species, attractive for frogs' legs
						trade
					•	in the 1980s, one of the dominating species in
						Indonesia's exports to Europe (Le Serrec 1988)
Limnonectes	BN, ID	LC (2018)	\downarrow	_	•	large body size make species attractive for food
ibanorum	(Kalimantan),					trade
Rough-backed	MY (Sarawak)				•	probably utilised locally and possibly also for
river frog						the international frogs' legs trade*
					•	life history traits make this species vulnerable to
						overharvest
					•	declining populations indicate over-exploitation
Limnonectes	BN?, ID	LC (2018)	?	_	•	large body size make species attractive for food
<i>ingeri</i> Inger's	(Kalimantan),					trade
wart frog	MY (Sabah,				•	potentially exported for the frogs' legs industry*
	Sarawak)				•	locally consumed in Kalimantan and Sarawak
					•	life history traits make this species vulnerable to
						overharvest
Limnonectes	ID	LC (2020)	\downarrow	_	•	cryptic taxon, species complex*
<i>kuhlii</i> Kuhl's		last			•	presence in several range States remains
broad-headed		assessment				uncertain (see IUCN SSC Amphibian Specialist
frog		of 2004!				Group (2022e))
					•	in some of these potential range States,
						populations are locally overexploited for
						consumption, for example, China
					•	the meat is highly priced in Vietnam (Nguyen
						2000)
					•	look-alike species of L. macrodon, included in
						EU imports (MNHN 2012; Ohler and Nicolas
						2017)
Limnonectes	BN, ID	LC (2018)	\downarrow	-	•	potentially exported for the frogs' legs industry*
<i>leporinus</i> Giant	(Kalimantan),				•	regionally > overharvest of large individuals >
river trog	MY (Sabah, Sarawak)					suggesting demographic change

Species	Distribution	RLA (year)	Pop.	CITES /		Information on taxonomy, threat, trade,
			Trend	EU WTR		farming operations & exploitation levels
<i>Limnonectes</i> <i>macrodon</i> Giant Javan frog	ID (Sumatra, Java)	LC (2017)	Ļ	D	•	locally, nationally exploited as food; Javan populations are exploited for the international market
					•	has been heavily harvested for the frogs' legs trade (Kusrini and Alford 2006) and between 1988 and 1991, 17 tonnes were traded for their skins and meat (Kusrini 2017 in IUCN SSC Amphibian Specialist Group (2018))
					•	according to Ohler and Nicolas (2017), the species was not traced in the international frogs' legs market
Limnonectes	ID, MY, SG,	LC (2021)	\downarrow	-	•	significant decline initially reported in 2004
malesianus	TH	last			•	overharvest is considered a major threat
Malesian river frog		of 2004!			•	collected for subsistence use and trade & utilised locally, nationally
					•	sympatric occurrence with the larger <i>Limnonectes blythii</i> that is favourably collected; however, harvest impact is not well understood due to a lack of harvest/trade data (IUCN SSC Amphibian Specialist Group 2022f).
					•	look-alike species of <i>L. macrodon</i> , included in EU imports (MNHN 2012; Ohler and Nicolas 2017)
<i>Lithobates</i> <i>catesbeianus</i> American	CA, US	LC (2020)	ſ	_	•	introduced in many other countries across the globe (IUCN SSC Amphibian Specialist Group 2022h)
bullfrog					•	commercially farmed for food (in non-range countries, for example, in Thailand, Vietnam and Brazil)
					•	considered a pest & invasive species, for example, in large parts of Europe, Central and South America, East and Southeast Asia
					•	considered a vector of pathogens* (Fisher and Garner 2007)
Lithobates	CA, US, PA,	LC (2021)	\downarrow	-	•	taxonomy unresolved; species complex
<i>pipiens</i> Northern leopard frog	MX?	last assessment of 2004!			•	previously commercial overexploitation was considered a major threat (Hammerson et al. 2004)
					•	information on international trade is vague, other than trade for research/educational purposes (IUCN SSC Amphibian Specialist Group 2022i)
Pelophylax	CY, EG, GR;	LC (2021)	\rightarrow	_	•	harvest/exports for food from Turkey to western
<i>beariagae</i> Bedriaga's marsh	IL; JO; LB, SV TR	last			_	Europe > considered a significant threat
frog	51, 1K	of 2008!			•	(Şereflişan and Alkaya 2016; Çiçek et al. 2021) and Egypt
					•	high extinction risk in Turkey until 2032 if exploitation level continues (Çiçek et al. 2021).
					•	utilised local and internationally for consumption) (IUCN SSC Amphibian Specialist Group 2022j)

Species	Distribution	RLA (year)	Pop.	CITES /		Information on taxonomy, threat, trade,
			Trend	EU WTR		farming operations & exploitation levels
<i>Pelophylax</i> <i>caralitanus</i> Beyşehir frog	TR	NT (2008, outdated)	Ļ	_	•	largest edible frog in Turkey; commercially overexploited for the frogs' legs trade in France, Italy and Switzerland (Şereflişan and Alkaya 2016; Çiçek et al. 2021) > have caused its rapid
					•	decline so that the species is now considered endangered (Erismis 2018) high extinction risk until 2032 (Çiçek et al.
Pelophylax epeiroticus Epirus water frog	AL, GR	NT (2019)	Ļ	_	•	locally, nationally utilised for food intensively utilised in Albania for consumption, at present no evidence for excessive collections in Albania <i>Bd</i> -infected populations in Albania Potential hybridisation with the sympatric <i>P</i> . <i>ridibundus</i>
Pelopbylax kurtmuelleri Balkan frog	AL, BG, GR	LC (2019) last assessment of 2008!	Ļ	_	•	nationally and internationally utilised for consumption in northern parts of its native range > significantly threatened through commercial overexploitation for consumption (IUCN SSC Amphibian Specialist Group 2022k) another threat is considered in the unintentional introduction of commercially traded non-native water frogs
Pelophylax ridibundus Eurasian marsh frog	Western Europe across the Arabian Peninsula, Central Asia to Russia	LC (2008, outdated)	Î	_	•	harvested for educational and medical research and food populations extensively collected for food in Turkey (~ 1,000 t/yt) (Alkaya et al. 2018) trade for frogs' legs may detrimentally impact populations in Turkey* (Şereflişan and Alkaya 2016: Çiçek et al. 2021) frogs' legs trade has led to declines in populations in eastern Asia, former Yugoslavia and possibly in Romania* <i>Rana (Pelophylax) kl. esculenta</i> considered a synonym
Pelophylax shqipericus Albanian water frog	AL, ME,	VU (2019)	Ļ	D	•	introduced to Italy and Croatia nationally and internationally utilised for consumption no management plan in Albania; significantly threatened by overexploitation potentially threatened by unintentional introduction of commercially traded non-native water frogs

It is noteworthy to mention that 57 species of the respective 187 species, have a decreasing population trend, but categorised as "Least Concern" (see Suppl. material 4).

Assessments of 28 species were re-evaluated in 2022/23, in seven species, the Red List status was changed (see Suppl. material 4). Uncertainties outlined in this review remain unevaluated and a resolution of these for individual species assessments would likely influence the categorisation of the threat status and population trends.



Figure 5. Red List status of 187 amphibian species globally utilised for consumption that have been assessed in eleven assessment periods between 2004 and 2021. Source: IUCN (2022); cf. Suppl. material 4).



Figure 6. Population trends assessed in 187 amphibian species, consumed for food, in eleven assessment periods between 2004-2021 (cf. Suppl. material 4). Source: IUCN (2022).

CITES species and their trade

The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) currently lists 220 amphibian species in their appendices, equating to ca. 2.6% of all amphibian species (8,386 spp.; Frost (2021)) recognised by science. The CITES trade database (https://trade.cites.org/, accessed January 2022, see Suppl. material 3) merely lists seven anuran species that are traded for the purpose of consumption (Table 3). Nonetheless, the majority of species involved in the frogs' legs trade are not listed in the appendices of CITES (cf. Table 3, Suppl. materials 2, 4): thus, trade in these non-CITES listed species is not documented in the CITES trade database, making information on species volumes traded per annum or analysis of specific trends not possible.

All seven CITES listed anuran species are utilised on a local/national scale and three (i.e. *Pelophylax shqipericus, Limnonectes macrodon* and *Hoplobatrachus tigerinus)*, are involved in the international frogs' legs trade (cf. Table 4) as well. All seven species have been evaluated in the IUCN Red List and three other species, (i.e. *Conraua goliath, Laotriton laoensis* and *P. shqipericus)* are not listed in the appendices of CITES, but appear in the annexes of the European Wildlife Trade Regulations (EU WTR). All but two of these species have a decreasing population trend and two species were last assessed in 2004 and 2008.

Four species are listed in CITES App. II and one in CITES App. III that are consumed either locally/nationally and/or internationally traded for consumption, while another four species are only listed in the annexes of the EU-WTR (Table 4, Suppl. material 4).

1. *Calyptocephalella gayi.* – Since 2011, the species is listed on CITES App. III in Chile. In 2012–2016, reported exports of 114 live individuals were recorded at the same time that 550 live individuals were imported. In 2012, 14 live individuals were seized in Japan and the 550 animals were sourced from captivity in Chile and imported by the US and Japan. International trade for the purpose of consumption is not explicitly documented, despite the fact that the species is nationally and internationally involved in the food trade (IUCN SSC Amphibian Specialist Group 2019a).

2. *Conraua goliath.* – This species is not listed in the appendices of CITES, but in Annex B of the EU-WTR. However, eight transactions 1998–2019 of wild-sourced individuals were documented in the CITES trade database. All exports were from Cameroon, with 19 live individuals commercially exported by Cameroon and 65 individuals claimed as commercial imports by EU importing countries. In 2004, Cameroon exported 199 specimens to the United States for scientific purposes. International trade for the purpose of consumption is not documented despite the species being locally/ nationally consumed (IUCN SSC Amphibian Specialist Group 2019b).

3. *Euphlyctis hexadactylus.* – International trade has been documented since 1985 (date of CITES listing), with India as the major supplying country until 1986, documenting the export of roughly 1215 tonnes of meat, while importing countries documented the import of at least 871.5 tonnes meat (https://trade.cites.org, see Suppl. material 3). In 1986, the United States imported another ~ 80 tonnes meat indicating India as the country of origin.

4. *Hoplobatrachus tigerinus.* – Exports are documented since 1985 (date of CITES listing) and transactions have been reported until 2019. However, the largest quantities were shipped in 2007. Analysis of trade data of this species is particularly challenging because quantities are misleadingly indicated and non-range States of the species export large quantities, including meat of wild-sourced individuals (for example, from Vietnam and Madagascar, documented in the CITES trade database).

5. *Limnonectes macrodon.* – This species is not listed in the appendices of CITES, but in Annex D of the EU-WTR. However, a single transaction was documented in the CITES trade database. In 2016, Germany reported the import of two live individuals from Indonesia, sourced from the wild. The species is intensively involved in the local, national and international food trade (IUCN SSC Amphibian Specialist Group 2018). It is remarkable that the Annex D records do not reflect an intense EU import of frogs' legs officially labelled as "*Limnonectes macrodon*", as noted by Dittrich et al. (2017), since this is almost a certainty.

6. *Pelophylax shqipericus.* – This species is not listed in the appendices of CITES, but is in Annex D of the EU-WTR since 2009 because there was concern regarding the numbers imported into the EU, with monitoring of this trade warranted and a distinct lack of a rigorous non-detriment finding (https://www.speciesplus.net/species#/taxon_concepts/5193/legal, see Suppl. material 3).

7. *Telmatobius culeus.* – Commercial trade of the species was suspended in 2017, with listing in CITES Appendix I and EU-WTR Annex A. In the period 2010–2022, the CITES trade database indicates only two transactions: the import of 20 live individuals to Canada and 150 live animals to the UK. In both cases, the animals were destined for zoos and sourced as "farmed" from the USA. According to the IUCN SSC Amphibian Specialist Group (2020g), it is estimated that > 15,000 animals/year are used to prepare frogs' legs.

Disease, pesticides and veterinary drug residues, genetic pollution

The farming and regional/international trade activities involving amphibian species for consumption purposes is associated with numerous risks. Here, we outline these more specifically.

Disease

Evidence clearly demonstrates that the commercial trade of amphibians infected with pathogens contributes to the spread of diseases within and between countries, on a global scale and involves species traded for food (Fisher and Garner 2007; Miller et al. 2011; Rodgers et al. 2011; Olson et al. 2013; O'Hanlon et al. 2018).

The intercontinental spread of two fungal pathogens i.e. *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*), has led to the decline of more than 500 amphibian species and currently more than 1000 species are known to be infected

Table 4. Seven anuran species listed on the appendices of CITES (I-III) and annexes of the European Wildlife Trade Regulations (A-D) that are currently known to be consumed locally/nationally and those utilised within the international frogs' legs industry. Country codes follow acronyms provided in the CITES Trade Database (https://trade.cites.org/cites_trade_guidelines/en-CITES_Trade_Database_Guide.pdf); RLA: Red List Assessment and year, when the species was assessed, LC: Least Concern, VU: vulnerable, EN: endangered, CR: critically endangered; Pop. trend: population trend (→: stable; ↓: decreasing). CITES: listed in either the appendices I-III or in the annexes of the European Union Wildlife Trade Regulations (EU-WTR) A-D; Sources: IUCN (2022) and therein published Red List assessments of the species concerned (https://www.speciesplus.net).

Species	Distribution	RLA(year)	Pop.	CITES & EU-WTR	Consumption &
			Trend	(year when listed)	Trade
Calyptocephalella gayi	CL	VU (2018)	\downarrow	III (2011) C (2012)	National,
Helmeted water toad					international (likely
					only pet trade)
<i>Conraua goliath</i> Goliath	CM, GQ, GA?	EN (2018)	\downarrow	B (1997)	National
frog					
Euphlyctis hexadactylus	BD, IN, NP?, LK	LC (2004,	\rightarrow	II (1985) B (1997)	National
Indian green frog		outdated)			
Hoplobatrachus tigerinus	AF, BD, BT?, CN?,	LC (2008)	\rightarrow	II (1985) B (1997)	National,
Asian bull frog	IN,MM, NP, PK				international
Limnonectes macrodon	ID(Sumatra, Java)	LC (2017)	\downarrow	D (2009)	National,
Giant Javan frog					international
Pelophylax shqipericus	AL, ME	VU (2019)	\downarrow	D (2009)	National,
Albanian water frog					international
<i>Telmatobius culeus</i> Titicaca	BO, PE	EN (2019)	\downarrow	I (2017) A (2017)	National,
water frog					international

by one of these two emergent infectious diseases (Scheele et al. 2019; Monzon et al. 2020). The spread of infectious diseases may also be exacerbated by global warming (e.g. Lampo et al. (2006); Bosch et al. (2007); Seimon et al. (2007)). With new climate projections, models predict expansion of Bd into new areas both in higher altitudes and elevations (Xie et al. 2016) which might impact with current farms in those areas. Other pathogens (e.g. ranaviruses) also could expand their range as a consequence of climate change (cf. Price et al. (2019)), highlighting the need for better biosecurity measures in the commercial trade.

Interactions between ecological factors and amphibian-pathogen dynamics are extremely complex and pose major challenges for management decisions (Lips 2016; Bienentreu and Lesbarrères 2020). The commercial farming of anuran species poses challenges in terms of hygiene and proactive biosecurity and disease prevention measures. In the past (Kanchanakhan 1998; Zhang et al. 2001; Mauel et al. 2002; Weng et al. 2002), as well as more recently (Gilbert et al. 2013; Aktaş et al. 2019), many bacterial, viral and fungal pathogenic diseases have been reported affecting mass-produced farmed frogs. A mycobacterium-associated disease has been detected in *Hoplobatrachus rugulosus* animals in Vietnam that may pose a public health risk and highlights the need for improved biosecurity measures in the breeding and trade of frogs (Gilbert et al. 2013). Already in the 1970s (Andrews et al. 1977) and 1980s,

Salmonella was detected in samples of frozen frogs' legs. Out of 304 samples, *Salmonella* was detected in 121 samples (39.8%), with 25.4% from India and 51.5% of the positive samples from Indonesia. In France, frogs' legs are a significant source of *Salmonella* and are undoubtedly a source of multiplication (Catsaras 1984). In a long-term study 1990–1998, *Salmonella* of the serotype C1 was isolated from domestically available frogs' legs from New York State previously imported from Indonesia (Heinitz et al. 2000).

Exports of *Pelophylax* [*Rana*] *esculentus* from Albania for consumption to foreign markets also revealed *Salmonella*, *Vibrio cholerae*, *Listeria* spp. and *Aeromonas* spp., the latter two being clearly more common (Vergara et al. 1999).

One internationally commercialised species for consumption is particularly striking: the North American bullfrog (Lithobates catesbeianus), a known vector of ranavirus detected in cultured specimens in South American exports to the USA (Galli et al. 2006; Miller et al. 2007; Schloegel et al. 2009) and the fungal disease Bd (Garner et al. 2006) translocated within farming operations in South America (Mazzoni et al. 2003) and in China and Singapore, where cross-infections from farmed individuals to native amphibians have been suggested (Bai et al. 2010; Gilbert et al. 2013). The danger that L. catesbeianus, as a carrier of Bd, can threaten naïve populations of other amphibian species has been emphasised by Rödder et al. (2013) who clearly highlight the link between the spread of Bd and bullfrogs. Additionally, novel chytrid genotypes have been identified and linked to the trade with *L. catesbeianus* (Schloegel et al. 2012). However, with regard to live imports of L. catesbeianus into the EU since 2016, the species is subject to a stricter legal regime and has, therefore, been deleted from Annex B (http:// eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32016R2029&from= EN, accessed March 2022, see Suppl. material 3); in 2013 and 2014, L. catesbeianus listing in Annex B referred to the import of live specimens.

Two more species involved in the food trade (see Table 1, 3, Suppl. material 2) were tested *Bd*+: *Lithobates megapoda* (Frías-Alvarez et al. 2008) and Albanian populations of *Pelophylax epeiroticus* (Vojar et al. 2017; IUCN SSC Amphibian Specialist Group 2020c, d).

Challenges with regard to the spread of diseases with live animals intended for the food trade are multi-layered. On one hand, trade of live amphibians poses a potential risk of cross-infection into naïve wild populations via escape and contamination through waste water disposal. On the other hand, commercial breeding farms also pose risks of escaped animals and disposal of water and housing materials that can be carriers of pathogenic diseases. This demonstrates two predominant pathways for spreading pathogenic diseases: translocation and commercial farming operations (cf. Travis et al. (2011); Jaÿ et al. (2019)). To what extent processed frogs' legs pose a hygiene risk (see issues described above) appears to be a largely understudied topic. However, skinned and frozen meat seems to present less risk with regard to the spread of infectious diseases, such as *Bd* (Gratwicke et al. 2010). In the case of *Salmonella*, however, more care is needed to avoid contamination (Grano 2020) in any substrate, individual or tissue, frozen or fresh.

Pesticide and veterinary drug residues in wild and farmed frogs

We cannot provide comprehensive information on residues and effects (on the end consumer) of toxins used in regional agriculture and ingested indirectly (via the nutrient cycle) by frog species. Nor are we able to tease apart the effects of ingestion of veterinary cocktails of commonly-used antibiotics, i.e. oxytetracycline and doxycycline (see Nguyen and Tran (2021)) used in commercially-farmed frog species for international consumption. Instead, we would like to illustrate existing health risks for humans as end consumers with a collection of circumstantial evidence. Many of the studies mentioned provide initial results of research projects, but many more follow-on studies do not exist due to the lack of interdisciplinary studies, opacity of supply chains and distances and conditions of transportation of fully or partially processed frogs' legs.

Here, we address the questions: (1) What are the most common habitat types and species that are captured for the international consumption trade? (2) How are these habitats managed with regard to the use of pesticides, herbicides and other agricultural chemicals? (3) Do these agrochemicals negatively affect faunal assemblages and their ecosystems? (4) Are these chemicals detectable in imported frogs' legs? (5) Have veterinary drug residues been detected in aqua-cultured frogs' legs? and finally, (6) Is there evidence that the consumption of frogs' legs contaminated with medicinal or pesticide residues can be hazardous to human health?

Probably the most common frog involved in the global frogs' legs industry is Indonesian *F. cancrivora* (75% of reported species). This species is considered the most abundant frog species inhabiting rice fields in Indonesia (see Kusrini and Alford 2006) and references therein).

It appears that Javan populations of *F. cancrivora* are predominantly harvested for the international frogs' legs trade (cf. Kurniati and Sulistyadi (2017)). The intense use of pesticides is prominent in Indonesia and, according to Ardiwinata et al. (2018), highest pesticide residues are found in Central Java. Quality of freshwater in terms of pesticide input and, hence, the contamination of semi-aquatic communities (e.g. amphibians), in rice plantations on Java, is problematic (Iskandar 2014). Disruption of the food web has led to an increase in populations and population densities of the brown locust (Nilaparvata lugens) which damages rice plantations and causes significant crop losses. West and Central Java farmers, therefore, feel compelled to use more pesticides and create their own mixtures of these chemicals (Prihandiani et al. 2021). The use of pesticides in various agro-ecosystems (incl. freshwater ecosystems) negatively affects food webs (see Relyea and Hoverman (2008)), shifts species composition and abundance and leads to severe declines of some species in these systems (cf. Pingali and Roger (1995) and references therein). Furthermore, exposure of frogs to pesticides also leads to an increased risk of infection due to the weakening of the immune system (Kiesecker 2011). According to Quaranta et al. (2009), absorption of herbicides, such as atrazine through the skin of amphibians, is "300 times higher than in mammals". Herbicides were found to negatively affect larval stages of *F. limnocharis* populations in Taiwan (Liu et al. 2011) and health status was likewise reduced in populations of *F. limnocharis* in pesticide-contaminated rice fields (as residues in soil and direct exposure) in the Western Ghats and Kerala (India) (Hedge and Krishnamurthy 2014; Kittusamy et al. 2014). A study by Kittusamy et al. (2014) also found pesticide residues in *F. limnocharis* and *H. crassus* that led to malformations in some individuals. However, other pathogenic influences besides pesticides, as well as synergistic effects of pesticides, are also considered to be causing these malformations (also see Wijesinghe (2012)). The harmful effects of pesticides on anuran species have been confirmed in populations of *Pelophylax perezi* in France as well (Mesléard et al. 2016).

The question now arises whether pesticide residues or other toxins have been detected in traded animals or parts thereof for commercial consumption by humans. Information on the potential of bioaccumulation has rarely been analysed and more work is needed (Mani et al. 2021). It was found that some populations of pig frogs (*Lithobates* [*Rana*] grylio) harvested in south-eastern United States (for local consumption) contain a high level of mercury (Ugarte et al. 2005). According to a study performed by Turnipseed et al. (2012), drug residues could be detected in aqua-cultured samples of frogs' legs. The combination of different residues in the examined frogs' legs was striking and leads to the conclusion that varying chemotherapeutic agents (including those harmful to human, for example, chloramphenicol; Turnipseed et al. (2012)) are apparently used indiscriminately in frog aquaculture. More recently, a study highlighted a variety of antibiotics applied at commercial frog aquaculture facilities in Vietnam and uncontrolled dosage of drugs (Nguyen and Tran 2021).

The question of whether pesticide residues and other potentially toxic substances in frogs that are imported into the EU have been monitored could not be determined in the course of this work. This in itself is shocking and in view of the situation in exporting countries and the lack of transparency and management in the application of agrochemicals and veterinary medicinal substances within commercial farms, we strongly recommend that this monitoring become an urgent near-future task for importing countries.

Genetic pollution

In 2010, Holsbeek and Jooris reported that, in the preceding decade, humans translocated individuals of *Pelophylax* spp. either unintentionally (for example, escaped animals from nurseries and markets) or intentionally (for example, for stocking garden ponds and for local culinary harvest) almost everywhere they exist. A study conducted by Dufresnes et al. (2018) showed that the presence of individuals of the *Pelophylax ridibundus* species complex derive from varying genetic lineages that correlate with registered frogs' legs industry imports in Switzerland, implying that individuals were also released/translocated for commercial purposes (regionally and internationally), revealing hybridisation events in several cases. Thus, the harvest of East European frog species involved in the frogs' legs industry and subsequent introduction into western Europe has led to genetic pollution and threatens to damage their native congeners (Dubey et al. 2014; Dufresnes et al. 2018). It has also been suggested that the introduction of the invasive *P. kurtmuelleri* from the south-western Balkans to southern Italy was also due to the frogs' legs trade (Bisconti et al. 2019).

Another example that does not explicitly address commercial trade of frogs' legs in the EU, but names taxa that are traded regionally for this purpose (see also Table 3), is the unregulated trade of frogs for ornamental ponds in Belgium. This has led to non-native *Pelophylax* spp. displacing native species or hybridising with them and is due to inefficient legislation at national and EU level, lacking regulation for the import of potentially invasive species (Holsbeek et al. 2010).

Furthermore, the commercial frogs' legs industry already contributes to the unintentional release of specimens into naïve habitats and displacing native species (e.g., Ribeiro et al. (2019) and references cited therein). Amongst these myriad species are American bullfrogs (L. catesbeianus), which, including their larval stages, detrimentally impact many other anuran species (cf. Kiesecker (2011)). Escapees of Hoplobatrachus rugulosus (originating from Thailand, referred to as "Thailand tiger frogs") have been reported and are kept in Chinese frog farms and may lead to hybridisation with Chinese populations of *H. rugulosus* (referred to as "Chinese tiger frogs") (Yu et al. 2015). The authors suggest improving management of these farms to avoid further release of Thailand tiger frogs because a cryptic species complex is suspected and, thus, species may unwittingly be driven extinct because they have not been recognised. These issues are also pertinent for other amphibian species complexes. For example, in the case of the Chinese Giant salamander (Andrias davidianus), recent assessments show that multiple species exist across China, but farming and release of one of these species outside its range has virtually eliminated other Chinese Giant salamander species (Turvey et al. 2018; Yan et al. 2018; Lu et al. 2020). Additionally, the introduction of the Chinese Giant salamander in Japan resulted in hybridisation with populations of the Japanese Giant salamander (Andrias japonicus) (Fukumoto et al. 2015).

Taxa traded with uncertain taxonomic status

The use and trade of species in their country of origin and whose taxonomic status is uncertain affects at least four species involved in the international frogs' legs industry as well. Amongst these, three are designated as species complexes (i.e. more than one species under one current scientific name) and species with unresolved taxonomy in IUCN Red List assessments. They are: Fejervarya cancrivora, Hoplobatrachus tigerinus and Limnonectes blythii. There are many other species complexes, wherein the taxonomy is extremely complex and uncertainties are even more fraught with problems. Fejervarya moodiei was described from "Manila", Luzon Island Philippines and, hence, taxonomic studies should initially be conducted on that population. In another two species (Limnonectes grunniens and L. kuhlii), where the impact of international trade for frogs' legs has not been explicitly ascertained within their assessments (but is very high), taxonomy remains unresolved. In these species of *Limnonectes*, both their geographic range and number of cryptic species 'hiding' under one scientific name are still unclear (IUCN SSC Amphibian Specialist Group 2020b; IUCN SSC Amphibian Specialist Group 2022e). To what extent populations assigned to *L. kuhlii* are involved in the international frogs' legs industry is not indicated in the species' Red List assessment.

Since all but two assessments are from 2004, *H. tigerinus* in 2008 and *L. grunniens* in 2019 (Table 3, Suppl. material 2), recent research findings sometimes provide more clarity regarding the unsettled taxonomy of the aforementioned species/taxa.

Of the three "species" that clearly represent complexes of many different species, we highlight what is known here, but reiterate that the dearth of data is staggering, considering that these are the most economically valuable species in terms of the known trade in commercial frogs' legs.

Fejervarya cancrivora. - An initial molecular analysis, six years after F. cancrivora was evaluated in the IUCN Red List (IUCN SSC Amphibian Specialist Group 2022a), revealed three geographically distinct clades/subclades: one confined to Bangladesh, Thailand and the Philippines; another representing Malaysia and Indonesia (Greater Sundas); and the remaining one from Sulawesi (incl. one population in southern West Java, as a result of human introduction) (Kurniawan et al. 2010). A second study by Kurniawan et al. (2011) examined the species' morphological traits and crossing experiments through artificial insemination that resulted in three distinct taxa: 1) populations of West Java, peninsular Malaysia and Bangladesh assigned to *F. cancrivora*, 2) populations from the Philippines and China previously referred to as *F. moodiei* and 3) a new species endemic to Sulawesi. However, findings of a more recent study delimit F. cancrivora to Thailand, peninsular Malaysia and Indonesia (Sumatra, Kalimantan, western and central Java, Bali), with introduced populations occurring in Papua New Guinea and Guam (Yodthong et al. 2019; and refs therein). According to Dubois and Ohler (2000), F. moodiei was considered a valid species and almost 20 years later, the species was confirmed from mainly coastal areas of South Asia (eastern India, Andaman and Nicobar Isl.,), East Asia (southern China) and Southeast Asia (Vietnam, Thailand, Myanmar, Malaysia and the Philippines [Luzon Isl.]) (Yodthong et al. 2019; and references therein).

Clear taxonomy is the foundation of efficient and sustainable species conservation and so is the naming of the species or parts thereof that are to be traded. Examination of 209 frozen frogs' legs sold in supermarkets in France listed exclusively as *Limnonectes [Rana] macrodon* (based on product labelling), revealed that almost all (206 of the 209 or 98.6%) were in fact legs of *F. cancrivora* and only 2 (0.96%) could be attributed to *L. macrodon*, while one sample was revealed to be *F. moodiei* (Ohler and Nicolas 2017). Such forensic studies clearly highlight the importance of competent species identification, especially when it comes to evaluating current use in terms of sustainability, as the lack of such information precludes accurate monitoring of trade as a consequence of misidentification. Many more members of both the Dicroglossidae and Ranidae families are commercially involved in the frogs' legs industry and their taxonomic status remains blurry at best.

Hoplobatrachus tigerinus. – In their Red List assessment, Padhye et al. (2008) indicate *H. tigerinus* reflects a species complex including an unknown number of morphologically very similar (cryptic) species. This was confirmed by Hasan et al. (2012). Most recent research identified populations of *H. tigerinus* from Pakistan and Bangladesh as genetically identical to those from Nepal (Khatiwada et al. 2017), but genetically different from Indian populations (Akram et al. 2021). Clearly, this is a complex issue with much more clarity needed before the trade becomes sustainable.

Limnonectes kuhlii. – The taxonomic status of *L. kuhlii* associated with the species' currently known distribution range has been described as particularly uncertain within the Red List assessment (IUCN SSC Amphibian Specialist Group 2022e) and many more new taxa have been assumed with some revealing range-restricted distributions. Following genetic research, this complex now includes a minimum of 22 "distinct evolutionary lineages" (McCleod 2010). Again, the real biological entities that are involved in the commercial frogs' legs trade clearly are not well understood, much less studied to the degree to which we can provide realistic plans or guidelines for sustainable trade.

Ecological impact of trade

Sixteen of the 30 anuran species listed in Table 3 and Suppl. material 2, (i.e. Fejervarya cancrivora, Limnonectes blythii, L. grunniens, L. kuhlii, L. leporinus, L. macrodon, L. malesianus, L. microtympanum, Lithobates pipiens, Pelophylax bedriagae, P. caralitanus, P. kurtmuelleri, P. ridibundus, P. shqipericus, Rana amurensis and R. chensinensis) have commercial (regional) overharvest/overexploitation as a significant/main threat (both for food) indicated as assumed or known threat in their respective Red List Assessments. Species that were previously intensively exploited were not included (i.e. Hoplobatrachus tigerinus and Leptodactylus fallax), as former legal trade was banned in the mid-1990s (Padhye et al. 2008) and other utilisation has been banned since the 2000s (IUCN SSC Amphibian Specialist Group 2017). It is important to note that the conservation status of most species involved in the food trade (Table 3; Suppl. material 2) is not up to date (53% or 16 species last assessed 2004–08) and re-assessments of some species might indicate overexploitation, adding more species where commercial exploitation for international consumption is considered unsustainable.

Prior to export for international trade, a considerable number of live animals die on arrival to the processing facilities. For Indian exports, this loss has been estimated at 10–20%, in Indonesia it is 40–50% because quality is not sufficient for export and some frogs are killed prior to being exported (Niekisch 1986 and references therein). Information on pre-export mortality rates in countries of origin were not easy to obtain within the scope of our study. These figures are also relevant when it comes to evaluating the ecological impact of harvest and more clear understanding of how these losses could be lowered would benefit both the people involved in the trade and the frog populations.

Initial reports on the sustainability of this trade were published more than 20 years ago; however, large-scale ecological studies to assess offtake rates and their sustainability appear severely lacking. Here, we highlight studies that indicate amphibian declines associated with harvest for the food trade both regionally and internationally. Historically, overharvest was detected in Californian populations of *Rana aurora draytonii* (Jennings and Hayes 1985). In Florida, harvest regimes of *Lithobates [Rana] grylio* affect population structure and survival rates (Ugarte 2004). The increasingly intense regional harvest of frogs in West Africa, particularly in Nigeria where trade has moved

across borders (e.g. Benin), clearly demonstrates overexploited species and populations (Mohneke 2011). The harvest of populations of *Quasipaa spinosa* in Hong Kong is also detrimental to populations in the long-term (Chan et al. 2014). Below, we highlight case studies that report on overexploitation of species/populations from Indonesia and Turkey involved in the international commercial trade.

Indonesia

In 2005, Kusrini noted that current harvest levels of *Fejervarya cancrivora* and members of the *F. limnocharis-iskandari* complex (*F. iskandari* was separated from the *F. limnocharis* complex through allozyme data; Veith et al. (2001)) appear to be sustainable; however, offtake of *Limnonectes macrodon* may detrimentally affect populations more than those of *F. cancrivora*.

The majority of frog hunters in East Java reported that the number of harvested frogs has decreased and this was also perceived by middlemen (in West and East Java) and exporters, who argued that, depending on the season, supplies were sometimes scarce (Kusrini and Alford 2006). To explain declines in frog populations, hunters reported a combination of three reasons, "1) increasing numbers of harvesters; 2) increasing numbers of middlemen, allowing harvesters to go to other middle-men; and, 3) habitat change, as more rice fields have been developed for other uses" (Kusrini and Alford 2006). However, overharvest synergistically promotes decline of amphibian populations happening simultaneously from habitat loss and degradation, pollution, disease and invasive exotic species (Kusrini 2007).

Several regional field studies have been conducted in Indonesia to assess population densities of frog species involved in the food trade, and these clearly show these synergistic effects. In a 20 × 20 m paddy field in West Kalimantan, the density of *E. cancrivora* was measured at 1.01 individuals/ m^2 (Saputra et al. 2014). According to Iskandar (2014), populations of Limnonectes blythii in West Sumatra have largely been decimated by export of frogs' legs (though once again monitoring is absent) and, hence, the harvest of populations has shifted to other provinces like Riau, Jambi and South Sumatra. The Karawang District, on the other hand, is the largest producer of frog meat in West Java. In order to determine the sustainability of hunted populations of F. cancrivora, in May 2016, an approximately 10-day population survey was conducted in a rice field in eastern Karawang. Average density for juveniles was 0.33 individuals/ m², 0.04 for subadults and 0.005 for adults. In contrast, average density in watered paddy fields was 0.89 individuals/m² for juveniles, 0.08 for subadults and 0.01 for adults (Kurniati and Sulistyadi 2017). Depending on the season and the status of the rice fields (state of cultivation, amount of water), an average of 3-10 kg of adult frogs can be caught per night since frog hunters have an agreement not to capture juveniles and subadults to maintain viable breeding populations (Kurniati and Sulistyadi 2017). Populations of F. cancrivora in the rice fields of the Karawang Region are considered unhealthy, most likely due to unsustainable exploitation and setting export quotas for frogs' legs should be done with care (Kurniati and Sulistyadi 2017). The main threat

to *F. cancrivora* is the large-scale harvest for trade and consumption, although habitat destruction and degradation also play a role and further impair population recovery following collection of individuals from the wild (Amin 2020).

Limnonectes macrodon is also regionally impacted and preferred for their better taste (compared to *F. cancrivora*; Kusrini and Alford (2006)). In addition, *L. macrodon* has slower reproduction rates, [~ 1000 eggs per clutch (Iskandar 1998) as opposed to > 18,000 eggs in one spawning for *F. cancrivora* (Saputra et al. 2014)] and is, therefore, more vulnerable to overharvest. According to Ohler and Nicolas (2017), populations of *L. macrodon* are in rapid decline.

Turkey

Overharvest of frog populations in Turkey (intended for export to France, Italy, Greece, Spain, Switzerland and Lebanon) has been reported by Şereflişan and Alkaya (2016), who note that a reduced weight in frog populations has been attributed to overharvest and that had a negative effect on the export value. Regional overharvest in Turkey has been shown for *Pelophylax caralitanus* populations in south-western Anatolia (Erismis 2018).

A very recent study, by Çiçek and others in 2021, on the sustainability of Anatolian water frogs, is by far one of the most comprehensive studies to analyse commercial trade in frogs' legs for the EU market. In 2013–2015, > 13,000 *Pelophylax* spp. (cf. Red List assessments of *Pelophylax bedriagae*, *P. caralitanus* and *P. ridibundus*) from two regions were tagged for population and density estimation. A population viability analyses was conducted over a 50-year period, based on catch and export data from Turkey. If this trade were to continue at the same harvest rate, extinction risk would be 90% in 50 years, affecting two to five species of the *Pelophylax* species complex (Çiçek et al. 2021 and references therein). Accordingly, a reduction of harvest rates would be advisable in order to be able to ensure the viability of these frog populations and a long-term source of income for the harvesters/frog catchers (Çiçek et al. 2021).

Discussion

During the course of this study, it became clear just how difficult it is to obtain concrete data on the current international trade in frogs' legs. Specifically, relevant data are scattered across different unconnected databases (for example, national databases, FAO, EUROSTAT or information/services that can only be obtained/provided via payment, for example, Infofish International (http://infofish.org/v3/, Suppl. material 3). Another problem is data reliability with the competence of sourcing agencies and institutions having conflicts of interest and little expertise in frog identification. While the USA primarily imports live frogs and frog products for human consumption originating from frog farms, frogs and their processed legs imported into the EU are mostly sourced from the wild. The EU trade also includes far more species than are officially declared, potentially including many cryptic species of conservation concern. Our findings highlight the central role of the European Union as the main importer of frogs' legs derived from wild individual anuran populations, the urgent need for stricter trade regulations, better monitoring and data integrity to prevent further declines of wild frog populations and to help create a more sustainable commercial trade.

A long road to EU accountability

The high uncertainty of the assumed number of individual frogs within total imports throughout the study period impressively illustrates the opacity of the trade. Actual harvest numbers imported into the EU for annual consumption remain unknown and very difficult to quantify. This is undoubtedly due to the fact that they are non-CITES species and, thus, international trade data (species/volumes) remain undocumented. Listing species in the appendices of CITES is justified when international trade poses a severe threat to the conservation status of a species. The scientific authority of a CITES member state must review the harvest/export for Appendix II in terms of compatible offtake numbers/quotas in order to maintain the species' ecological function in its native habitat (https://cites.org/eng/disc/text.php#IV, accessed May 2022, see Suppl. material 3). Complete transparency of annual quotas and the quantification of numbers of individual frogs per kilo must be ensured if a kilo value is to represent the number of affected individuals. It remains unclear for what reasons the calculations of the number of individuals per kilo have been reduced by seven animals as of 2018 (Table 2) and we remain sceptical of these numbers.

Anurans involved in the international frogs' legs trade are all r-strategists, which means that they have large numbers of offspring, a rapid developmental rate and a high reproductive output. This also makes these species more amenable to regular (monitored) harvest while remaining viable. However, r-strategists also define themselves in having highly variable population sizes over time and mortalities may be density-independent or even catastrophic (Pianka 1970). Despite relatively high individual densities of some species in agroecosystems, regular removal of thousands of individuals still raises questions about the extent that the ecosystems can compensate for this intervention. For example, negative ecological shifts may have already occurred (for example, can ecologically more flexible species outcompete more specialised species and how have populations of insect pests been affected by fluctuations in frog populations?). There is also no doubt that trophic interactions in certain agro-ecosystems such as rice fields are very complex and we still do not have a grasp of the main drivers of the complexities. For example, type of cultivation and human impact can have severe implications on biodiversity. Abrupt regular removal of rice plants in a wet paddy, for instance, results in a considerable sudden loss of energy for the entire biotic community (cf. Bambaradeniya et al. (2004)). A decline in pond frogs (Pelophylax nigormaculatus) in rice field-dominated landscapes in Japan has been noted as a result of the modernisation of drainage systems which also led to the decline of the grey-faced buzzard (Butastur indicus) (Fujita et al. 2015). It is clear that human impacts on nutrient supply and food web structure have strong and interdependent effects on biodiversity and ecosystem functioning and it is, therefore, essential to monitor/these both (see Worm et al. (2002)).

These considerations may, however, be too complex to be actively explored within the framework of the EU. We highlighted that there are many internationally traded species/species groups with sales in the EU where unsustainable trade has been detected (cf. Symes et al. (2018)), that could be regulated more easily. Governmental priorities within transnational cooperation projects should develop common methodological approaches that include genetics (species identification and origin) and biosecurity measures to prevent the spread of disease.

However, in the context of amphibians that are, for example, imported live into the EU for the exotic pet trade industry, amongst which many are traded that are also known to be infected with *Bd/Bsal*, (see Wombwell et al. (2016); Nguyen et al. (2017); Fitzpatrick et al. (2018)), even here, biosecurity measures prior to the import into the EU (incl. non-EU- European States) have not been implemented to prevent cross-infections, despite the fact that *Bd* was listed as a notifiable disease by the World Organisation for Animal Health (OIE) in 2008 (Schloegel et al. 2010) and *Bsal* in 2017 (https://www.oie.int/app/uploads/2021/03/a-bsal-disease-card.pdf, see Suppl. material 3).

IUCN Red List assessments

Required data for the IUCN Red List are crucial for assessing the conservation status of species. In Red List assessments, trade in a species can either: (1) be mentioned at the national/ international level, (2) go unmentioned (despite the fact that trade occurs) or (3) if mentioned, in some cases be designated as an acute threat to a species/population. In such cases, it is particularly problematic when Red List assessments are up to 19 years old (Table 3, Suppl. materials 2, 4) and for species utilised domestically or traded internationally where overexploitation was already identified in 2004, but impact on the local populations has not been well assessed (e.g. *Limnonectes blythii, L. kuhlii* or *L. malesianus*; see Table 3). Our query in August 2022 retrieved 187 species (see Methods); a query in January 2023 resulted in 219 species; however, the selected query parameters do not cover all relevant species, for example, *Fejervarya moodiei* (cf. Table 3).

Taxonomic uncertainties, interbreeding

Several *Pelophylax, Limnonectes* and *Fejervarya* spp. are morphologically very difficult to distinguish and many taxa are taxonomically treated as cryptic species complexes (see Bickford et al. (2007)) within their genera (Kurniawan et al. 2011; Dufresnes et al. 2018; Yodthong et al. 2019). Therefore, challenges of quantifying actual harvest of each species are substantial if these taxa are harvested in the hundreds of thousands to millions of individuals per year. Accurate identification of species is the foundation for any management plan and trade and conservation need to go hand in hand. Disregard of this basic knowledge and trading activity can cause fundamental damage to the species and, in the worst case, to respective ecosystems (Estes et al. 2011). Unfortunately, it is precisely this taxonomic uncertainty that is exploited by companies, for example, as done in Turkey, labelling frogs as the hybrid *Pelophylax esculentus* which does not

occur in Turkey but does in other parts of Europe (Çiçek et al. 2021). Evidence provided by genetic methods could reveal incorrect labelling in Indonesian exports of frozen frog's legs destined for European markets with packages indicating Limnonectes [Rana] macrodon rather than as Fejervarya cancrivora, but rigorous assessments of accuracy of species identification have not been conducted (Dittrich et al. 2017; Ohler and Nicolas 2017). In 2001, Veith and colleagues could separate F. iskandari as a valid species from the *F. limnocharis*-complex through allozyme data. Another clear example is *F. iskandari* (restricted to the island of Java) which was previously traded undetected within the F. limnocharis-complex (Kusrini 2005) and could be negatively impacted by overharvest. Apart from these examples of harvested taxa included in species-complexes with uncertainty in their taxonomic status (e.g. Holsbeek et al. (2008); McLeod (2010); McLeod et al. (2011); Dehling and Dehling (2017); Yodthong et al. (2019); Stuart et al. (2020)), introduction of exotic species that interbreed with closely related species or crossbreeding incidences of farm escapees into other ecosystems (Yu et al. 2015), may lead to a replacement of formerly native species (cf. Leuenberger et al. (2014)). In addition to these concerns is the potential for an invasive species (e.g. Lithobates catesbeianus) to become a driver of ecological trophic cascades in naive ecosystems (e.g. Gobel et al. 2019). Such issues are well known from other taxa, yet the lack of monitoring and the number of cryptic species underscores the under-appreciated risks associated with hybridisation of these as yet unrecognised frog species. Species identification of skinned or frozen frogs' legs is impossible without genetic techniques, thus mislabelling may not have been strategic, but an indication that processors and exporters in Indonesia are not trained in frog species identification. This knowledge was not considered a prerequisite for the export of frogs' legs and, as there are no strict checks, the trade of potentially misidentified species has been allowed to continue. Of more concern, it may also be that maintaining consistent supplies would not be possible if adequate scrutiny of what is in the trade, where it is from and how availability fluctuates, are taken into account. In fact, it must be clearly emphasised that the prerequisite, "we only eat/trade what we know", has not yet been met and relevant stakeholders (including government agencies) have not made an adequate effort to address this issue.

Ecological impact and economic uncertainties

Sustainable international trade can only be ensured if the use and movement of species within national borders is managed in such a way that species or populations maintain their viability and do not show shifts in physical traits due to bias in selection of key traits (cf. Leader-Williams 2002). In fact, differences in body size in intensely harvested populations of *Lithobates [Rana] grylio* are probably due to selective harvesting pressure on larger size classes (Ugarte 2004). Kusrini (2005) found that body sizes of captured adults are smaller than those of the same species in other un-harvested regions and capturing larger adults may lead to lower recruitment rates. Similarly, the pronounced sexual dimorphism in species attractive to hunters (e.g. *F. cancrivora* and *L. macrodon*), leads to reduction in the number of those larger individuals. As females
are usually larger in anurans (Duellman and Trueb 1994), the collection of breeding females, in particular, significantly reduces the potential reproductive effort and, thus, will detrimentally impact populations. For the maintenance of viable populations, sexual dimorphism traits should, therefore, be considered in harvest regimes to sustain populations. According to Kusrini (2005), one important criterion for monitoring is the recording of body size. These worrying, but prescient data from 17 years ago do not seem to have been properly considered until now and viability of harvested frog populations has largely been overlooked.

In this context, governments are called upon to use resources in an adaptive and sustainable manner. Furthermore, EU commitments to Environmental Impact Assessments (EIAs) of imported wildlife mean that the EU is obligated to monitor what is in trade as well as the impact it is likely to have on source populations. As soon as the species triggers international demand and sales, importing countries are equally held accountable to take responsibility, whereby relevant stakeholders must ensure that their consumption of exotic species does not lead to population declines. Clearly, this will entail other anthropogenically induced threats affecting these species/populations (e.g. Chen et al. (2019)). It is worrying to note that there are very few studies reviewing current trade in terms of sustainability and the little information that is published, implies very strongly that current harvest/trade is unsustainable. For example, populations of Pelophylax caralitanus are still locally widespread in Turkey, but the species is considered endangered (Öz et al. 2009), not only because of habitat loss, but also because of local overexploitation for trade with the EU (Erismis 2018; Cicek et al. 2021). Further, overharvest of Pelophylax shqipericus has been noted in the species' Red List assessment (IUCN SSC Amphibian Specialist Group 2020e) and the unsustainable trade of this species has been highlighted (Gratwicke et al. 2010). However, populations of *P. shqi*pericus in Albania (core distribution of the species) have not yet been considered within a conservation management plan (Eco Albania 2019).

Numerous examples of overexploited species assessed in the IUCN Red List assessments are detailed (see Table 3, Suppl. materials 2, 4) and examples of unsustainable trade at the regional level (for example, in western Africa and that of species and species complexes in Southeast Asia) have also been presented. However, there is a severe shortage of established field studies (cf. Auliya et al. (2016); Morton et al. (2021)) over longer periods of time to provide not only snapshots of single localities, populations and their harvest status, but also long-term studies (for example, use of pesticides and potential residues on populations in trade, impact of local population declines, if populations can maintain their role as pest control etc.).

According to Raghavendra et al. (2008), comprehensive ecological field studies in India investigating the function of anuran communities and their control of pests such as mosquitoes are still in their infancy. Local knowledge in West Java (Indonesia) reveals that at least *Fejervarya limnocharis* is perceived in functioning as pest control (Partasasmita et al. 2016).

A two-year field study in the Philippines compared prey items of the native Luzon wartfrog (*Fejervarya vittigera*) with that of the introduced cane toad (*Rhinella marina*)

to determine the proportion of rice pests in their diets and which of the two species was more efficient feeding on rice pests. It turned out that the proportion of pests eaten by *F. vittigera* was significantly larger than that of *R. marina*, which mainly preyed on beneficial arthropods in the rice-ecosystems. The authors conclude that adult *F. vittigera* may provide effective pest control services and suggest protecting and promoting *F. vittigera* populations (as opposed to reducing *R. marina* populations) to minimise the use of insecticides (Shuman-Goodier et al. 2019).

Is frog farming a sustainable alternative?

Due to problems of sustainability caused by the removal of species from their ecosystems (see Table 3), various authors suggest a focus on commercial frog farming (e.g. Nguyen (2014); Şereflişan and Alkaya (2016); Ribeiro et al. (2019)). Indeed, commercialisation of frog farming appeared to be the way forward for a promising industry in many countries (first attempts at breeding *Lithobates catesbeianus* in the US and Canada are dated before 1900), but continuing efforts to implement these plans have proved less successful (Helfrich et al. 2009; Dodd and Jennings 2021). Such ventures have been discouraged since the 1930s and many problems (for example, live food and water quality availability, risk of spreading disease, slow mass increase or growth and economic start-up constraints) were known to the early proponents of such ventures. However, because investments are relatively low and profits can be many times higher, this branch of business creation continues.

Globally, *Lithobates catesbeianus* is the most widespread species involved in farming operations and has been introduced for the purpose of commercial farming into more than 40 countries (FAO 2021).

In other parts of the world, initiatives to commercialise frog farming are also being publicised as a result of increased demand. For example, under EU funding, the CaPFish Capture and Aquaculture programmes were launched to promote aquaculture in 10 provinces of Cambodia, primarily to promote food security in line with national government plans for fisheries development. Specifically, the Minister of Agriculture, Forestry and Fisheries, "Veng Sakhon", encouraged farmers to raise frogs due to an increased market demand (https://en.khmerpostasia.com/2020/10/16/frog-farming-encouraged-as-market-demand-rising/, accessed, June 2022, see Suppl. material 3). However, this programme is explicitly designed for national needs, not international export.

Likewise, in Thailand, establishment of commercial frog breeding facilities has been described and limited for national consumption (Pariyanonth and Daorerk 1994).

A major problem underlying establishment of commercial frog farming facilities is that there are no international standards or hygiene guidelines (see Dittrich et al. (2017)). In some of EU's major supplying countries, i.e. Vietnam, frog farms remain being insufficiently controlled (Nguyen 2014; Nguyen and Tran 2021) implying that no health controls are imposed on farms and processing into frogs' legs, as well as testing for disease. As a result, the risk of international trade spreading diseases, such as ranavirus and Bd into naive amphibian populations, is ever-present (cf. Gratwicke et

al. (2010); Gilbert et al. (2013)). However, unfavourable conditions are present, for example, the lack of appropriate management measures, resulting in the (unintentional) release of disease-infected *L. catesbeianus* into the environment of supplier countries (cf. Ribeiro et al. (2019)). Species escaping from breeding farms may also hybridise with congeners and here the problem of genetic pollution needs to be addressed.

An additional complicating factor for international control is that species harvested for frogs' legs are exclusively non-CITES species, implying that there is no documentation across international borders.

Conclusions and recommendations

The complexity of issues underlying the frogs' legs trade is not a priority policy item for the EU, despite several important issues reviewed herein. This neglect strongly suggests that the EU, as the main consumer of wild-harvested frogs' legs, has deliberately shirked responsibility in addressing the many issues facing the frog's legs trade. The important precondition for such trade must be that consumers in the EU can have a guarantee that their actions will not contribute to the decline of species they consume or cause the spread of pathogens to native species. However, to achieve this goal, all stakeholders have to work together to remove existing loopholes and implement new regulations to control the trade in the foreseeable future. Full transparency of current supply chains, including information on sourced populations or commercial breeding farms, is also critically needed. Otherwise, we suggest temporarily suspending trade in certain species until such data are available and assurances made by all stakeholders. These measures result from the uncertainties highlighted here and are to ensure maintenance of viable populations in the countries of origin. Accompanying these should be awareness campaigns and education to help foster information for consumers to help them make decisions. The role of the EU should, therefore, be guided by the problematic conditions of this trade (unclear taxonomy, unsustainable offtakes, no disease control/biosecurity measures, re-introduction of exotic and invasive species and lack of a centrally established checkpoint for imports into the EU) in order to develop a more responsible and sustainable framework of the frogs' legs trade. The only measure the EU has in place for non-CITES species at present is TRACES and it generally fails to list species. In addition, the World Trade Organisation (WTO) does not require amphibian species to be clearly listed in trade, which makes monitoring of international trade activities almost impossible.

One fact, in particular, became clear in this review: the lack of knowledge about species conservation and factors to promote implementation of sustainable harvest. The establishment of strictly supervised commercial farming according to industry-set protocols and hygiene measures (especially in the main supplier countries) and the difficulty in implementing these, is ignored by the EU. On both sides of the trade, short-term economic benefit is more important than long-term sustainability of the trade itself. Unsustainable trade prevents continued harvest and, therefore, long-term economic viability and ultimately ecological costs will also mount unrealised until severe non-linear results accrue (for example, crop failure due to pest outbreaks because predators have gone, as in India in the 1970s). This observation is particularly sobering because the international trade in frogs' legs has been ongoing for decades (Le Serrec 1988; Warkentin et al. 2009; Altherr et al. 2011).

It is irrefutable that the international frogs' legs trade into the EU is riddled with uncertainties (no biosecurity measures, species identity is opaque, reported source is absent or doubtful etc.). The EU, as the main consumer of frog's legs, does not assume any obligation to responsibly solve problems listed in this review, but herein is challenged to address the problems identified. We can only presume that many departments and agencies within the EU are aware of the extreme complexity of this trade with its diffuse network and various databases, but clearly put economics before the conservation of natural resources or the long-term benefits and livelihoods of people involved in the trade internationally.

Gratwicke et al. stated in 2010 that additional CITES listings could help reduce negative impact of international commercial trade. As stated earlier, IUCN Red List assessments of several trade-relevant anurans highlight the need for improved monitoring and creating a more regulated trade. Intensively traded species should also be reevaluated for IUCN Red List status at more frequent time intervals in order to add upto-date information on the conservation status of vulnerable species. More specifically, we propose that the IUCN SSC Amphibian Specialist Group designate a new working group that monitors and evaluates the conservation/threat status of particularly intensively harvested/traded species involved in the frogs' legs trade at regular annual intervals. This information is critical to be implemented into CITES for timely decisions.

The increasing incidence of infectious diseases (both within a species as well as zoonotic spill-overs) via the wildlife trade correlates closely with the loss of biodiversity in source countries and is considered a worrying environmental problem that must be counteracted as a matter of urgency (see Kiesecker (2011)).

More science required

Modern innovative scientific methods are required to ensure a fully transparent, legal, traceable and sustainable trade. We will need to implement scientific methodologies to distinguish farmed vs. wild individuals (cf. Dittrich et al. (2017)) and to obtain sufficient data on all source populations to ensure that harvest levels fall below annual population replacement levels. Basically, taxonomic uncertainties need to be clarified and the formation of specific research groups (for example, taxonomists, field ecologists, experts of current legal frameworks etc.) is highly recommended.

To prevent the spread of infectious diseases, biosecurity measures need to be established at distinct points along the trade chain. Interestingly, such measures were already proposed at the 37th Standing Committee of the Convention on the Conservation of European Wildlife and Natural Habitats, in December 2017 (https://rm.coe.int/recommendation-on-biosafety-measures-for-the-prevention-of-the-spread-/168075a4b0, accessed May 2022, see Suppl. material 3), but never implemented. Therein, recom-

mendation No. 197 refers to "biosafety measures for the prevention of the spread of amphibian and reptile species diseases". This document lists 10 recommendations for contracting parties, none of which includes information on species traded either alive or processed for the frogs' legs trade. The majority of recommendations encourage support for increased research. However, recommendation 5, "Using the most appropriate legal framework and, at the earliest opportunity, implement immediate restrictions on the amphibian and reptile species trade when an emerging pathogen spread with significant impact on wild populations has been identified until necessary preventative and management measures are designed, based on evidence, throughout the entire commercial chain", does not reflect an expansion of the regulatory framework, but describes a direct suspension of trade in an infected species. With regard to the prevention and spread of known diseases identified by OIE (such as Bd), we reference a document from 2015 by the Standing Committee to the Convention on the Conservation of European Wildlife and Natural Habitats on the Recommendation on the Prevention and Control of the Bsal fungus (https://rm.coe. int/1680746acf, accessed May 2022, see Suppl. material3). The implementation of these recommendations, however, cannot be verified. The need for supervision of hygiene and veterinary inspections for edible frogs (also those farmed and are non-native) in the Asian region has been indicated (Grano 2020; Borzée et al. 2021), given the tight links observed between market locations and detection of *Bd* in wild amphibian populations.

Hardouin (1997) stated that authorities in countries that import frogs' legs should be encouraged to regulate international trade more closely by banning products that cannot be sourced from farms where they are subject to official controls. He further notes that Europe cannot ignore risk of wild harvests that may lead to declines in local frog populations as a result of overexploitation. We also recommend the listing of some if not all species in trade on CITES App. II. International trade should be regulated for those species that are already documented in an IUCN Red List threat category and those for which there is published evidence that trade has depleted local or regional populations. Taxa in species complexes whose morphological differentiation is not readily possible or are processed only as frogs' legs are particularly vulnerable, so standardised use of molecular approaches to verify and monitor trade would be particularly useful.

Results outlined in this review provide strong clear recommendations for both source and consuming countries. Promptly counteracting abuses in the international trade of frogs' legs by adapting existing legislation and applying the precautionary principle to prevent irreversible damage to populations or species will help to promote the sustainability of the trade in the long-term. Recommendations for source and consuming countries are listed separately below.

We recommend that source countries should:

• conduct field surveys at comparative study areas to estimate size and trends of wild frog populations and of the impact of harvest for both national consumption and international trade.

• validate species identity through centralised authorities to check and certify trade exports through the use of genetic tools.

• include analyses of trade data and standardise documentation of volumes (number of individuals must be considered, not an estimate of the number of individuals by means of weight).

• establish long-term field studies in selected areas (where regular harvest takes place) to assess biotic communities in relation to the application of pesticides.

• make non-detriment findings (NDFs) a result of CITES listings at regular time intervals.

• examine the domestic/national use of frogs' legs versus exports to decipher the complexity of this resource use and improve equity and fairness within each source country.

• study mortality rates of frogs in transport and processing prior to export. When identifiable loopholes exist, source countries should make every effort to minimise mortality and economic loss.

• accurately and regularly verify harvest rates, including both local as well as harvest for international trade. As highlighted earlier, it has been estimated that offtakes of edible frogs on a national level can be seven times as much as that of annual exports (Kusrini 2005).

• establish conservative, but reasonable harvest and export quotas, based on high quality data for targeted species/populations and taking into account other threats that affect species/populations.

• ban harvest during the mating season. Specific management measures have been highlighted for the harvest of *Pelophylax* spp. in Turkey and claim, "that further harvest restrictions are essential for the sustainability of Anatolian water frog populations" (Çicek et al. 2021).

• evaluate and implement adaptive management measures for all harvested species, i.e. the ban of certain size classes for a given period/season as a default to help ensure sustainability.

• define and implement stricter regulations for farming operations to ensure closed systems, prevent re-stocking from the wild, release of farmed animals back into the environment, as well as avoiding farming of non-native species when possible.

• register and monitor all export companies and their suppliers and require that exporters identify processed frog products by DNA analysis.

Consumer countries have the obligation to take appropriate responsibility for the consumption of a resource. Accordingly, it would be obligatory to transparently inform relevant societies on which information basis trade is permitted.

We recommend that consumer countries should:

• implement a centralised database to document all imports of all wildlife and list species and quantities in the Annexes of the EU Wildlife Trade Regulation, using the USFWS-LEMIS Database (2023) as a model.

• list all species in trade in CITES to regulate international trade and enforce restrictions.

• implement NDFs for the import of species from the wild, regardless of CITES status.

• provide captive breeding guarantees for species claimed to be of captive origin.

• push for improved standards (based on revised guidelines), such as import bans on wild harvested species that have been evaluated in one of the IUCN Red List threat categories.

- impose trade suspensions if trade data are not provided in full transparency.
- check all imports for pesticides and other pollutant residues.

• assist range states in conducting surveys of wild frog populations and to create a biobank with references samples from species/populations of major harvest regions to cross-check genetic identities of shipments imported.

• conduct random DNA analysis of frogs' legs shipments to determine if shipment labelling is correct and ban imports for persistent mislabelling.

• allow only positively identified, skinned, processed and frozen frogs' legs to be imported to avoid the introduction and spreading of diseases and invasive species.

• rigorously catalogue all imported species with standards parallel to those implemented under the USFWS-LEMIS Database (2023).

• improve regional monitoring schemes with joint-efforts between stakeholders and governments to bolster the sustainability of the trade along multiple facets.

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Supplementary material I

Three world map distribution images and 2 world trade graphs

Authors: Mark Auliya, Sandra Altherr, Charlotte Nithart, Alice Hughes, David Bickford Data type: docx file

- Explanation note: Export and import data of frogs' legs by country/regions from 1996–2009. Source: Atlas of Economic Complexity (2023). Richness of frog species in trade for consumption based on IUCN species ranges. Richness of frog species traded internationally for consumption based on IUCN species ranges. Number of species native to each country in international trade for consumption based on IUCN species ranges (see Methods).
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

Link: https://doi.org/10.3897/natureconservation.51.93868.suppl1

Supplementary material 2

Anuran species assessed in the IUCN Red List with uncertainties mainly prevailing in national/international trade routes, and level of exploitation

Authors: Mark Auliya, Sandra Altherr, Charlotte Nithart, Alice Hughes, David Bickford Data type: docx file

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Link: https://doi.org/10.3897/natureconservation.51.93868.suppl2

Supplementary material 3

Online sources and those useful with explanatory information

Authors: Mark Auliya, Sandra Altherr, Charlotte Nithart, Alice Hughes, David Bickford Data type: docx file

- Explanation note: Useful data sources available online (URLs), with data type(s), and management authority for data and websites. Other relevant information accessible online referred to in the main text.
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Link: https://doi.org/10.3897/natureconservation.51.93868.suppl3

Supplementary material 4

Amphibian species assessed in the IUCN Red List that are utilized either doemticalyy/nationally and or internationally

Authors: Mark Auliya, Sandra Altherr, Charlotte Nithart, Alice Hughes, David Bickford Data type: excel file

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RESEARCH ARTICLE



The trail less traveled: Envisioning a new approach to identifying key food resources for threatened Hawaiian arboreal snails

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Abstract

Our understanding of Hawaiian arboreal snails' diets remains rudimentary, hindering the development of effective conservation strategies. To identify important food resources, we tested the hypothesis that epiphytic microbial assemblages differ on plant species preferred and avoided by snails at Mt. Kaala Natural Area Reserve, where snail plant preferences are known from previous studies. Comparing microbial assemblages on plants that snails both prefer and avoid was identified as a potentially key step to moving research away from characterizing which microbes snails encounter, towards testing if microbial assemblages are driving snail plant preferences. We found that fungal and bacterial assemblages differed between plant species preferred and avoided by snails, indicating that Hawaiian arboreal snails may be selecting plants based on their epiphytic microbial assemblages. Previous microbes thought to be important, *Cladosporium* spp., propagated in captive rearing facilities, and *Botryosphaeria* spp., preferred fungi in a feeding experiment, were both rare and had similar abundances on preferred and avoided plant species in Mt. Kaala. Our approach, conducting preference studies before isolating microbes, is key to identifying arboreal snail food resources and improves our ability to identify microbes that form the foundation of Hawaiian arboreal snails' diet. If we can identify important food resources, it greatly expands our ability to: (1) assess and monitor habitat quality, (2) make informed restoration recommendations, and (3) improve rearing efforts for highly endangered captive reared populations.

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Keywords

bacteria, conservation, diversity, epiphytic, fungi, gastropod, microbe, Pacific, trophic

Introduction

The Hawaiian archipelago is a land snail diversity hotspot with high richness (759 described species), endemism (> 99%), and extinction rates (Cowie 1995; Cowie et al. 1995; Yeung and Hayes 2018). Most estimates of extinction suggest that > 65% of the Hawaiian land snail species have already been lost and families have already been extirpated from the islands (Solem 1990; Cowie 1998, 2001; Lydeard et al. 2004; Yeung and Hayes 2018). Since many of the remaining species continue to experience reductions in range and population sizes, effective conservation strategies are urgently needed (Solem 1990; Yeung and Hayes 2018). Unfortunately, developing effective management strategies for Hawaiian snails, and most threatened invertebrates, is often hindered by a lack of key ecological information (Lydeard et al. 2004; Cardoso et al. 2011; Yeung and Hayes 2018).

While many factors contribute to the limited ecological information on threatened invertebrates (see Cardoso et al. 2011; Regnier et al. 2015; Cowie et al. 2022), unraveling the ecology of many invertebrate species can be difficult. For example, our understanding of what Hawaiian arboreal snails, i.e., snails that live primarily on trees and shrubs, eat remains rudimentary despite significant research effort (see references below). Hawaiian arboreal snails graze on the fungi, bacteria, and algae on the surface of plants, hereafter, referred to as the phyllosphere. As the phyllosphere is composed of extremely small (often single-celled) organisms and phyllosphere assemblages are hyper-diverse (i.e. thousands of different fungi and bacteria on a single plant), determining which food resources are important can be extremely difficult. However, identifying important food resources is key to assessing habitat quality, informing restoration efforts aimed at preserving the remaining Hawaiian land snail species, and developing cultures to enhance fitness and limit exposure to pathogens and/or toxins for captively reared endangered snails (Sischo et al. 2016; Strouse et al. 2021; Meyer et al. 2022).

Previous research has used a variety of approaches to identify important arboreal snail food resources, but each has shortfalls. One approach examines differences in survivorship, growth, and reproductive output when snails are offered different food resources (Holland et al. 2017; Strouse et al. 2021). For example, Holland et al. (2017) found that while survivorship was not impacted, egg production by *Auriculella diaphana* was reduced by 15 to 20% when snails grazed on non-native plant surfaces as opposed to native plants. Similarly, Strouse et al. (2021) found that *A. diaphana* produced 2.4 and 30.1 times more eggs when provided both native vegetation and a lab reared fungus relative to treatments where snails were provided just native vegetation or lab reared fungus alone. While these experiments suggest that phyllospheres on native plants can enhance the fecundity of *A. diaphana*, we are left with little information on which components of the phyllosphere are important, if patterns hold for other native and non-native plants, and if this snail species is a good model for other Hawaiian land snails. For example, the non-native ginger, Hedychium coronarium, used by Holland et al. (2017), was preferred by native succineids on the island of Hawaii, and reproduction on ginger in the wild seemed robust (Brown et al. 2003, 2006; Meyer 2012). Similarly, Metrosideros polymorpha, a widespread native tree species used by both Holland et al. (2017) and Strouse et al. (2021), was avoided by all snail species in native wet cloud-forests on Maui, Hawaii, and Oahu (Meyer 2012; Meyer et al. 2014, 2022), though *M. polymorpha* was a preferred plant for two endangered tree snails, Achatinella sowerbyana and Achatinella lila in forests with native and non-native vegetation on Oahu (Price et al. 2017). A second approach uses metagenomic techniques to characterize phyllospheres on plants used by native snails and to compare the microbial assemblages on leaves to those in fecal pellets in an effort to determine what they are eating (O'Rorke et al. 2015; Price et al. 2017). Assessing the phyllosphere of plants that act only as snail hosts has limited utility as comparisons are required to explore why certain plants and phyllospheres may be chosen. Comparing microbial assemblages between fecal and leaf samples also has limitations, as elevated abundances of some microbes in the fecal samples may suggest that microbes are important food resources targeted by the snails, or that snails are unable to assimilate those microbes. Alternatively, snail feces may act as a colonizing substrate that is facilitating the growth of microbial assemblages that have nothing or little to do with their diet. Feeding trials have also been used in a limited capacity. O'Rorke et al. (2016) explored preferences among 11 microbial (bacterial and fungal) isolates obtained from leaf and fecal samples using feeding trials. While isolating targeted microbes is difficult, extrapolating O'Rorke et al.'s (2016) findings that Potato Dextrose Agar (PDA) media and Cladosporium spp., a fungus used as a food source in the snail rearing facility, were preferred resources, is difficult, especially since snails given these resources had significantly lower fecundity (see Strouse et al. 2021).

These results highlight the large knowledge gaps in defining the key food resources for Hawaiian arboreal snails. Such knowledge shortfalls are a major hindrance to our ability to conserve biodiversity effectively (Hortal et al. 2015). Here, we explore the utility of integrating field studies that examine which plants snails prefer and avoid as hosts with metagenomic analyses which might form the foundational first step to identifying important food resources for Hawaiian arboreal snails. Three recent studies have explored which plants native arboreal snails prefer as hosts in montane wet forests on three islands: Oahu, Maui, and Hawaii (Meyer 2012; Meyer et al. 2014, 2022). While this research was conducted on three separate islands and explored the preferences of many island endemic snails, concordant patterns emerged with snails across islands having relatively similar plant preferences (Meyer et al. 2022). Across islands and species, snails preferred a subset of understory plants. *Hydrangea arguta* was preferred by all snail species across all three islands. *Ilex* spp. and *Clermontia* spp. were preferred on Oahu and Maui, respectively, though *Ilex* spp. were avoided on Hawaii where nonnative ginger (*Hedychium coronarium*) were abundant (Meyer 2012; Meyer et al. 2014, 2014, 2022). In contrast, snails avoided the two most abundant plants at all three sites: the dominant tree, Metrosideros polymorpha, and the mid-story ferns Cibotium spp. (Meyer 2012; Meyer et al. 2014, 2022). Using these results as a guiding framework, we employed a metagenomic approach to test the hypothesis that epiphytic fungal and bacterial assemblages differ between preferred and avoided plant species at Mt. Kaala, a site on Oahu where Meyer et al. (2014) previously examined plant preferences of arboreal snails. We also tested if relative abundances of Cladosporium spp. and Botryosphaeria spp. differ between preferred and avoid plant species. We chose these two fungi, because Cladosporium spp. has been used in the Hawaiian Tree Snail captive rearing facility and both fungi were preferred by snails in O'Rorke et al.'s (2016) preference study. Differences in bacterial and fungal richness, diversity, and evenness between preferred and non-preferred plant species were examined, but we had no a priori predictions about patterns of richness and diversity between preferred and avoided plants. If differences in microbial assemblages exist between preferred and avoided plant species, it provides a mechanism to: (1) develop hypotheses about which bacteria and fungi enhance arboreal snail survivorship and fitness, and (2) explore if concordant patterns with some microbes consistently having higher or lower abundances on preferred plant species exist across sites and islands.

Materials and methods

Sample collection

This study was conducted in the Mt. Kaala Natural Area Reserve (NAR), Honolulu, Hawaii (21.5064418°N, 158.1453868°W). The Mt. Kaala NAR is one of the few remaining intact, protected areas in the Hawaiian Islands. This site was chosen as arboreal snail plant preferences were previously described and the diverse flora on Mt. Kaala is composed primarily of native plants (Meyer et al. 2014). Studies in areas that harbor both native snails and are composed primarily of native plants are critical because high abundances of non-native species can modify snail behaviors and change the utility of the findings (Meyer et al. 2014, 2022). For example, identifying that snails prefer native plant species in areas that have lost significant native plant diversity may mean that sub-optimal plants are currently being used as hosts. Also, in sites where snails prefer non-native plants (see Meyer 2012), native plants with beneficial phyllospheres may be avoided, limiting restoration recommendations.

To test our hypothesis that phyllospheres differ between preferred and non-preferred plant species, we collected samples from five plant species: three plants preferred by snails, *Hydrangea arguta, Ilex anomala, Clermontia* sp.; and two plants avoided by snails, *Metrosideros polymorpha* and *Cibotium* spp. (Meyer 2012; Meyer et al. 2014, 2022). Sampling consisted of swabbing the stems, leaves (both top and bottom sides), and nodes of each plant with sterile cotton swabs which were placed in sterile 1.5 ml microcentrifuge tubes and stored in cold blocks (-20 °C) for transport back to the Bishop Museum for processing. Swabs were collected from one individual of each plant species at three sites in July 2018 and seven sites in November 2020 in the Mt. Kaala NAR. *Clermontia* spp. samples were only collected in 2020. Sites are used loosely here as we collected samples from the closest individual to our stopping point and not within a defined area, but all sites were at least 100 m apart along a transect that followed the boardwalk through the NAR. Our goal was to collect samples from individuals that span multiple microhabitats within the Mt. Kaala NAR and to make sure that location was not responsible for differences in phyllospheres between preferred and avoided plants. All samples were stored in an ultracold (-80 °C) freezer prior to DNA extraction.

DNA extraction and sequencing

DNA was extracted with Qiagen's DNeasy PowerSoil Kit following the manufacturer's instructions with modifications for swabs. Briefly, all swabs were maintained in a cold block after removal from the -80 °C freezer and prior to being processed for extraction. Microbiome samples recovered on three swabs of each plant (node, stem, and leaf) were cut away from the swab using sterile scissors, being careful to minimize how much swab material was included. All three samples from each plant were combined in a single 2 ml tube containing Powerbeads and 60 µl of C1 solution provided with the kit. The microbiome cells were disrupted and lysed via homogenization in a Mini-Beadbeater 96 (BioSpec Products Inc., Bartlesville, OK) at 2600 rpm for 10 min. Following homogenization, tubes were centrifuged at 10,000× g for 30 s. Supernatant $(-450 \ \mu l)$ was transferred to a clean, sterile 2 ml tube provided with the kit, and 250 μl of C2 solution were added to each. Samples were vortexed for 5 s and incubated at 4 °C for 5 min followed by centrifugation at 10,000× g for 1 min. Approximately 750 µl of supernatant was transferred to a clean 2 ml tube, and 1200 µl of c4 solution added to each tube. Samples were vortexed for 5 s to mix and 650 µl of the solution added to the MB Spin Column supplied with the kit. Samples were centrifuged at 10,000× g for 1 min to bind the DNA to the column and the flow through discarded. This was repeated twice with an additional 650 µl of sample each time until all the sample had been run through the column. The column filter was washed by adding 500 µl of C5 solution to each column and centrifuging at 10,000× g for 30 s. The flow through was discarded and the columns centrifuged an additional 1 min at $10,000 \times g$. The column was placed into a clean 2 ml collection tube and 100 µl of C6 solution added to the filter. Extracted DNA was eluted via centrifugation at 10,000× g for 1 min. Eluted DNA was quantified using the Qubit 3 fluorometer and the high sensitivity DNA assay (Thermo Fisher, USA) and stored at -20 °C prior to sending to Molecular Research LP (https://www.mrdnalab.com/) for sequencing. Bacterial 16S DNA was amplified using primers 515F-Y and 926R from Parada et al. (2015), and fungal ITS2 regions were amplified using primers ITS1-F (Gardes and Bruns 1993) and ITS2 (White et al. 1990).

The 16S and ITS regions were amplified and sequenced by Molecular Research LP using the following protocol: The HotStarTaq Plus Master Mix Kit (Qiagen, USA)

was used for PCR with the following cycle: 5 min at 95 °C, then by 30 cycles of 30 s at 95 °C, 40 s at 53 °C and 1 min at 72 °C, followed by a final elongation step of 10 min at 72 °C. Amplification success and relative quantity were verified via visualization on a 2% agarose gel. Samples were pooled in equal proportions using unique dual indices and purified using calibrated Ampure XP beads. The pooled and purified PCR products were used to create an Illumina DNA library. Sequencing was performed using Illumina MiSeq following manufacturer guidelines (Illumina, San Diego, CA, USA).

All sequences used in this paper are available at GenBank's SRA database under Bioproject PRJNA880325.

Bioinformatics

Reads were processed using Qiime2 (Bolyen et al. 2019). For fungi, all samples were imported into Qiime2 and the ITS1 region was extracted from each read using the Q2_ITSxpress plugin (Rivers et al. 2018). For the bacterial reads, we used the cuta-dapt Qiime2 plugin (Martin 2011) to filter reads without the primer sequences in the forward and reverse reads.

For both bacteria and fungi, the reads were processed using DADA2 (Callahan et al. 2016) (using the q2-dada2 qiime plugin) to generate a table of unique amplicon sequence variants (ASV) and their counts per sample. Taxonomy for each ASV was determined using the q2-feature-classifier plugin (Bokulich et al. 2018) classifysklearn naïve Bayes taxonomy classifier against the Silva database version 138 for bacterial ASVs (Quast et al. 2013), and the Unite database version 8.3 for fungal ASVs (Abarenkov et al. 2021).

The bacterial samples were rarefied to 8,000 reads and the fungal samples to 19,000 reads based on the number of reads of the sample with fewer reads, and alpha and beta diversity measures were calculated using Qiime's diversity plugin. The high sequence variability of the ITS region makes multiple sequence alignment of this region highly unreliable for distantly related groups of fungi, which result in unreliable phylogenetic trees (Fouquier et al. 2016). Because of this, phylogenetic based measures of alpha and beta diversity are not recommended for ITS based fungal amplicon samples.

For each sample we calculated α -richness (number of different ASVs), diversity (Shannon Entropy), and evenness (Pielou Evenness index). For the bacterial samples we also calculated phylogenetic diversity (Faith's phylogenetic diversity metric). To estimate the differences between samples we used the Bray-Curtis index.

All the commands used in the Qiime2 analyses are available on the GitHub repository: https://github.com/aroc110/Meyer-et-al-2022.

Statistical analyses

To test if bacterial and fungal assemblages differed between preferred and avoided plants and among the five plant species, we ran four PERMONOVA tests. Bray-Curtis distance matrices for both bacteria and fungi assemblages were calculated in Qiime2 and were uploaded into PRIMER-E with the PERMONOVA+ add on (Clarke and Gorley 2006). First, we ran a two-factor PERMONVOA for both bacterial and fungal assemblages using plant preference (preferred and avoided) and sampling date (July 2018 and November 2020) as factors. Second, we ran a two-factor PERMONVOA using plant species and sampling date as factors for both bacteria and fungi. Following significant tests for plant species, we ran pairwise permutation-based t-tests to assess which plant species harbored different phyllosphere assemblages using a Bonferroni correction to account for multiple testing. Corrected α -values were 0.005 for the ten pairwise tests. We constructed MDS plots for both bacteria and fungi to visualize differences in microbial assemblages between preferred and avoided plants and among the five plant species.

We intended to test if relative abundances of fungal species (*Cladosporium* spp. and *Botryosphaeria* spp.) used in captive rearing efforts and found to be preferred fungi in lab feeding trials (O'Rorke et al. 2016; Strouse et al. 2021) differed on preferred and avoided plant species. However, because most samples did not contain either of these genera, we were not able to test the hypotheses that these two genera are more abundant on preferred plant species. Instead, we created box plots to show relative abundance of both genera between preferred and avoided species. Because median values for the relative abundances of both *Cladosporium* spp. and *Botryosphaeria* spp. on both preferred and avoided plants were zero, meaning that most samples did not contain sequences from either genus, we also reported the proportion of samples that contained each genus.

To test if fungal and bacterial ASV α -richness, diversity (Shannon), evenness (Pielou), and phylogenetic diversity (Faith) differed between preferred and avoided plant species and among the five plant species, we ran fourteen univariate PERMONOVAs using PRIMER-E with PERMANOVA+ add on. Faith phylogenetic diversity was not calculated for fungal samples as the ITS region used is well suited for distinguishing species, but it is too fast evolving to create reliable trees (Fouquier et al. 2016). Similarity matrices for each metric were created using the Euclidian similarity index. For each metric for bacteria and fungi, seven and six, two-factor PERMONOVAs were run, respectively. Half examined differences using preference status (preferred and avoided) and sampling date (July 2018 and November 2020) as factors, while the others used plant species and sampling date as factors. Following significant tests for plant species, we ran pairwise permutation-based t-test to assess which plant species harbored assemblages with higher richness, diversity, or evenness using a Bonferroni correction to account for multiple testing (α -values were 0.005 for the ten pairwise tests).

Results

Bacterial ($F_1 = 3.51$; P = 0.0001) and fungal ($F_1 = 2.46$; P = 0.0001) assemblages differed among preferred and avoided plant species (Fig. 1). While bacterial assemblages did not differ between sampling dates ($F_1 = 1.26$; P = 0.066), fungal assemblages



Figure 1. MDS ordination showing relationships between preferred and avoided plant species and among the five plant species sampled according to the composition and relative abundance of bacterial and fungal ASVs on each plant. Similarity was determined using the Bray–Curtis distance coefficient. Sites that are closer together are more similar in terms of ASV composition.

 $(F_1 = 1.46; P = 0.007)$ did. No significant plant preference by sampling date interactions were observed. We also found that bacterial $(F_1 = 2.92; P = 0.0001)$ and fungal $(F_1 = 2.46; P = 0.0001)$ assemblages differed among plant species (Fig. 1). When plant species was used as a factor, both bacterial $(F_1 = 1.31; P = 0.030)$ and fungal $(F_2 = 1.46; P = 0.006)$ assemblages differed between sampling dates. For analyses that explored differences among plant species, no significant plant species by sampling date interactions were observed. Pairwise comparisons revealed that all plant species harbored unique bacterial and fungal assemblages (Table 1).

We found that fungal taxa used in captive rearing efforts (*Cladosporium* spp.) and identified as potentially preferred taxa (*Cladosporium* spp. and *Botryosphaeria* spp.) in feeding trials (O'Rorke et al. 2016; Strouse et al. 2021) were present in less than 30% of the samples and that abundances of these two genera were generally low, although one sample contained > 10% *Cladosporium* spp. (Fig. 2).

Bacterial and fungal α -richness, evenness (Pielou), and diversity (Shannon) did not differ between preferred and avoided plant species (Fig. 3). However, preferred plant species had higher bacterial phylogenetic diversity than avoided plant species and phylogenetic diversity was higher on *Hydrangea arguta* than *Cibotium* spp. (Fig. 3). Differences in sampling date were only observed for fungal α -richness (analyses with preference categories, F₁ = 9.54; P = 0.0041; analyses examining differences among plant species, F₁ = 7.36; P = 0.0107) with fungal richness elevated in November 2022. Sampling date was non-significant for all other metrics examined.


Figure 2. Relative abundances of *Cladosporium* spp., a fungus used in captive rearing facilities in Hawaii, and *Botryosphaeria* spp., a fungus preferred in lab feeding trials on preferred (green) and avoided (black) plant species. The numbers below each bar report the proportion of samples that contained each genus.

Table 1. Pairwise comparisons in fungal and bacterial assemblages among plant species. All pairwise
comparisons were significant. Corrected <i>a</i> -values were 0.005 for the ten pairwise tests. ^P preferred plant
species, ^A avoided plant species.

	Bac	teria	Fu	ıngi
Pairwise comparison	t	P _(perm)	t	P _(perm)
<i>B. arguta^P</i> vs. <i>Clermontia</i> sp. ^P	1.53	0.0004	1.39	0.0006
B. arguta ^P vs. I. anomala ^P	1.77	0.0001	1.55	0.0001
B. arguta ^P vs. M. polymorpha ^A	1.87	0.0001	1.50	0.0001
B. arguta ^p vs. Cibotium spp. ^A	1.84	0.0001	1.64	0.0001
Clermontia sp.vs. Ilex spp. ^P	1.34	0.0026	1.34	0.0031
Clermontia sp. ^p vs. M. polymorpha ^A	1.59	0.0002	1.28	0.0010
Clermontia sp. ^p vs. Cibotium spp. ^A	1.77	0.0001	1.56	0.0006
I. anomala ^P vs. M. polymorpha ^A	1.68	0.0001	1.50	0.0001
I. anomala ^P vs. Cibotium spp. ^A	1.87	0.0001	1.72	0.0001
M. polymorpha ^A vs. Cibotium spp. ^A	1.53	0.0001	1.50	0.0001

Discussion

Our approach, comparing phyllosphere assemblages between plants preferred and avoided by Hawaiian arboreal snails, may form a foundation to identify which microbes are key components of arboreal snail diets. For decades, it has been observed that Hawaiian arboreal snail distributions are patchy and that snails are often clustered on a few native plant species (Hadfield 1986; Meyer et al. 2014, 2022; Price et al. 2017). This specificity contrasts with the idea that arboreal land snails have been described as generalist grazers, consuming what is on leaf surfaces and not selecting specific bacteria or fungi (O'Rorke et al. 2015), and the idea that Hawaiian land snails represent a non-adaptive radiation (Rundell 2011). However, these observations are not mutually exclusive, as snails may be selecting plants with preferred phyllosphere assemblages. Unfortunately, previous research has not allowed us to test this hypothesis



Figure 3. Fungal and bacterial ASV α -richness, diversity (Shannon), evenness (Pielou), and phylogenetic diversity (Faith) for preferred (green) and avoided (black) plants species and the five plant species sampled: Ha, *Hydrangea arguta*, Ia, *Ilex anomala*, Cl, *Clermontia* sp.; Mp, *Metrosideros polymorpha*; C, *Cibotium* spp. Capital letters with horizontal lines indicate differences between preferred and avoided plants and under case letters indicate differences among plant species.

as plants that did not serve as snail hosts were excluded from analyses (O'Rorke et al. 2015; Price et al. 2017). When designing this study, inclusion of avoided plant species as a comparison was identified as a key step, moving research away from characterizing which microbes snails encounter to testing if snails may be choosing plants based on their phyllosphere assemblage. The finding that preferred and avoided plant species have different phyllosphere assemblages indicates that Hawaiian arboreal snails are potentially selecting plants based on their phyllospheres, highlighting that their feeding behaviors are more nuanced than the classification as generalist grazers suggest.

Our approach is species (both plant and snail) and location specific. First, much of the confusion in synthesizing previous research lies in how we classify and choose which plant and snail species to study and where to conduct our studies. For example, Holland et al. (2017), using ginger (Hedychium coronarium) and jasmine (Cestrum nocturnum), concluded that egg production by Auriculella diaphana was reduced when snails grazed on non-native plant surfaces. In contrast, O'Rorke et al. (2015) found that phyllosphere assemblages on non-native strawberry guava (Psidium cattleianum) and coffee berry (Schinus terebinthithifolius) did not differ from native host plants. Consequently, classifying plants based on their native origin may have little utility in determining which plants harbor key phyllosphere resources. Second, it is important to realize that abiotic and biotic changes across sites impact phyllosphere assemblages (O'Rorke et al. 2015), meaning extrapolating snail preference patterns from one site to another may be inappropriate. Therefore, understanding how changes in conditions across sites impact phyllosphere assemblages on various plant species and how these changes influence preferences of different Hawaiian snails is key to developing informed conservation practices. For example, though reducing snail fitness for snails on Oahu, ginger (H. coronarium) was preferred by native succineids on the island of Hawaii (Brown et al. 2003, 2006; Meyer 2012; Holland et al. 2017). Understanding how phyllospheres differed between the two sites would help elucidate if differences were due to the various snail species being studied or because phyllospheres differed on ginger from these two sites. In addition to understanding how phyllospheres vary spatially, understanding how various abiotic conditions influence phyllospheres is important to predicting how a changing climate could influence these important resources and impact snail persistence.

We recognize that different arboreal snail species may require different phyllosphere resources. Previous research has primarily focused on protecting the remaining Hawaiian Oahu Tree Snails in the genus Achatinella, of which all 42 species are either extinct (33) or listed as endangered (9) (USFWS 1981). Observations and empirical evidence suggest that Achatinella spp. prefer ohia (M. polymorpha), a widespread native tree species, and that phyllospheres from this plant species enhance snail fitness (Hadfield 1986; Holland et al. 2017 Price et al. 2017; Sato et al. 2018; Strouse et al. 2021). In contrast, all snail species, none of which are Achatinella spp., in examined montane wet forests on Oahu, Maui, and Hawaii were found to avoid ohia (Meyer 2012; Meyer et al. 2014, 2022). Two scenarios are possible: (1) different conditions in montane wet forests mean that phyllospheres on ohia in these forests differ to the wet, but drier, forests in which Achatinella spp. are found, or (2) Achatinella spp. have evolved to use different phyllosphere resources from the snail species in the montane wet forests. Understanding the subtleties is important when collecting plant material for captive reared endangered snails, or when translocating snails to areas protected from predators, and when new snail species are added to conservation efforts (Sischo et al. 2016; Strouse et al. 2021).

It is also important to explore how phyllospheres differ among seasons and years. We found that fungal assemblages on preferred and avoided plants differed between our two sampling dates, July 2018 and November 2020, but bacterial assemblages did not. When differences in phyllospheres among different plant species were examined, both fungal and bacterial assemblages differed among sampling date. These differences were slight relative to preference and species effects. Still, understanding seasonal and interannual variation, in concert with examining how phyllospheres differ across sites, can provide insights into how phyllospheres may change over various gradients and will allow us to predict how climate change may impact the remaining Hawaiian arboreal snails (Ovando et al. 2019; Teles et al. 2022). Our data provide baseline data for the Mt. Kaala NAR, and O'Rorke et al. (2015, 2017) and Price et al. (2017) provide valuable baseline data for other sites throughout Oahu. A concerted and collaborative effort by snail biologists and conservationists across the archipelago to identify long term study sites and explore how phyllosphere assemblages differ across sites and seasons/years using key plant species (maybe *M. polymorpha* for achatinellines, and *H. arguta* for other snails) and how changes in phyllospheres influence snail fecundity would enhance our ability to make informed conservation actions.

Knowing that preferred and avoided species have different phyllosphere assemblages allows us to develop hypotheses about which microbial species may be important snail food resources. However, we caution that this may be the most difficult step in the processes. Are abundant fungi and bacteria on preferred plants important for snail survival and fecundity, or are snails avoiding certain fungi and bacteria that may be pathogenic, less palatable, or reduce fitness? Hypothesis development is easy, but feeding trials like those conducted by O'Rorke et al. (2016) and experiments that examine differences in fitness like those conducted by Holland et al. (2017) and Strouse et al. (2021) are required to confirm that certain plants and microbial isolates enhance snail fitness. However, these trials need to incorporate taxa that are likely influencing preference and snail fitness. While Cladosporium spp. has been used in captive rearing facilities and in subsequent feeding experiments, we found that this genus is rare in most of our samples and has relatively low abundances on preferred and avoided plants in Mt. Kaala. Similarly, Botryosphaeria spp., a preferred fungus in O'Rorke et al.'s (2016) study was also rare and had low abundances on preferred and avoided plant species. Both Cladosporium spp. and Botryosphaeria spp. were chosen for lab rearing and feeding preference studies because they were able to isolate and grow these fungi in culture (O'Rorke et al. 2016). However, these species do not enhance snail fecundity or survivorship (Strouse et al. 2021). Because of this, lab rearing still includes collection of enormous amounts of live plant material which is not sustainable. We respect that isolation and growth of targeted fungi and bacteria are difficult, but we argue that randomly selecting fungi and bacteria for feeding trials using the criteria of which can easily be cultivated in a lab setting is a heuristic approach that may not yield key food resources and may not be in the best interests of effective snail conservation.

We also tested the hypotheses that snails are choosing plants with elevated richness and diversity. As we found no differences in both bacterial and fungal α -diversity, evenness, or diversity between preferred and avoided plants and among plant species, richness and diversity are probably not influencing snail preferences. However, for bac-

teria, we did find elevated phylogenetic diversity on preferred plants, suggesting that richness and diversity calculated using metrics that do not account for phylogenetic relationships among microbial taxa, may not adequately address the diversity of food resources snails encounter. While we could not run these analyses for fungi, we provide preliminary evidence that snails may also select plant species that host a more phylogenetically diverse assemblage of microbes.

Conclusions

We argue that our framework, which consists of first identifying which plants are preferred and avoided by arboreal snails, and then examining differences in phyllosphere assemblages between preferred and avoided plant species, is key to developing hypotheses about which microbes are important food resources for the remaining Hawaiian arboreal snails. Subsequent laboratory analyses would be required to determine if these microbial taxa influence snail fitness. If we could identify important microbes that form a healthy diet for Hawaiian arboreal snails, it greatly expands our ability to: (1) assess and monitor habitat quality by swabbing plants and assessing phyllosphere assemblages, (2) make informed restoration recommendations that may enhance arboreal snail survivorship and fitness, and (3) improve rearing efforts for highly endangered captive reared populations by enhancing survivorship and fitness and reducing the probability of introducing pathogens and toxins (Sischo et al. 2016). To develop effective longterm conservation practices, we also recommend that transformative long-term studies explore how snail preferences and phyllospheres differ across sites, seasons and years, and how changes in phyllospheres impact snail fecundity. The Hawaiian snail conservation community is extensive, with a large group of researchers and conservationists dedicated to protecting a unique snail fauna. This community has been working hard to stem the tide of snail extinctions for decades (Yeung and Hayes 2018), and recently has been coordinating efforts across labs to address some of the most pressing issues. We argue that conservation of Hawaiian arboreal snails is significantly hindered by our lack of knowledge about what they eat. We hope a coordinated effort using this framework can help elucidate key information that can help stem further extinctions.

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Using drone imagery to obtain population data of colony-nesting seabirds to support Canada's transition to the global Key Biodiversity Areas program

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Abstract

Identifying of global or national biodiversity 'hotspots' has proven important for focusing and prioritizing conservation efforts worldwide. Canada has nearly 600 Important Bird and Biodiversity Areas (IBAs) identified by quantitative criteria to help guide avian conservation and management. Marine IBAs capture critical waterbird habitats such as nesting colonies, foraging sites, and staging areas. However, due to their remote locations, many lack recent population counts. Canada has begun transitioning IBAs into the global Key Biodiversity Areas (KBA) program; KBAs identify areas that are important for the persistence of biodiversity and encompass a wider scope of unique, rare, or vulnerable taxa. Assessing whether IBAs qualify as KBAs requires current data - as will future efforts to manage these biologically important sites. We conducted a pilot study in the Chain Islets and Great Chain Island IBA, in British Columbia, to assess the effectiveness of using drones to census surface-nesting seabirds in an IBA context. This IBA was originally designated for supporting a globally significant breeding colony of Glaucous-winged Gulls (Larus glaucescens). Total nest counts derived from orthomosaic imagery (1012 nesting pairs) show that this site now falls below the Global and National IBA designation criterion threshold, a finding consistent with regional declines in the species. Our trial successfully demonstrates a flexible and low cost approach to obtaining population data at an ecologically sensitive KBA site. We explore how drones will be a useful tool to assess and monitor species and habitats within remote, data-deficient IBAs, particularly during the transition to KBAs.

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Keywords

conservation, Important Bird and Biodiversity Areas (IBAs), population data

Introduction

Important Bird and Biodiversity Areas (IBAs) are sites that contain a recurring presence of one or more regionally or globally threatened species (BirdLife International 2010). These sites are designated using standardized selection criteria that apply numerical thresholds (e.g., occupied by a minimum % of a global breeding population) to determine the significance of a site for conservation; these thresholds are applied consistently to sites around the world. IBAs are continually recognized for their influence and significant use for conservation policies in multilateral environmental agreements, site protection and land-use planning, and for their involvement in driving locally-led stewardship programs (Wren and Couturier 2009; Waliczky et al. 2018).

The International Union for Conservation of Nature (IUCN) developed a process to identify Key Biodiversity Areas (KBAs) that was released in 2016; this conservation tool aims to identify sites that are important for the persistence of biodiversity and encompasses all taxa, as well as ecosystems in the assessment process. The criteria and thresholds for assessments of KBAs builds on the approach of the IBA program and other conservation framework tools, while bringing in other criteria based on vulnerability and irreplaceability (IUCN 2016). Species-specific KBA assessments require population data collected through field surveying and monitoring, and a gap analysis of the KBA program has identified the need for updated survey data to ensure sites originally designated as IBAs still meet their respective thresholds under KBA criteria (Langhammer et al. 2007). Although systematic and effective field-based protocols have been developed for monitoring IBAs (BirdLife International 2006), limitations to conducting surveys such as accessibility leaves some sites with a lack of updated and accurate data. Among these are remote or otherwise difficult to access marine sites worldwide.

As the world's second-largest nation (9.98 million square kilometres), Canada lacks the biodiversity of smaller tropical countries, but has many areas of critical importance for aspects of temperate biodiversity. There are currently 581 sites identified as IBAs throughout the country. Approximately 64% of these IBAs are considered data deficient (Evans 2022), meaning they cannot currently be established as KBAs due to the lack of consistent and reliable data for the given 'trigger' species (i.e., the species meeting KBA criteria at a given site). A significant number of these IBAs border Canada's coastlines and are designated for seabird breeding colonies, foraging areas, or coastal stopover sites for migratory shorebirds (Wren and Couturier 2009).

As with other nations, a high proportion (approximately 75%) of Canadian data deficient IBA sites are coastal and marine. Monitoring seabird colonies through field surveying is often expensive, difficult, and dangerous, and will invariably cause

disturbance to nesting birds (Burger 1997). Thus, new approaches to data gathering should be considered in order to obtain information sufficient to retain such remote and important sites if they do meet the quantitative criteria for KBA designation. Remotely Piloted Aircraft Systems (RPAS), or drones, are increasingly being used for ecological research in areas that are difficult or dangerous to access (Watts et al. 2008). The use of drones to survey seabird colonies provides an opportunity to access sites that are otherwise challenging or hazardous to access directly. Compared to ground-based survey methods, RPAS can also allow for increased spatial coverage and frequency of surveying, reduced risk of entering remote island locations, and increased accuracy of counts (McClelland et al. 2016; Hodgson et al. 2018). Additionally, RPAS can be of a similar cost to or less expensive than ground-based surveys, and, as capabilities are improved, costs will likely continue to decrease.

In this study, we used RPAS technology to estimate an IBA population count of a colonial-nesting seabird, the Glaucous-winged Gull (*Larus glaucescens*). Our objectives were to support the IBA program's evolution to a KBA-based initiative; to explore the overall feasibility of using drone-based applications to obtain reliable IBA or KBA data; and to assess the current status of a nationally important seabird colony. This study location has historically supported one of Pacific Canada's largest breeding colonies of Glaucous-winged Gulls, as well as migratory aggregations of as many as 2000 Brandt's Cormorants (*Urile penicillatus*) in the area. For this reason, it was categorised as an A4i IBA and D1 KBA site, one which is "known or thought to hold 1% or more of a biogeographic population of a congregatory waterbird species on a regular basis" (Moore and Couturier 2011). We wanted to use our counts to help determine if the area still meets the threshold that it was originally assessed for when it was designated an IBA 25 years ago, prior to the steep decline observed in this gull species beginning in the 1980s (Blight et al. 2015).

Methods

Study Site

Our study took place at the Chain Islets and Great Chain Island IBA, a cluster of small islets and rocky outcrops along the marine coastline south of the City of Victoria in the Strait of Juan de Fuca (48.42°N, 123.28°W), British Columbia (Fig. 1). This site also comprises the Oak Bay Islands Ecological Reserve. The IBA is 1.38 km² in size and consists of rocky flats and rocky shorelines with grass and sedge meadows, and sand flats throughout. The rocky islets within the IBA are sparsely vegetated, Great Chain Island is the largest and the most vegetated island with a herbaceous cover. Both Great Chain and the rocky islets are used by nesting and migrating seabirds. As this site lies within an introgression zone (Bell 1996), our references to Glaucous-winged Gulls include any Western Gull (*L. occidentalis*) × Glaucous-winged Gull hybrids.



Figure 1. Great Chain Island & Chain Islets IBA, British Columbia, Canada. Environment and Climate Change Canada, 2022.

Flight/drone specifics

RPAS flight operations and protocol were selected to be consistent with a previous study in the region (Blight et al. 2019). Drone imagery for this study was collected using a multi-rotor RPAS (DJI Mavic 2 Pro) with a 20-megapixel Hasselblad L1D-20c

aerial camera. Flights were performed on 21 June 2019 from 0859 to 1257 h, with operations run from the aft deck of a 42-foot former commercial fishing vessel. The survey date was chosen based on known local breeding phenology of Glaucous-winged Gulls and was meant to capture the maximum number of occupied nests, when breeding adults are likely either incubating eggs or brooding newly hatched chicks (Blight et al. 2014). Weather conditions on the day of the flight were clear skies with flat, calm water and minimal wind speed (~Beaufort 0–1).

Flights were pre-programmed through drone mapping software (DroneDeploy, www.dronedeploy.com) to run single straight-line paths across the colony, offset to achieve consistent sidelap and followed by a return to the stationary vessel. Due to strong tidal currents in the region, the vessel motored in place instead of dropping anchor, with all vessel operations occurring outside the Ecological Reserve boundary, approximately 650 metres from Great Chain Island; this distance was well beyond the 100 m launch distance recommended by Vas et al. (2015) for minimising effects on waterbird behaviour. The drone climbed vertically until survey altitudes were achieved and then flew horizontally over the colony. The altitude above ground level of the drone flights varied from 90–65 metres, with initial flights starting at 90 meters, a height at which we observed no disturbance in an earlier study in the region (Blight et al. 2019). Flight altitude was adjusted according to the natural topography of the islands and generally decreased for each subsequent flight after no Glaucous-winged Gull disturbance was observed.

Image capture was via a 28 mm lens on auto-exposure mode, certain flight specifics such as speed over ground and photo intervals were set by front-lap and side-lap requirements within the drone software. As flight altitudes and topography were variable throughout the six flights, ground sample distance (GSD) also varied. GSD ranged from 1.41 cm/pixel at 60 meters altitude to 2.11 cm/pixel at 90 meters. Drone flights were operated using one pilot and one visual observer, along with two additional observers monitoring the colony for any evidence of disturbance to nesting gulls during drone operations.

Data overview

We used photogrammetry software to process drone flight images and create orthomosaics of the study area. Clear weather on the day of the flight generally created optimal light conditions for subsequent visual assessment of the orthomosaic imagery. However, solar glare on the less-vegetated small islets (approx. 33% of the colony) rendered a subset of the images overexposed; these islets were excluded from subsequent analysis. Orthomosaics of Great Chain were enlarged and visually scrutinised on-screen for occupied gull nests; these were readily apparent as the white and pale grey plumage of an incubating gull contrasts strongly with its background of rock and vegetation, and its posture is distinctive (Fig. 2). Thus, we assumed that each incubating bird represents a nesting pair; as two mates may attend one nest, two birds sitting side by side in an incubation pose were counted as a single pair. The Glaucous-winged Gulls we



Figure 2. Gull nests detected within an enlarged portion of orthomosaic imagery.

identified were primarily on nests, birds flying overhead and apparently roosting along the shoreline were also visible but were not included in the total nest count. As each individual nest was counted, a pin was placed over it to eliminate duplicate counts. The orthomosaic encompassed Great Chain Island as well as several of the small islets situated within the IBA (Chain Islets; Fig. 1).

Results

We conducted six flights capturing the entire IBA in 537 raw images. In the orthomosaic of Great Chain Island (Fig. 1), 1012 active nests were counted in total. Imagery from the drone overflights was sufficient to census the breeding population of our target population over about 67% of its nesting colony. However, the smaller rocky islets proved difficult to survey, with little vegetation cover and a surface layer of white gull guano; they were subject to solar glare and resultant overexposed imagery. Survey data collected in 2009 indicated 464 nests within the small islets of the IBA (33% of the colony). Using these data, we then assessed the counts against the KBA criteria to determine if this site still reaches the specific thresholds to categorize it as a KBA.

At all flight altitudes, no reaction was observed from nesting gulls during drone overflights, with the only alarm-calling or flushing by gulls observed when a single Bald Eagle (*Haliaeetus leucocephalus*), a gull predator, flew low over the colony on one occasion. No other seabird species were detected through the imagery analysis. The only other vertebrate species observed remotely on land was Harbour Seal (*Phoca vitulina*).

Discussion

Our study successfully demonstrated an approach to using RPAS to survey and evaluate the population status of a colonial seabird at an ecologically sensitive and difficult-toaccess site, in the context of monitoring an existing Important Bird and Biodiversity Area. The area overflown during the flights is natural, open rocky topography which generally creates an ideal setting for this type of analysis. The imagery captured from the flights was sufficient to analyze the large island within the IBA, however, some of the smaller rocky islets with little vegetation proved difficult to assess due to the high sun glare off the rocks and therefore were excluded from this trial. However, we were able to make use of previous surveys to estimate the nest counts for this minor part of the colony. Our project budget did not extend to extensive post-processing work, but we suggest that under similar conditions it might be possible to adjust exposure on each image prior to stitching the orthomosaic together to allow for a clearer visual of overexposed tiles. Alternatively, for rocky seabird colonies on light-coloured and/or guano-coated rocky substrate, drone surveys might best be scheduled for overcast days. The smaller islets within the IBA were not counted during this study, however with the addition of a 33% count of 464 nests (928 individuals), the total population of 1476 nests (2952 individuals) still would not qualify the site under the existing KBA thresholds.

KBA assessment and population trends

Our count results were similar to ground-based survey data collected a decade earlier (Blight et al. 2015) as well as more recent estimates by Ecological Reserve wardens (J. Sirois and M. Lambert, pers. comm). Our drone-based count confirms that the breeding population now falls below the KBA threshold to designate this site, a finding consistent with regional declines in the species (Blight et al. 2015). The estimated total of 2952 individuals accounts for approximately 67% of the threshold needed to classify this site as a KBA. Currently, to reach a national threshold for Glaucouswinged Gull, an aggregation representing 1% or greater of the national population size over a season, and during one or more life history stages is required, resulting in a threshold of 4,400 individuals. Globally, the same requirements are necessary, resulting in a threshold of 4,700. While this specific site no longer qualifies for KBA status, there is potential to extend the boundaries of this site to create a larger KBA with the inclusion of a similar sized colony nearby, Mandarte Island. These two sites, combined with Mitlenach Island in the northern Strait of Georgia, account for a quarter of the breeding gulls in British Columbia (Booth 2001). In Pacific Canada, there are 10 other IBA sites with Glaucous-winged Gull as the trigger species that could be candidates for future RPAS analysis.

Canada is one of the first countries globally to transition IBAs to the KBA program. Two main challenges identified in the program crosswalk relating to survey data are data age and repeatability (Birds Canada, pers. comm.). "Lack of population data" was listed as a key methodological issue in six out of seven regions in an early assessment of KBA progress globally (Foster et al. 2012) and it remains a challenge in the development of the program. While drones and other remote technologies are not a panacea for this problem – particularly where lack of funding is a key issue – expanding the type of approaches available is important for some data-deficient sites, as demonstrated by this study. Recent literature explores the potential use of semiautomated counts on drone imagery of breeding waterbirds through free software and highlights the effectiveness and accessibility of this method as a low-cost alternative to manual counts for conservation use (Francis et al. 2020; Corregidor-Castro and Valle 2022). The cost of this survey was comparable to the 2-day field operation required to undertake this census as a ground-based survey. This semi-automated method can be especially cost-effective for areas such as IBAs that need accurate repeat surveys and for large areas that are difficult to access (Francis et al. 2020).

Drone applications

Though the use of RPAS technology has rapidly increased, gaps remain in establishing best practices for surveying sensitive species (Vas et al. 2015) and literature on methods and guidelines for this approach continues to evolve (Vas et al. 2015; Rush et al. 2018; Weimerskirch et al. 2018; Francis et al. 2020). The technique utilized in this study caused no evident disturbance to nesting birds, as was anticipated based on previous work in the region (Blight et al. 2019). However, our study's IBA is located less than 2 km from the City of Victoria and birds may be habituated to air traffic due to their proximity to the Victoria aerodrome. Though Glaucous-winged Gulls are considered a non-sensitive species in this context, lack of disturbance is an important survey factor for colonial bird studies, especially during sensitive life cycle stages including breeding. Geldart et al. (2022) studied the heart-rate response of drone surveys on colonial-nesting Eider ducks (Somateria mollissima), in Nunavut, Canada; their results indicated no physiological stress response from drones flying 30 m above the nesting colony. However, the authors highlight that other species may perceive a greater threat to drones, specifically avian species that may experience adult predation from other avian predators (Geldart et al. 2022). This study, among others, allows researchers to better understand the behavioural and physiological response of birds to drones and highlights that, when done properly and in accordance with available guidelines to avoid disturbance to seabird and waterbird colonies, RPAS can allow for a less invasive surveying method that provides adequate coverage of a site in significantly less time compared to land-based surveys.

Drone surveying can not only assist in the establishment of KBAs but can provide critical population and habitat monitoring which can indicate habitat loss or degradation. It is evident that climate change will continue to pose stressors that may lead to fluctuations in biodiversity, potentially impacting elements for which a conservation site was initially designated (KBA Canada Coalition 2021). Bollard et al. (2022) explore drone technology for mapping protected areas in Antarctica to provide baseline vegetation inventories and ecological data for future monitoring. This method proved useful in obtaining vegetation maps that show spatial cover with higher accuracy and resolution, though small intricate taxa such as lichen and cyanobacteria could not be accurately monitored through this method (Bollard et al. 2022). The low impact of this survey method at a sensitive site, combined with its repeatability and inexpensiveness, made this a successful demonstration of collecting baseline data for a protected area.

While our study only used RPAS to obtain a viable count of breeding gulls, the imagery could be used to indicate the area of occupancy, the extent of suitable habitat, and the range of such habitat for this and other species; these are all metrics that are considered acceptable to assess qualifying KBA sites (KBA Canada Coalition 2021). Further, such metrics can be monitored over time to indicate future changes to habitat and ecosystems. The drone imagery was high-resolution enough and the visibility on our survey day clear enough for us to view the extent of the underwater kelp (*Nereocystis luetkeana*) forests surrounding the archipelago, as well as macroalgae in the intertidal zone (cf. Cavanaugh et al. 2021; Tait et al. 2021), indicating the utility of drone imagery for assessing multiple species and habitats at a target site. This methodology could prove useful for assessing a range of species across various landscapes to support the KBA assessment process.

Conclusion

Obtaining updated population data is critical to understanding change and detecting risks to biodiversity. Drones provide the opportunity to significantly advance our understanding of environmental changes, especially within remote environments. The results of this study add to growing literature showing the effective use of drones in surveying surface-nesting birds in natural, hard-to-access settings. In a rapidly changing world where biodiversity continues to decline, obtaining accurate inventory data to capture that change remains a logistical challenge for many IBAs (Donald et al. 2018). Adapting to such rapid change with new technologies can allow for systematic monitoring and updated data for both wildlife and ecological communities as KBAs and protected areas are established and continue to evolve.

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The authors have declared that no competing interests exist.

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RESEARCH ARTICLE

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Changes in suitable habitat for the critically endangered Northern white-cheeked gibbon (Nomascus leucogenys) in the Western Nghe An Biosphere Reserve, Vietnam: Implication for conservation

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Abstract

Several recent studies have highlighted that change in land use and land cover (LULC) is the main threat causing the decline and extinction of certain species. Gibbons (Hylobatidae) could be excellent examples, on account of their potential for extinction in the near future under the effects of LULC changes due to their particular ecological traits. This study aims to model the current suitable habitat of the Northern white-cheeked gibbon (*Nomascus leucogenys* (Ogilby, 1840)) in the Western Nghe An Biosphere Reserve (BR), Vietnam and assess the changes in its suitable habitat following the changes in LULC from 1990 to 2020. The maximum entropy approach (MaxEnt) was used to predict the suitable habitat of the gibbon using its occurrence localities and environmental predictors. The model analysis showed that the "Distance to Agriculture" variable habitat of the gibbon species at approximately 4,022.42 km² (30.95% of the overall BR area) in three spatially separated areas inside the Western Nghe An BR. Furthermore, the suitable habitat areas of the gibbon in 1990, 2000, and 2010 were projected at roughly 4,347.68 km²,

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4,324.97 km², and 2,750.21 km², respectively, following a decreasing trend from 1990 to 2010, but a gradual increase between 2010 and 2020. The suitable habitat of the gibbon inside three core protected areas (Pu Mat National Park, Pu Huong, and Pu Hoat Nature Reserves) showed a continually increasing trend from 1990 to 2020. Our results highlighted the influence of LULC changes and the role of the protected area network in gibbon conservation. The information from the study provides a quantitative baseline for the future conservation of the critically endangered gibbon in the Western Nghe An BR.

Keywords

Gibbon, land use & land cover, MaxEnt, Nomascus, species distribution modeling, suitable distribution

Introduction

Southeast Asia contains approximately 15% of the world's tropical forests and is home to at least four globally important biodiversity hotspots (Myers et al. 2000). However, the region is also assessed as a deforestation hotspot. From 2005 to 2015, around 80 million ha (28%) of natural forests were lost in Southeast Asia (Estoque et al. 2019). Researchers also predicted that the forest in Southeast Asia would experience a significant change, either shrinking by 5.2 million ha or growing by 19.6 million ha, depending on the choice of the pathway (Estoque et al. 2019). Several recent studies have highlighted that the change in land use and land cover (LULC) is the main threat driving the decline and extinction of species, with loss in the habitat sustainability of wildlife species (Jetz et al. 2007; Díaz et al. 2019; Su et al. 2021), especially for large-bodied species. Gibbons could be excellent examples revealing the impacts of the extreme effects of LULC changes due to their particular ecological traits. The species are strictly arboreal and highly frugivorous, preferring closed-canopy broadleaved evergreen forests (Geissmann et al. 2000; Ruppell 2013). Territoriality and monogamy are observed in almost all gibbon species (Mitani 1987; Brockelman et al. 2014). Gibbons also respond sensitively to habitat degradation and fragmentation, as well as anthropogenic activities (Geissmann et al. 2000; Rawson et al. 2011; Tran and Vu 2020; Sarma et al. 2021).

The northern white-cheeked gibbon (NWCG) (*Nomascus leucogenys*) is classified as a Critically Endangered species on the International Union for Conservation of Nature (IUCN) Red List (Rawson et al. 2020). The species is native to northwestern Vietnam, northern Lao PDR, and southwestern China (Rawson et al. 2011; Rawson et al. 2020). The gibbon is believed to be extinct or functionally extinct in China (Fan et al. 2014), while the largest remaining population probably persists in Lao PDR, although its current status is still unclear (Rawson et al. 2011; Rawson et al. 2020). In Vietnam, the distribution of NWCG is restricted in the north by the Black River (Geissmann et al. 2000; Rawson et al. 2011) and limited in the south by the Rao Nay river (Thinh et al. 2010). The species' total population was estimated to be only around 300 groups remaining in Vietnam, most of them persisting in a few isolated forest blocks close to the Lao PDR border (Rawson et al. 2011).

The largest population of NWCG was found in Pu Mat National Park (NP), with 22 confirmed groups; the site was also considered as the highest priority area for

conserving the species (Rawson et al. 2011). The occurrence of the species was also confirmed in several other protected areas, although their population is likely low or very low, for example, in Muong Nhe Nature Reserve (NR), Sop Cop NR, Xuan Lien NR, Vu Quang NP, and Pu Huong NR (Rawson et al. 2011). It should also be noted that the species seems to have been extirpated from many other protected areas, including Hang Kia – Pa Co NR, Ngoc Son – Ngo Luong NR, and Pu Luong NR (Rawson et al. 2011). Hunting and habitat loss are currently considered as the major threats to NWCG, especially with the high rate of forest fragmentation and degradation pushing the species into a few isolated forest areas (Rawson et al. 2011; Rawson et al. 2020). To date, studies on gibbons in Vietnam have mainly focused on population size assessments (Luu and Rawson 2009, 2010, 2011; Hoang et al. 2010; Ha et al. 2011; Tran and Vu 2020). Gibbons require high-quality habitat with high food abundance and a dense canopy (Geissmann et al. 2000; Sarma et al. 2021). However, limited efforts have been made to monitor gibbon habitat, and no current studies report the changes in suitable habitat for gibbons in Indochina.

In this study, we aimed to predict the suitable habitat of NWCG in the Western Nghe An BR by using species distribution modeling (MaxEnt) based on identified presence localities of the species and environmental predictors. We also attempted to project suitable habitat fluctuation in relation to the changes in LULC from 1990 to 2020. Based on the results, we discussed the impact of LULC changes and the role of the protected area network in gibbon conservation in Vietnam. Our results are a baseline for researchers, conservationists, and wildlife and habitat managers to aid decision-making and plan future conservation strategies for NWCG in the Western Nghe An BR, Vietnam.

Methods

Study area

The Western Nghe An Biosphere Reserve (BR) was recognized by UNESCO as the 6th Biosphere Reserve of Vietnam in 2007. The Western Nghe An BR (18°34'43"–19°59'44"N, 103°52'28"–105°30'07"E) is located in the western part of Nghe An province, central Vietnam, covering an area of 12,997.95 km² with three functional zones: the core zone (1,683.01 km²), buffer zone (6,085.47 km²), and transition zone (5,229.47 km²). The Biosphere Reserve is expected to create a green corridor between the three protected areas: Pu Mat NP, Pu Huong NR, and Pu Hoat NR. The forest cover of the Western Nghe An BR is approximately 66.4% of the total area. The area is home to more than 3,000 vascular plants and more than 940 vertebrate animals, with several species listed as threatened, rare, and endemic. More than 900,000 people in five indigenous minority groups currently reside within Western Nghe An BR (Western Nghe An BR were detected in the three protected areas, including Pu Mat NP, Pu Huong and Pu Hoat NR. Pu Mat NP is one of the largest remaining natural forests in

Vietnam and is believed to be home to the largest population of the NWCG with an estimated 455 gibbons in 130 different groups (Luu and Rawson 2011). However, the forests in Pu Huong and Pu Hoat NR are isolated and probably not effectively linked ecologically to the Pu Mat NP. More than a decade ago, researchers confirmed at least seven groups of the gibbon remaining in the Pu Hoat NR (Luu and Rawson 2009). However, the last field survey in Pu Hoat NR and adjunction areas detected at least 40 gibbon groups (Pu Hoat NR 2021).

Occurrence data

To predict the suitable habitat of NWCG in the Western Nghe An BR, we collected the occurrence of the endangered species through our field surveys in Pu Mat NP, Pu Huong NR, and Pu Hoat NR from March-May 2021 (Phung and Dong 2021), and another published document (Pu Hoat NR 2020). The localities of gibbons in Pu Mat NP, Pu Huong, and Pu Hoat NR were used as presence data for the MaxEnt model in 2020. For presence data on the species for the 2010 model, we gathered data from published documents (Luu and Rawson 2009, 2010, 2011). Initially, we collected 36 presence localities for the 2010 model, and 98 localities for the 2020 model. To avoid spatial autocorrelation among localities that could cause an overestimation, we used the "spThin" package (Aiello-Lammens et al. 2015) in R version 4.1.2 to thin out the locations of gibbons within one km radius by randomly selecting one location and removing the others, similar to the models for the Southern white-cheeked gibbon (N. siki) in Tran et al (2023). One km was used as a criterion for thinning because gibbons in the genus Nomascus have a relatively small home range, around 0.45 km² for the Southern yellow-cheeked gibbon (N. gabriellae; Hai et al. 2020). Consequently, we used 25 and 40 occurrence localities of NWCG for the 2010, and 2020 models, respectively (Fig. 1).

Environmental predictors

To predict the suitable habitat of NWCG in the Western Nghe An BR, we obtained variables on climate, topography, and LULC. The variables were selected based on our knowledge of the habitat of the gibbon and consulting the available research sources. For the climatic variables, we downloaded 19 predictors with the highest available resolution (30 arcseconds) from the World Clim database (http://www.worldclim.com/; Fick and Hijmans 2017). The variables include 11 and eight layers of temperature and precipitation, respectively, which were derived from the monthly temperature and rainfall values in order to generate more biologically meaningful variables. According to Tran and Vu (2020), elevation might also highly contribute to the species distribution modeling of gibbons. Therefore, we used the Digital Elevation Model with a resolution of 30×30 m (available from https://earthexplorer.usgs.gov) as elevation variables. Then, we calculated the Slope and Aspect variables based on the Digital Elevation Model in ArcMap version 10.2 (ESRI). To assess the change of habitat suitability of the threatened gibbon, we extracted the LULC of the Western Nghe An BR from the LULC of Vietnam in 1990, 2000, 2010, and 2020 at 30×30 m resolution (Fig. 2)



Figure 1. The occurrence localities in 2020 (red) and 2010 (green) of NWCG in the Western Nghe An BR were used for predicting the suitable habitat by the MaxEnt model.

available at https://www.eorc.jaxa.jp/ALOS/en/dataset/lulc/lulc_vnm_v2109_e.htm (Phan et al. 2021). The distance to the settlement plays an important role in modeling suitable habitat for gibbons (Tran and Vu 2020). Here, we extracted the settlement area from the LULC layer (Phan et al. 2021). The agricultural land was also selected from the LULC layer (Phan et al. 2021), including rice paddies, woody crops, other croplands, and in-house crops' categories. To assess the effect of settlement and agricultural land on the suitable habitat of the gibbon, we calculated the distance to settlement and agricultural land by the Euclidean Distance tool in ArcMap version 10.2 to create two variables: Distance to Settlement, and Distance to Agriculture in 1990, 2000, 2010 and 2020. Due to the difference in the resolution of variables, we resampled all variables to a resolution of 30×30 m using the Resample tool in ArcMap version 10.2. The collinearity among environmental predictors might cause the overestimation of species distribution modeling. Thus, we calculated Pearson's correlation index (r) using ENMTools version 1.4.4 (Warren et al. 2010). With the highly correlated predictor pair (|r| > 0.80), we eliminated one variable and kept the remaining variables for final analysis (Nazeri et al. 2012). To select or remove variables, we considered our understanding of the ecology of gibbons, and other publications on predicting habitat



Figure 2. The LULC in the Western Nghe An BR in **A** 1990 **B** 2000 **C** 2010, and **D** 2020 extracted from Phan et al. (2021).

suitability, including Sarma et al. (2015, 2021); Tran and Vu (2020). Additionally, we also ran a model with all the variables and then considered the contribution of each variable. Finally, we used 13 predictors for the final models (Table 1).

No.	Name	Sources	Description
1	Bio01	WorldClim	Annual Mean Temperature
2	Bio03		Isothermality (BIO2/BIO7) (×100)
3	Bio07		Temperature Annual Range (BIO5-BIO6)
4	Bio12		Annual Precipitation
5	Bio13		Precipitation of Wettest Month
6	Bio15		Precipitation Seasonality (Coefficient of Variation)
7	Bio16		Precipitation of Wettest Quarter
8	Elevation	Earthexplorer.usgs.gov	Height above sea level
9	Slope		Degree of rise/run
10	Aspect		Direction a slope face
11	LULC	ALOS Research and Application	Land Use and Land Cover
12	Distance to settlement	Project	Distance to residential land
13	Distance to Agriculture		Distance to agricultural land

Table 1. The predictor variables used for generating the habitat suitability of NWCG in the Western Nghe An BR.

Ecological niche modeling processing

To generate the suitable habitat model for NWCG in the Western Nghe An BR, we applied the Maximum Entropy approach (MaxEnt) version 3.4.4 available from https://biodiversityinformatics.amnh.org/open_source/maxent/. The MaxEnt model predicts the habitat suitability of species based on the probable occurrence of species in distinct localities finding the maximum entropy distribution of environmental predictors (Phillips et al. 2006). The model was assessed as one of the most accurate species distribution models and has been broadly applied to predict suitable habitats for species, especially for the small sample size of presence data (Elith et al. 2006; Phillips et al. 2006; Raxworthy et al. 2007). Here, we applied the default setting for the MaxEnt models as suggested by the model developer (Phillips et al. 2006). The area under the receiving operator curve (AUC) has been widely used to evaluate the accuracy of the model performance (Elith 2000; Phillips et al. 2006; Nazeri et al. 2012). The higher AUC values indicate better model performance: inability to predict model (AUC < 0.5), poor performance (AUC = 0.5-0.7), moderate performance (AUC = 0.7-0.9), excellent performance (AUC = 0.9-1) (Peterson et al. 2011).

To assess the changes in the suitable habitat of NWCG from 1990 to 2020, we ran separately two models for 2010 and 2020 based on the presence localities and environmental data set of each year. For the model of 2020, we ran the model using 10-folds cross-validation to evaluate the model, while we applied the jackknife method due to the small sample size for the model 2010 (Pearson et al. 2007). Due to the lack of occurrence data of the gibbons before 2000, we applied the projection function of the MaxEnt model to project the suitable habitat into the past (2000 and 1990) based on the presence localities in 2010, 2020 and the available environmental data set in 2000 and 1990. Because 2010 was closer to 2000 and 1990 than 2020, we assumed that the suitable habitat models in 2000, and 1990 that were projected using presence localities in 2010, would be more accurate than the models projected using localities

in 2020. Therefore, we used the models projected from localities in 2010 to analyze the gibbon's suitable habitat in 2000 and 1990 instead of the projected models from localities in 2020. However, the results of models in 1990, and 2000 projected from localities in 2020, were also presented in the Suppl. material 1: SI–III, and IV. For the environmental variables, we kept topographic variables as unchanged while LULC, Distance to Settlement, and Distance to Agriculture variables were changed following the time. We also assumed that the climate did not change much during this time by keeping the climatic variables as unchanged due to the lack of climate data in the study area for each study period.

The relationship between the predicted suitable habitat of species and environmental variables in the MaxEnt model was shown by response curves. We presented the response curves of the most contributing variables to identify the main impact of the variables on the change of suitable habitat of NWCG in the Western Nghe An BR.

The result of the MaxEnt model is presented in a logistic format ranging from 0 to 1, in which the higher values mean higher suitability. The threshold selection should be determined according to the objectives of the study (Merow et al. 2013; Vale et al. 2014). To maximize the area for conservation purposes, we applied the threshold "Minimum training presence logistic threshold" to determine the suitable/unsuitable categories. To clearly show the changes in suitable habitat, we also categorized suitable areas into three sub-levels by dividing equally suitability values: High, Moderate, and Low suitability (Tran et al. 2020; Sarma et al. 2021).

Results

Performance of models and importance of variables

The MaxEnt model predicted the habitat suitability of NWCG based on the available presence localities and predictor data set of LULC, climate, and topography with mean test AUC at 0.896 ± 0.043 and 0.936 ± 0.084 for the model of 2020 and 2010, respectively (Fig. 3). The AUC values showed the high discrimination capacity of the model to predict the suitable habitat of NWCG in the Western Nghe An BR.

For the contribution of each environmental variable in the model of suitable habitat in 2020, the top three variables were "Distance to Agriculture" (39.2%), "Temperature Annual Range – Bio7" (25.3%), and "Precipitation of Wettest Quarter – Bio16" (23.3%). For the model of 2010, the highest contribution variables were "Distance to Agriculture" with 55.4%, followed by "Distance to Settlement" and "Elevation" with 29.3% and 6.6%, respectively (Table SI-II). The response curves in the MaxEnt model show the changes in habitat suitability in response to the changes in the predictors used in the model. The response curve of the highest contribution variables of both models (2020 and 2010) was mainly identical when the habitat suitability of NWCG increased significantly for the distance to agricultural land below around one km, and rose gradually for the distance above about one km (Fig. 4).



Figure 3. The results of AUC curves in developing the suitable habitat model of NWCG in **A** 2020, and **B** 2010. The red line showed the mean of AUC, and the blue area showed the standard deviation.



Figure 4. The response curve of Distance-to-Agriculture, the most contribution variables to predicting the suitable habitat of NWCG by the MaxEnt model in **A** 2020, and **B** 2010.

The suitable habitat of NWCG in the Western Nghe An Biosphere Reserve in 2020

The total suitable habitat of NWCG in Nghe An BR under the current land cover condition was estimated at around 4,022.42 km² (30.95% of the overall BR area; Table 2) and mainly concentrated in three areas including the (i) the northern part of Que Phong and Quy Chau district – close to Thanh Hoa province, (ii) Pu Huong NR, and (iii) in the southern Tuong Duong, Con Cuong district – within Pu Mat NP. However, the high habitat suitability with around 380.64 km² only occurred in the northern part (adjacent to Thanh Hoa province) and the southern part of Nghe An BR (within Pu Mat NP; Fig. 5).



Figure 5. The suitable habitat of NWCG under present landcover condition (2020) predicted by the MaxEnt model.

Table 2. Area of suitable habitat of NWCG projected by the MaxEnt model in 1990, 2000, 2010, 2020 (unit: km²).

Habitat suitability	Year							
	1990	2000	2010	2020				
Low	3,091.51	3,221.81	1,719.21	2,488.18				
Moderate	871.28	793.30	708.17	1,173.40				
High	384.89	309.86	322.83	360.84				
Total	4,347.68	4,324.97	2,750.21	4,022.42				

Habitat suitability changes of NWCG in the Western Nghe An Biosphere Reserve from 1990 to 2020

Using the MaxEnt model, we projected the suitable habitat of NWCG in 1990 and 2010 (Fig. 6A–C). The predicted suitable habitat of NWCG in the Western Nghe An BR under the changes in LUCL showed a fluctuating trend from 1990 to



Figure 6. The suitable habitat of NWCG in the Western Nghe An BR in **A** 1990 **B** 2000 **C** 2010, and **D** 2020 (Pu Mat NR was established in 1995, and upgraded to be national park in 2001; Pu Huong, and Pu Hoat NRs were established 2003, and 2013, respectively).

2020. In particular, the total suitable habitat gradually declined by around 22.72 km² from 1990 to 2000. The moderately and highly suitable habitat shrank by around 77.98 km² (8.95%) and 75.04 km² (19.49%), respectively. Between 2000 and 2010, there was around 1,574.76 km² contraction of suitable habitat for the gibbons in the Western Nghe An BR. The majority of habitat lost lay within the low to moderate suitable habitat types. The predicted suitable habitat of NWCG increased significantly from 2750.21 km² to 4,022.42 km² between 2010 and 2020. All suitable categories of habitat showed an expanding trend in the period. The high, moderate, and low habitat suitability categories were estimated to increase by around 38.01 km² (11.77%), 456.23 km² (65.69%), and 768.97 km² (44.73%), respectively (Table 2).

The extracted LULC in the Western Nghe An BR from 1990 to 2020 constituted seven main types, including Settlement, Agriculture, Barren land, Broadleaf forest, Plantation, Water, and Bamboo forest (Table 3). The Broadleaf Forest type occupied the largest area and showed different trends between 1990 and 2020: $6,366.36 \text{ km}^2$ (46.01%), 7,145.05 km² (51.64%), 6902.46 km² (49.89%), and 7695.72 km² (55.62%) in 1990, 2000, 2010, and 2020, respectively (Table 3). On the other hand, the Settlement area was the smallest category but presented an increase from around 0.56 km² (0.004%) in 1990 to 34.75 km² (0.25%) in 2020 (Table 3).

No	LULC	Year				Change		
	categories	1990	2000	2010	2020	1990-2000	2000-2010	2010-2020
1	Settlement	0.56	1.86	13.79	34.75	1.30	11.93	20.97
2	Agriculture	1,789.05	2,008.77	1,835.63	1,707.34	219.72	-173.14	-128.29
3	Barren land	791.70	869.97	899.11	549.95	78.27	29.13	-349.16
4	Broadleaf Forest	6,366.36	7,145.05	6,902.46	7,695.72	778.69	-242.59	793.26
5	Plantation	3,930.07	2,681.34	2,733.05	2,275.61	-1,248.73	51.71	-457.43
6	Water	68.27	83.86	69.91	125.89	15.59	-13.94	55.96
7	Bamboo forest	889.56	1,044.72	1,381.62	1,446.31	155.16	336.90	64.68

Table 3. Area and change of the LULC categories in the Western Nghe An BR from 1990 to 2020 extracted from Phan et al. (2021) (unit=km²).

Table 4. Area of suitable habitat inside and outside borders of protected areas (Pu Mat NP, Pu Huong NR, and Pu Hoat NR*) of NWCG projected by the MaxEnt model in 1990, 2000, 2010, 2020.

Suitability	1990		2000		2010		2020	
-	Inside	Outside	Inside	Outside	Inside	Outside	Inside	Outside
Low	0	3,091.51	382.32	2,839.49	437.10	1,282.11	723.43	1,764.75
Moderate	0	871.28	348.16	445.15	444.19	263.99	613.29	560.11
High	0	384.89	137.57	172.28	228.84	93.99	236.99	123.85
Total	0	4,347.68	868.05	3456.91	1,110.13	1,640.08	1,573.71	2,448.70

*: Pu Mat NR was established in 1995, and upgraded to national park in 2001; Pu Huong, and Pu Hoat NR were established 2003, and 2013 respectively.

Habitat suitability changes of NWCG within existing protected areas inside the Western Nghe An BR.

The overlay between the existing protected areas within the Western Nghe An BR, including Pu Mat NP, Pu Huong, and Pu Hoat NRs, and the suitable habitat of NWCG predicted by the MaxEnt model was only approximately 868.05 km² (25.11%) of suitable habitat lies within protected areas (PAs) in 2000. In 1990, three protected areas in the core zone of the BR were not yet established. In 2010, the areas of suitable habitat inside PAs increased to 1,110.13 km². Then, the predicted suitable habitat rose to approximately 1,573.71 km² in 2020 (Table 4).

Discussion

The AUC value of both models in 2010 and 2020 shows a high capacity to predict the suitable habitat of NWCG with a good performance. The MaxEnt model has been broadly employed to predict the potential distribution of primates (Sarma et al. 2015, 2021; Tran and Vu 2020; Widyastuti et al. 2020; Yang et al. 2021), and also to assess the changes in the suitable habitats of several species under changes in land cover or climate (Tran et al. 2020; Vu et al. 2020; Sarma et al. 2021; Trinh et al. 2021; Blair et al. 2022; Ngo et al. 2022). The predicted suitable habitat of the 2020 model of NWCG in the Western Nghe An BR covered all of the known presence points within Pu Hoat, Pu Huong NR, and Pu Mat NP and adjacent areas (Fig. 5). The predicted suitable habitat of the 2010 model also fitted with the known occurrence of the species in Luu and Rawson (2009, 2010, 2011), and Rawson et al. (2011). Our result indicates that the model could reliably predict the change of suitable habitat over the years 1990–2020 of NWCG in the Western Nghe An BR.

In the present study, we tried to predict the suitable habitat changes of NWCG in the Western Nghe An BR. Modeling a partial distribution could not provide a complete species range because a part of the environmental conditions and presence records of species outside of the target area of the model are not included (Carretero and Sillero 2016; Sillero et al. 2021). Models for the entire distribution of a species can provide a better result than models of partial distribution (Barbet-Massin et al. 2010; Carretero and Sillero 2016). However, it also should be noted that a model on a partial range of a species can forecast other distribution constraints on this species, which may fluctuate in distinctive parts in the whole range (Martínez-Freiría et al. 2008; Vale et al. 2015; Sillero et al. 2021). Our study focused on a small part of the species' overall distribution range that spans Vietnam, northern Lao and southern China (Rawson et al. 2011). Thus, the results could reveal the distinctiveness of environmental variables in the Western Nghe An BR, resulting in changes in suitable habitat of the Western Nghe An BR under changes in LULC between 1990 and 2020. The results also provided valuable insights that could assist in predicting changes in habitat suitability in the entire distribution range of the species.

Our models indicated that the "Distance to Agriculture" was the most significant variable for both predicted models 2020, and 2010 of NWCG, with the highest contributions to the models. The habitat suitability was likely to increase when the distance to agricultural land value was high (Fig. 3), which was also shown in a model of the Northern yellow-cheeked gibbon by Tran and Vu (2020). Gibbons are very sensitive to human disturbances (Geissmann et al. 2000) and habitats near agricultural land can be more easily accessed. Therefore, these areas are considerably impacted by anthropogenic activities such as illegal logging or hunting. Previous studies also emphasized that habitat loss is an important issue and has caused a dramatic reduction of gibbon populations throughout their distribution (Rawson et al. 2011; Rawson et al. 2020).

The suitable habitat generated by species distribution modeling could be very useful in suggesting areas to find new populations, especially for rare and threatened species (Pearson et al. 2007; Thorn et al. 2009; van Schingen et al. 2016; Ngo et al. 2019, 2022). Our model for the present suitable habitat of NWCG in the Western Nghe An BR showed a concentration in three distinctive areas, including the northern part of Que Phong and Quy Chau districts, Pu Huong NR, and the southern Tuong Duong, Con Cuong districts - within Pu Mat NP (Fig. 4). Our result also revealed that the predicted suitable habitat area lies outside the existing protected areas at around 2,448.70 km². This area is primarily located in the northern region of Pu Hoat NR, near Thanh Hoa province, and in the northwest and southeast areas of Pu Mat NP, Nghe An province (Fig. 5). Rawson et al. (2011) indicated that the majority of NWCG populations in Vietnam were detected within existing protected areas, while a large forest area outside the established protected area system has not been surveyed. Recently, researchers also found at least 40 groups of NWCG, mainly inhabiting an area to the north of Pu Hoat NR, outside the current protected systems (Pu Hoat NR 2021). Thus, we believe that a large population of NWCG outside protected areas probably have not been detected yet. We recommend that more survey efforts on gibbons should be spent on the higher suitable habitat areas outside protected areas that were predicted by our model, besides the priority for gibbon surveys within Pu Hoat NR, Pu Huong NR, and Pu Mat NP. Our suitable habitat results given by the MaxEnt model can allow managers, conservationists, and researchers to easily plan field surveys for exploring the unrecorded population of NWCG in the Western Nghe An BR with greater confidence.

Our current study indicates that LULC changes may significantly impact the distribution of gibbons in the Western Nghe An BR, central Vietnam, as projected from 1990 to 2020. Knowledge of habitat transformation plays a crucial role in making decisions regarding biodiversity conservation (Fletcher et al. 2018; Su et al. 2021). Additionally, monitoring gibbons and their habitat is extremely important for conservation due to their shrinking populations globally (Sarma et al. 2021). The Western Nghe An BR was assessed as the most important site for the conservation of NWCG (Rawson et al. 2011), while Pu Mat NP might hold the largest population of the species, with an estimated population of 130 groups through gibbon surveys in 2010 (Luu and Rawson 2011). Our models showed that the habitat suitability for NWCG slightly declined from 1990 to 2000, and greatly decreased between 2000 and 2010. On the other hand, from 2010 to 2020, the predicted suitable habitat increased by around 1272.20 km². It showed that
the suitable habitat area of the NWCG strongly depended on the change of LULC. The main LULC types of the Western Nghe An BR constituted Agriculture land, Broadleaf forest, and Plantation, while changes in LULC in the area showed complex trends consistent with a case study in the highland area of Nghe An Province (Leisz 2009).

The Distance to Settlement variable was the second most important variable for the habitat suitability model of NWCG, although the Settlement was the smallest area among seven main LULC types in the Western Nghe An BR, probably indicating the high impact of human residence settlement areas to habitat suitability of NWCG. The settlement area in the transition zone of the Western Nghe An BR continually increased from 1990 to 2020, probably due to urbanization and population expansion. Between 1990 and 2000, the agricultural land area increased, leading to a slight decrease in the suitable habitat of NWCG. The broadleaf forest areas significantly decreased between 2000 and 2010, reflecting the ineffective activities for habitat conservation in the area. In contrast, there was an increase in the broadleaf forest from 2010 to 2020 that was probably facilitated by government policies, notably the closing of natural forest policy in the late 2010s, and the National Action Programme on REDD+ in the period 2011–2020 (Government of Vietnam 2012). Gibbons are known to be sensitive to human disturbance and to prefer pristine broadleaf forests. Based on our model results on habitat suitability, we strongly recommend protecting the suitable habitat for NWCG and increasing the broadleaf forest areas.

Although the Western Nghe An BR has been established since 2007 with only three core protected areas, including Pu Mat NP, Pu Huong, and Pu Hoat NR, most areas of the Biosphere Reserve have remained currently intact even with the increasing settlement, agricultural land, and the fluctuation of broadleaf forest areas. As a positive human policy, establishing protected areas for protecting all environmental components, including populations, habitats of wild species and natural ecosystems, is crucial because of its mitigations of negative impacts on biodiversity at a local scale (Margules and Pressey 2000; Estrada and Real 2018). Through the efforts of the Vietnamese government, Pu Mat NR was established in 1995 and upgraded to the national park level in 2001; Pu Huong and Pu Hoat NR were established in 2003 and 2013, respectively. The establishment is probably the major reason for the increase of suitable habitats for NWCG inside protected areas. In the general planning for biodiversity conservation of the country to 2020, orientation to 2030 in Degree 45/QD-TTg/2014 (Government of Vietnam 2014), the government also proposed three biodiversity corridors inside the Western Nghe An BR to support the movement of wildlife species under the impact of climate change. However, connectivity habitat for gibbons among the three core protected areas (Pu Mat NP, Pu Luong, and Pu Hoat NR) is very poor when the suitable habitat within these protected areas seems to be isolated. We suggest that areas for connecting three core protected areas inside the Western Nghe An BR also should receive high priority in habitat monitoring of the gibbon.

In this study, land-use change has a strong relationship with the suitable habitat of NWCG, which resulted in the contraction of the suitable habitat of the endangered gibbon from 2000 to 2010. Interestingly, an expansion of suitable habitat of NWCG was revealed in the period 2010 - 2020, which provides a positive sign for habitat conservation in the study areas. Additionally, a limitation of the study is the lack of infor-

mation on specific habitat parameters that might be critical for defining the selection habitat of gibbons, such as the height of tree canopy, food preference, and availability. We recommended that more intensive surveys should be focused on the suitable habitat areas suggested by our model to reveal the forest quality of these areas.

Vietnam is located in the Mekong region, which was assessed as one of the 11 areas experiencing the largest forest loss. The average rate of deforestation in the Mekong region was approximately 0.4% per year (Leinenkugel et al. 2015). Researchers also predicted that the Mekong area will have lost about 15-30 million hectares of natural forest by 2030 if there are no effective urgent measures from governments (WWF 2018). In the best scenario, countries have strong reforms toward green and clean development, and the natural forest of countries in Southeast Asian countries can increase by around 19.6 million hectares (Estoque et al. 2019). However, we have not yet had any fine-scale forecasts on forest status scenarios for the region in the future. With an increasing trend in forest areas, and the efforts and commitments of the Vietnamese government in recent times (De Jong et al. 2006; Government of Vietnam 2017), we predict that the natural forest area in the Western Nghe An BR will continually show a slight increasing trend in the next few decades, leading to an increase in the suitable habitat of gibbons in the study area. But it also should be noted that the increased forest area comes mainly in the form of young forests, which cannot meet the habitat needs of gibbons. Gibbons are specialized in arboreal life and require good-quality forests with a high canopy closure and large trees for their survival (Geissmann et al. 2000; Nadler and Brockman 2014). Therefore, we strongly recommend that activities to enrich young forests are also necessary for the conservation activities of NWCG and its habitats in the Western Nghe An BR.

Finally, we highly recommend the implementation of the following actions for protecting existing populations of NWCG and its habitat in the Western Nghe An BR: 1) implement population surveys, monitoring and conservation awareness programmes not only in three core protected areas (Pu Mat NP, Pu Hoat, and Pu Huong NR) but also in other highly suitable habitats that are predicted by our model, especially for the northern Pu Hoat NR, northern and southern Pu Mat NP; 2) upgrade the Pu Hoat watershed protection forest, the highly suitable habitat in the north of Pu Hoat NR, to be a Nature Reserve (Fig. 4). 3) improve green corridors connecting three core protected areas inside the Western Nghe An BR and other highly suitable habitats; 4) predict the suitable habitat of the NWCG in its whole distribution range, including northern Vietnam, Laos, and southern China, then, propose transboundary conservation programs, especially between Vietnam and Laos; 5) conduct surveys to obtain specific habitat parameters at the suitable habitat area and confirmed the presence of gibbon, then implement the enrichment activities in young forest areas that do not meet the requirements of gibbons for living.

Conclusion

There is currently a lack of research on the changes in suitable habitats for gibbons, leading to limited efforts to protect and conserve their habitats. Here, we applied the species distribution modeling to predict the suitable habitat of NWCG in the Western

Nghe An BR, and assessed the impact of changes in land use and land cover from 1990 to 2020 on the suitable habitat of this species. The current suitable habitat for the gibbon in the Western Nghe An BR was estimated at approximately 4,022.42 km², mainly concentrated in three distinctive areas (Pu Mat NP, Pu Huong, and Pu Hoat NR), while the predicted suitable habitat area lies outside the existing protected areas at around 2,448.70 km². Our result also indicated that due to the changes in LULC, the predicted suitable habitat of the gibbon decreased from 1990 to 2010 but gradually increased from 2010 to 2020. Based on our findings, we suggested that more survey efforts should be focused on areas of predicted suitable habitat areas within protected areas. We also highly recommend enriching young forests and protecting the preferred habitat for the gibbon, specifically broadleaf forests. Finally, we proposed several actions to safeguard NWCG and its habitat in the Western Nghe An BR.

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Supplementary material I

Supporting information

Authors: Khoa Van Phung, Dung Van Tran, Hai Thanh Dong, Vinh Quang Luu, Van Bac Bui, Thinh Tien Vu

Data type: figures and tables

- Explanation note: SI–I. Map of each variable that was used for the final model. SI–II. The percent contribution of environmental variables. SI–III. The suitable habitat of *N. leucogenys* were predicted from occurrence localities in 2020. SI–IV. Area of suitable habitat of *N. leucogenys* projected by MaxEnt model in 1990, 2000, 2010, 2020 from the model of 2020 (unit: km2).
- Copyright notice: This dataset is made available under the Open Database License (http://opendatacommons.org/licenses/odbl/1.0/). The Open Database License (ODbL) is a license agreement intended to allow users to freely share, modify, and use this Dataset while maintaining this same freedom for others, provided that the original source and author(s) are credited.

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RESEARCH ARTICLE



Local perception of the current state and threat factors of a critically endangered species, *Celtis toka* (Forssk.) Hepper & J.R.I. Wood, in Burkina Faso: implications for species conservation

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Abstract

Celtis toka, the only species of the genus *Celtis* (family Cannabaceae) encountered in the flora of Burkina Faso, is critically endangered in the country. To engage the public for the future conservation and domestication of the species, knowledge of the factors threatening *Celtis toka* survival is necessary. Thus, the study objective was to identify the perceptions of local people concerning the current state and conservation strategies of *Celtis toka* in Burkina Faso.

To investigate potential solutions to the threats posed to *Celtis toka*, we randomly surveyed 405 consenting participants using a selected semi-structured interview. Moreover, field observations were performed to assess the threat drivers cited by local people of the Sudanian and Sudano-Sahelian climatic zones. Descriptive analyses (relative frequency and fidelity level) and generalized linear models (GLMs) were used to highlight the impact of sociodemographic factors and climate zones on the current state, threat drivers, and potential solutions. The chi-square test was used to assess whether to plant *C. toka*.

GLM analyses revealed that local knowledge about the current state, threat factors and potential solution to the threat as related to natural stand varied significantly according to ethnolinguistic group (P < 0.000), sex (P = 0.01) and age (P = 0.01). Rural people had varying perceptions of the current state

of *C. toka*. Sixty-eight percent reported a decrease in population, ten percent reported scarcity, and five percent reported extinction. The views of local people were that the factors affecting *C. toka* were pruning (25%), climate change (14%), deforestation (10%), ageing (10%), debarking (9%), and agriculture (7%). Potential solutions included planting (45%), conservation of *C. toka* and its habitat (27%), sustainable use of *Celtis toka* (14%), promotion of education and awareness about *Celtis toka* (10%) and tree/crop association (5%).

The study concluded that the ethnobotanical knowledge of *Celtis toka* may play an important role in its conservation and domestication in Burkina Faso. Furthermore, its incorporation into reforestation and restoration programs is critical to species survival.

Keywords

climate, conservation strategies, COVID-19, decline, ethnobotany, extinction, West Africa

Introduction

Climate change is defined as a change in the weather pattern of a location or region that is related to average weather components such as temperature, wind patterns and precipitation (Ifeanyiobi et al. 2012). For instance, extreme weather events devastate the land and terrestrial ecosystems and exacerbate food insecurity for humanity. These changes, according to the United Nations' Intergovernmental Panel on Climate Change, have a large impact on many people who are thought to be disproportionately vulnerable to the effects of climate change (IPPC 2021). To cope with global change and mitigate its consequences, rural communities rely on forests, primarily in terms of multipurpose plants for medicine, food, fodder, shade, renewable energy, windbreaks, erosion control, carbon sinks, soil improvement, fertility restoration and conservation (Ifeanyiobi et al. 2012).

Ethnobotany, a branch of ethnobiology (Houéhanou et al. 2016), is the study of the relationships between plants and culture as well as the local perception of the use, management (Bridges and Lau 2006; de Albuquerque and Hanazaki 2009) and the state of plant resources. Moreover, ethnobotanical knowledge is critical for species conservation, mainly for endangered species that have been recognized by the Convention on Biological Diversity in Rio de Janeiro (CBD 1992). Furthermore, promoting endangered plants is critical to ensuring their sustainable conservation, domestication, and inclusion in reforestation and restoration programs. Therefore, plant promotion, conservation, domestication, and introduction through restoration and reforestation, necessitate a scientific approach based on local perceptions of the current state, threats, and potential solutions.

Celtis toka (Forssk.) Hepper & J.R.I. Wood belongs to the Cannabaceae family. The genus *Celtis* includes approximately 60–70 species worldwide (Mahre et al. 2017) and 12 species in Africa (Sattarian and Van Der Maesen 2005). This species is a very large tree with a compact crown (Sattarian 2006) and is usually monoecious (Alfaifi et al. 2021). It reaches 25 m in height and 2.2 m in diameter at breast height. The bole is either short or tall with ramifications (Dabré et al. unpublished). *Celtis toka* (*C. toka*) is

an open gallery forest, dense dry forest, savannah, sacred grove, and rocky area species (Neumann 1992; Shepherd 1992; Rabeil 2003; Akoegninou et al. 2006; Savadogo and Thiombiano 2010; Höhn and Neumann 2016; Arbonnier 2019). It thrives in areas with low rainfall (110–960 mm yr.⁻¹) and high temperatures (26–30 °C) (Watrin et al. 2007). It is widely distributed and found throughout Benin, Burkina Faso, Central African Republic, Chad, Côte d'Ivoire, Eritrea, Ethiopia, Gambia, Ghana, Guinea (Conakry), Mali, Mauritania, Niger, Nigeria, Senegal, South Sudan, Sudan, Togo, Uganda, and Yemen (Sattarian 2006; IUCN 2019).

The species provides a variety of products used for food (leaves, fruit); medicine (leaves, barks, roots, flowers, seeds); construction (wood); firewood and charcoal for human cooking activities; and fodder (leaves) for animals and handicrafts (Blench 2000; Lykke et al. 2004; Teklehaymanot and Giday 2010; Seignobos 2014; Höhn and Neumann 2016; Piqué et al. 2016; Arbonnier 2019; Badiane et al. 2019). The fruits and leaves are considered edible in Sudan (Arkell 1947), Ghana (Irvine 1961), Senegal (Lericollais 1990), Nigeria (Kiee et al. 2000; Harris and Mohammed 2003; Kubmarawa et al. 2011), Cameroon (Nakagawa 2008; Neba 2009; Betti and Yemefa 2011), Benin (Djègo-Djossou et al. 2015) and Burkina Faso (Dabré et al. unpublished). Mballow et al. (2020) stated that *C. toka* is the most commonly used species by communities in west and east Africa. Moreover, tannins, glycosides, alkaloids, saponins, phenols, coumarins, flavonoids, mucilage, triterpenoids and steroids are phytochemicals extracted from leaves of *C. toka* (Fall et al. 2017; Abba Idris and Halima Mohammed 2020).

In addition, all organs of *C. toka* are used to cure diseases such as measles, chickenpox, malaria, back and eye aches, ringworm, fever sore, mycosis, headache, and mental diseases (Hahn-hadjali and Thiombiano 2000; Betti et al. 2011; Arbonnier 2019; Diatta et al. 2019). The roots, leaves and bark are used in Nigerian traditional medicine to treat various diseases, including epilepsy (Muazu and Kaita 2008). Additionally, the plant organs are used to treat trypanosomiasis (Osue et al. 2018). In Cameroon, the bark is also used to cure epilepsy (Tsabang et al. 2016). The medicinal use of *C. toka* was also highlighted by Bizimana et al. (2006) in Mali and Badiane et al. (2019) in Senegal. In Burkina Faso, leaves, bark, flowers, fruits, grains, and roots were used to heal 29 ailments, such as madness, yellow fever, eye ache, malaria, casting, vitamin deficiency, ringworm, diarrhea, backache, toothache, ulcer, measles, and chickenpox (Dabré et al. unpublished).

In Ethiopia, *C. toka* is a wild tree that provides an important economic service because it is preferred for the hanging of beehives (Bareke 2018), for timber, and for making local boats (Rolkier and Abebe 2015). In addition, *C. toka* has a mystical value in Burkina Faso (Savadogo and Thiombiano 2010). Cultural and mystical values were also highlighted by Dabré et al. (unpublished).

The International Union for Conservation of Nature (IUCN)'s forest work addresses the role of trees and forests in building resilience to climate change (IUCN 2021). Additionally, the IUCN sustainable use initiative has been successful in delivering conservation outcomes that benefit society (IUCN 2020). *C. toka* was assessed on the IUCN Red List of Threatened Species in 2023 as being of Least Concern (LC), and its current population trend is stable at present (IUCN 2019, accessed on January 25, 2023). According to the IUCN (2019), *C. toka* has a very broad distribution and a large population, with no major threats currently existing and no significant future threats being identified.

Even though C. toka is classified as LC at the global level (IUCN 2019), this species is either threatened, rare, critically engendered and even extinct in some temperate regions and the Sahelian region in Africa (Hahn-hadjali and Thiombiano 2000; Garzuglia 2006; Al-Khulaidi 2018; Alfaifi et al. 2021; Tchobsala et al. 2022). Nonetheless, the species seems to be lacking in Senegal, Guinea Sudan, and Uganda (Hauman 1942). Moreover, C. toka is threatened in Benin, northern Cameroon and the Sahelian region of Africa (Gonzalez et al. 2012; Dansi et al. 2013; Moksia et al. 2019). In Chad, the species is either extinct or endangered (Tchobsala et al. 2022). Although the species is important in Yemen, it is currently rare and must be evaluated and monitored regularly (Al-Khulaidi 2018). In Saudi Arabia, C. toka is thus a very rare and threatened species (Alfaifi et al. 2021). The reasons for the rarity and near local extinction of this species in some parts of Africa are understandable. This is because of the enormous dependency on this species and its products for ecological and economic purposes in some parts of African countries. This, leads to overexploitation, thus predisposing the species to a high risk of local extinction in Africa. This phenomenon is prevalent in west Africa, especially Burkina Faso, where the species is subject to overexploitation because of its medicinal, fodder, food, and mystical uses (Dabré et al. unpublished).

Only C. toka, one of the twelve species of the genus Celtis in Africa, is found in Burkina Faso, and it is on the verge of extinction. Several pieces of empirical evidence suggest that C. toka is rare, threatened, critically endangered or extinct in Burkina Faso. Authors have shown that in Burkina Faso, C. toka is a one-of-a-kind critically endangered species (Garzuglia 2006) and is threatened to the point of one day disappearing (Hahn-hadjali and Thiombiano 2000; Thiombiano et al. 2010; Bayala et al. 2011). Vodouhe et al. (2007) also stated that C. toka has been on the verge of extinction in Burkina Faso since 2007. For instance, Hahn-hadjali and Thiombiano (2000) also stated that since 2000, C. toka has disappeared in eastern Burkina Faso, except in sacred groves. Furthermore, Bayala et al. (2011) discovered that C. toka was the most threatened species in the agroforestry parkland of Burkina Faso. Additionally, Savadogo and Thiombiano (2010) specified that the wild plant C. toka is endangered in the Northern and Southern Sudanian zones of Burkina Faso. More recently, the studies of Savadogo et al. (2017) have shown that C. toka is rare in the communities of the strict Sahelian, southern and northern Sahelian regions of Burkina Faso. Therefore, given the socio-economic and ecological importance of C. toka to the teeming African populace, and given its potential high risk of local extinction in Africa, there is a need for its conservation. However, an effective conservation strategy of the natural resources must take into account the knowledge and opinions of the stakeholders, especially the local people who are the direct beneficiaries of the resources so conserved

(Amoutchi et al. 2021). These authors argued that indigenous people all over the world have preserved distinctive understandings, rooted in cultural experience, that guide relationships among human and non-human beings in specific ecosystems. Thus, their perception and knowledge of threats to any natural resources, and their subsequent contribution to designing a conservation action will produce a positive outcome. The local people (especially in Burkina Faso) depend directly on the services provided by C. toka for their livelihoods. Therefore, they are expected to be the most affected if the species becomes extinct, and also, they are expected to be well aware of the nature of the disturbances and threats the species is facing given their indigenous knowledge of it. Their perception of the threats (the impact and causes) and knowledge of anthropogenic activities impacting this species, being part of their indigenous knowledge, are essential for making and implementing decisions and policies related to the mitigation of these threats, and the management of this species in the ecosystem. Furthermore, the knowledge and perception of the local people represent the baseline information for motivating and directing any research projects regarding any conservation action targeted at this species.

Despite its socioeconomic and cultural importance and its role in mysticism, knowledge of the threat factors of *C. toka* in Africa in general and Burkina Faso, in particular, is lacking. In this regard, this study aimed to assess the diverse local knowledge and perceptions of the dynamics and threats to *C. toka* in west Africa (using Burkina Faso as a case study), with the view to recommending conservation strategies to mitigate the local extinction of this species in west Africa where the species is already reported to be critically endangered. Specifically, we seek to answer the following questions: (a) What are the perceptions of rural people regarding the status of *C. toka* in Burkina Faso? (b) What are the threats to the survival of *C. toka* in Burkina Faso? And (c) what are the perceptions of the local population concerning the potential solutions to the threats posed to *C. toka* in Burkina Faso?

We explicitly tested three hypotheses:

H1: Interviewees perceived the extinction of *C. toka* in the study sites.

H2: Anthropogenic activity is the main driver of species decline.

H3: Planting and the conservation of the species and its habitat are two of the most important solutions to the threats posed to the species.

Description of the study site

This study was conducted from November 2020 to January 2022 in villages near Kou, Dinderésso, Sourou, Sâ and Koulbi classified forests located in the Sudanian and Sudano-Sahelian climatic zones of Burkina Faso (Fig. 1). In both climatic zones (BCZ), the climate is tropical with two separate seasons: rainy and dry periods. The principal rivers are Kou and Mouhoun, and the main soils are leptosols, vertisols, ferralsols, luvisols,



Figure 1. Localization of the study sites in Burkina Faso. (Studied PAs: studied protected areas).

lithosols and hydromorphic soils (Savadogo and Thiombiano 2010). The vegetation types include various savannahs, dry forests, and gallery forests.

The flora consists of some Sahelian, Sudanian and Guinean species, such as *Vitex chrysocarpa* Planch, *Antiaris africana* Engl., *Parkia biglobosa* (Jacq.) R.Br. ex G. Don, *Lannea microcarpa* Engl. & K. Krause, *Dialium guineense* Willd., *Cola laurifolia* Mast., *Carapa procera* DC., *Vachellia seyal* (Delile) P.J.H Hurter, *Detarium microcarpum* Guill. & Perr., *Balanites aegyptiaca* Del. (Nacoulma et al. 2018). The sociolinguistic groups are Dafing, Gourmantché, Gourounssi, Bobo, Bozo, Dioula, Sambla, Senoufo, Bambara, Marka and Bwaba. The key livelihoods are traditional subsistence farming, principally of cereals (millet, sorghum, and maize), livestock breeding and trade.

Materials and methods

Materials

Celtis toka is a wild plant that thrives in a variety of environments. It has different local names around Africa (Table 1).

Countries	Dialect	Vernacular names	Sources
Benin	Dendi	Séékossou	Dansi et al. 2013
	Yoruba	Afoufe	Diègo-Diossou et al. 2015
		Bousamsambou	Achigan-Dako et al. 2010
Burkina Faso	Mooré	Pargandé	Thiombiano et al. 2012
	Mooré	Silsaka	Thiombiano et al. 2012
Cameroon	Arabe	Ngouso	Betti and Yemefa 2011
		Aboum gatou	Vivien 1990
		Hala	
	Fulfuldé	Djiho	
	Haoussa	Douki	
	Toupouri	Likan	
	*	Loubour	
	Kanouri	Ngouzo	
	Mofu	Sabak	
	Mafa	Shéshébé	
		Wanka	
	Fulfuldé	Ganki	Gilbert et al. 2019
	Fulfuldé	Wanko	Gilbert et al. 2019; Seignobos and Tourneux 2002
	Mofou	Mebed	Gilbert et al. 2019
	Arabe	Falmaro	Betti et al. 2011
Ethiopia	Anywaa	Laero,	Awas 1997
	Kara (people)	Zuguay,	Teklehaymanot and Giday 2010
	Kwego (people)	Lompo	Teklehaymanot and Giday 2010
Nigeria		Aápe	Ogungbenro et al. 2018
Mali		Kamaua, Gamya	Bizimana et al. 2006
Senegal	Wolof	Mbul	Gonzalez 2001
	Diola	Busingilit	Diatta et al. 2019
	Sereer	ngan	Lericollais 1990
South Sudan	Mabanese	Shaw	Bloesch 2014
	Arabe	Tekey	Bloesch 2014
South Kordofan Sudan		Mohagria	Ismail and Elawad 2015
Sudan		Mohagria, Lipingo	Hamid and Kordofani 2015

Table 1. Some local names of *Celtis toka* in Africa.

Methods

Sampling strategy and data collection

A preliminary assessment was carried out in November 2020 to obtain an overview of the availability and distribution of *C. toka*. Furthermore, this assessment allowed us to obtain approval from authorities and village leaders, as well as to fine-tune the questionnaire. The preliminary assessment and survey were carried out while maintaining social distancing and other preventive measures (wearing a nose mask and using hand sanitizer) to avoid the spread of COVID-19. From the three climatic zones of Burkina Faso, two climatic zones (the Sudanian climatic zone (**SCZ**) and the Sudano-Sahelian climatic zone (**SCZ**)) were chosen considering the accessibility of the areas and the occurrence of *C. toka*. A discussion was held with the administrators, environmental officers, forest officers, farmers, fishermen/women, hunters, traditional healers, and



Figure 2. Map of the ethnolinguistic groups questioned in the study areas.

elders. Next, field observations were made cooperatively with field guides, farmers, village leaders and elders to look for *C. toka* availability, accessibility, distribution, threat drivers and potential solutions within the study sites. Thirty-four villages were included in the survey, comprising twenty-five ethnolinguistic groups (Fig. 2).

The ethnolinguistic groups Bambara and Bozo represent immigrants from Mali.

Villages were chosen based on the presence of the multipurpose species *C. toka*. Overall, 405 (148 female and 257 male) consenting local people who knew *C. toka* were randomly selected. Data were collected using a selected semistructured interview (Sop and Oldeland 2011; Theodory 2016) and direct field observation. It was impossible to interview an equal sex ratio in each village because the investigations were based on the knowledge of *C. toka*. All respondents were at least 30 years old because they were the only ones who knew about *C. toka* and its status. Data for age, sex, career, education level and ethnolinguistic group of the informants were recorded. Ethnolinguistic groups that were represented by few individuals were classified as "other" for the purpose of performing the statistical analysis. Proficient local translators were used to translate French into the local languages.

Photographs of the leaves (Fig. 3), the trunk (Fig. 4), and the fruits (Fig. 5) of *C. toka* were taken during the prospection, kept, and shown to each household in both climatic zones to ensure that local communities were familiar with *C. toka* (Arbonnier 2019). The respondents were questioned about the following:

- current state of *C. toka* in their community,
- factors affecting *C. toka* survival,
- potential solutions to the threat.



Figure 3. Pictures of leaves used for quick identification by local people during the survey. Pictures: Z. Dabré, 2020.



Figure 4. Pictures of the trunks used for quick identification by local people during the survey. Pictures: Z. Dabré, 2020.



Figure 5. Pictures of fruits used for quick identification by local people during the survey. Pictures: Z. Dabré, 2020.

Data analysis

Before the analyses, the interviewees were divided into two generations: adults (30–55 years) and older adults (\geq 55 years) (Sop et al. 2012). Three and four Sudanian and Sudano-Sahelian ethnolinguistic groups, respectively, were considered major ethnolinguistic groups, with the remainder classified as "other" (Fig. 2, Table 3).

The relative frequency and fidelity level (Table 2) were employed to analyse the most destructive threats to *C. toka*. GLMs with Poisson errors and chi-square tests at a threshold equal to 0.05 were used to detect sociodemographic parameter (ethnolinguistic groups, age, and sex) effects on the current state, threat, and potential solutions in the BCZ. Chi-square analysis was also used to determine whether to plant *C. toka* in the study areas. All statistical analyses were performed using R version 4.1.1 (R Core Team 2021).

Results

Sociodemographic characteristics and local perception of the status of *C. toka* in Burkina Faso

Sociodemographic characteristics of local populations

In total, 405 people were interviewed in this study, with 203 in the SCZ and 202 in SSCZ (Table 3). Most of the interviewees were male (72.77%), autochthonous (81.19%), and farmers (75.74%) with no formal education (80%). Bozo (8.37%) were immigrants, and those classified as "other" were autochthons (Table 3).

Index	Computation	Explanation	R
$RF = (FC/SF) \times 100$	Frequency of citation (FC) quoted by a given	Measures the ratio of the number of times a use	А
	informant divided by the sum of uses times 100.	pattern of C. toka appears in the set of overall use	
		patterns. The value ranges from 0 to 100.	
$FL = (Ip/Iu) \times 100$	Number of informants (Ip) connected to a specific	Measures the grade of consensus between	В
	use divided by the total number of informants (Iu)	informants. FL is significant when this is directly	
	times 100.	above 5% (FL > 5%).	

Table 2. Formulas related to relative frequency (RF) and fidelity level (FL).

R: References, A: (Thiombiano et al. 2016), B: (Friedman et al. 1986).

Table 3. Sociodemographic characteristics of informants and study design.

Demographic parameters	Variables	Sudanian	Sudano-Sahelian
Sex	Female	40.95	27.23
	Male	59.05	72.77
	Total	100	100
Residential status	Autochthon	65.02	81.19
	Migrant	34.98	18.81
	Total	100	100
Ages	Adult (30-55)	46.80	58.91
	Old (> 55).	53.20	41.09
	Total	100	100
Ethnolinguistic groups	Bobo	71.44	13.86
	Bozo	8.37	_
	Bwaba	-	31.68
	Dafing	-	23.27
	Dioula	10.34	_
	Mossi	-	12.87
	Others	9.85	12.87
	Total	100	100
School level	None	79.59	74.26
	Primary	15.75	21.28
	Secondary	4.37	3.47
	University	0.29	0.99
	Total	100	100
Main activity	Farming	61.44	75.74
	Trade	21.61	8.91
	Breeding	11.65	11.39
	Handwork	5.3	-
	Hunt	-	3.96
	Total	100	100

Local perception of the status of C. toka in Burkina Faso

Overall, 68% of informants (77.94% in the SCZ and 58.28% in SSCZ) (Fig. 6) widely expressed that the multiuse species *C. toka* was greatly decreasing (SCZ: 41% < FL < 90% and SSCZ: 52 < FL < 96) (Table 4). Thirteen percent of the participants expressed that the species is stable. In SSCZ, approximately 19% and 11% of the interviewees confirmed that the sacred tree *C. toka* was either rare or extinct, respectively. However, 4% of the local people (5.88% in the SCZ and 2.86% in SSCZ) highlighted that the species was increasing in abundance (Fig. 6, Table 4). As the most surveyed communities,



Figure 6. Local perception of the current state of the multiuse species C. toka in Burkina Faso.

Bobo, Bozo, Dioula, Bwaba, Dafing, Mossi, and Dioula represent the ethnolinguistic groups that perceived the most recent status of *C. toka* in the study areas. Ethnobotanical knowledge of the state of *C. toka* varied greatly across ethnolinguistic groups. However, Dioula (FL: 90%) and Bobo (FL: 62%), belonging to SCZ, perceived the decline of *C. toka*. However, in the SSCZ, Mossi (FL: 96%) and Dafing (FL: 81%) reported a decrease in the species. Respondents in the SCZ with an age above 55 years identified more of a declining status of the species than others. Regarding sex, men (SCZ) and women (SSCZ) reported more threatened status based on their traditional knowledge when compared to others. The rarity of the species was perceived by the Bwaba culture (FL: 42%), and extinction features of *C. toka* were mostly perceived by the Mossi (FL: 35%) and the "other" ethnolinguistic groups (FL: 35%) in the SSCZ (Table 4).

The GLM analysis revealed that local knowledge of *C. toka*'s status varied greatly across ethnolinguistic groups in terms of extinction and stable status, across sexes for all status levels, and across ages for all status levels except decreasing status (*p*–value < 0.05). Adults and elderly people in the BCZ had dissimilar perceptions of the declining, scarcity, and extinction aspects of *C. toka* (*p*–value < 0.05) (see Table 5).

Local perception of the threat factors of C. toka in Burkina Faso

According to the respondents, the sustainability of *C. toka* is threatened by some unfavourable factors, such as anthropogenic and natural factors. However, 4% of respondents indicated that there are no threats to the sustainability of *C. toka*. Globally, the threat features to *C. toka* were perceived as pruning (25%) for food and fodder, climate change (14%), deforestation (10%), ageing (10%), debarking (9%), agricultural expansion (7%), bushfires (6%), and "other" (6%) (Fig. 7). The "other" category referred

Criteria /	Variants		S	udar	nian cl	imat	ic zon	e				Suda	ano-Sa	ahelia	an clir	natic	zone		
Patterns		Bobo		Be	Bozo		oula	Ot	hers	Bo	obo	Bw	aba	Da	fing	M	ossi	Ot	hers
		(n=	145)	(n=	=17)	(n=	21)	(n=	=20)	(n=	28)	(n=	64)	(n=	47)	(n=	26)	(n=	26)
		F	FL	F	FL	F	FL	F	FL	F	FL	F	FL	F	FL	F	FL	F	FL
			(%)		(%)		(%)		(%)		(%)		(%)		(%)		(%)		(%)
Current state of	Extinct	0	0	0	0	0	0	0	0	1	4	13	20	2	4	9	35	9	35
C. toka	Rare	2	1	1	6	1	5	1	5	2	7	27	42	2	4	8	31	6	23
	Decreasing	90	62	7	41	19	90	21	70	15	54	33	52	38	81	25	96	16	62
	Stable	37	26	6	35	1	5	4	20	8	29	5	8	5	11	0	0	1	4
	Increasing	10	7	1	6	0	0	1	5	3	11	0	0	2	4	0	0	0	0
	ΣF	139	-	15	-	21	-	27	-	29	-	78	-	49	-	42	-	32	-
Threat factors	Pruning	56	39	8	47	13	62	13	65	4	14	62	97	33	70	6	23	15	58
	Bushfire	20	14	0	0	2	10	1	5	1	4	2	3	7	15	0	0	0	0
	Ageing	21	15	2	12	2	10	1	5	0	0	13	27	13	28	8	31	7	26
	Debarking	35	24	2	12	3	14	15	75	6	21	4	6	12	26	4	15	3	12
	Climate	23	16	3	18	4	19	3	15	2	7	20	31	15	32	5	19	2	8
	change																		
	Deforestation	19	13	1	6	3	14	3	15	2	7	15	23	5	11	9	35	7	27
	Farming	7	5	2	12	5	24	3	15	1	4	17	27	4	9	10	38	7	27
	Lack of	9	6	0	0	0	0	1	5	2	7	7	11	4	9	0	0	0	0
	regeneration																		
	Settle of	8	6	0	0	1	5	3	15	0	0	12	19	2	4	1	4	1	4
	infrastructures																		
	Overgrazing	6	4	1	6	0	0	0	0	0	0	15	23	3	6	1	4	0	0
	Others	3	2	1	6	0	0	0	0	3	11	5	8	2	4	0	0	0	0
	Failure of <i>C. toka</i>	4	3	1	6	3	14	0	0	0	0	1	2	0	0	0	0	0	0
	ΣF	211	-	21	-	36	-	43	-	21	-	173	-	100	-	44	-	42	-
Potential	Tree/crop	9	6	3	18	1	5	1	5	0	0	2	3	2	0	2	8	0	0
solutions to the	association																		
threats	Planting	38	26	4	24	9	43	4	20	5	18	36	56	2	49	19	73	11	42
	Conservation	47	32	3	18	1	5	0	0	5	18	8	13	28	58	16	62	5	19
	of <i>C. toka</i> and																		
	its habitat																		
	Sustainable use of <i>C. toka</i>	22	15	3	18	1	5	0	0	3	11	7	11	4	9	2	8	1	4
	Promoting	13	9	1	6	0	0	1	5	1	4	11	17	36	2	1	4	1	4
	education,																		
	and awareness																		
	about C. toka	1.00		1/		1.2		,		1/		~ /		70		(0		10	
	ΣF	129	-	14	-	12	-	6	-	14	-	64	-	72	-	40	-	18	-

Table 4. Local knowledge of *C. toka* in two climatic zones of Burkina Faso.

n: number of individuals interviewed, F: frequency, FL: fidelity level, and Σ F: sum of frequencies.

to the specific habitat of the species, infertility of soil, production of charcoal, diseases, attacks by parasites, fungi, epiphytes, termites, and invasion of *Azadirachta indica* and *Ficus*. In the SCZ, the main causes were pruning (21%), deforestation (13%), climate change (13%), debarking (12%), bushfire (9%), ageing (8%) and agriculture (8%) (Fig. 7). Pruning (FL:47%), climate change (FL:18%), debarking (FL:12%), and age-ing (FL:12%) were emphasized by the Bozo, and debarking (FL:75%) was emphasized by others in the SCZ (Table 4). In the SSCZ, pruning (28%) and climate change (15%) were reported as the key drivers (Fig. 7). Pruning (FL: 70%), climate change

CZ	Variables	n	Extinct	Rare	Increasing	Decreasing	Stable
Ethno	linguistic grou	ıps					
S	Bobo	145	0 ± 0	0.014 ± 0.12	0.23 ± 0.42	0.47 ± 0.5	0.26 ± 0.44
	Bozo	17	0 ± 0	0.035 ± 0.22	0.47 ± 2.75	0.92 ± 5.63	0.48 ± 3.08
	Dioula	21	0 ± 0	0.048 ± 0.22	0.51 ± 0.51	1 ± 0.51	0.53 ± 0.22
	Others	20	0 ± 0	0 ± 0	0.3 ± 0.47	0.45 ± 0.51	0.25 ± 0.44
SS	Bobo	28	0.037 ± 0.19	0.074 ± 0.26	0.088 ± 0.27	0.56 ± 0.50	0.30 ± 0.47
	Bwaba	64	0.20 ± 0.41	0.42 ± 0.50	0.047 ± 0.21	0.47 ± 0.50	0.078 ± 0.27
	Dafing	47	0.043 ± 0.20	0.043 ± 0.20	0.064 ± 0.25	0.79 ± 0.41	0.11 ± 0.31
	Mossi	26	0.267 ± 0.46	0.133 ± 0.35	0.067 ± 0.26	0.67 ± 0.49	1.333 ± 0.35
	Others	26	0.207 ± 0.46	0.103 ± 0.25	0.067 ± 0.26	0.57 ± 0.39	1.303 ± 0.32
	X^2		7.83	2.1	1.15	2.98	6.08
	P value		0.00468	0.138	0.295	0.0804	0.02
Sexes							
S	Female	91	0 ± 0	0.011 ± 0.11	0.269 ± 0.44	0.314 ± 0.47	0.337 ± 0.47
	Male	112	0 ± 0	0.027 ± 0.16	0.230 ± 0.42	0.566 ± 0.50	0.168 ± 0.38
SS	Female	55	0.15 ± 0.36	0.1 ± 0.30	0.025 ± 0.16	0.7 ± 0.46	0.175 ± 0.38
	Male	147	0.123 ± 0.33	0.254 ± 0.44	0.078 ± 0.27	0.561 ± 0.50	0.114 ± 0.32
	X^2		23.406	39.03	15.41	4.67	10.64
	P value		0.00028	< 0.0000	0.00022	0.0309	0.00169
Age							
S	Old	108	0 ± 0	0.020 ± 0.14	0.215 ± 041	0.479 ± 0.50	0.243 ± 0.43
	Adult	95	0 ± 0	0.017 ± 0.13	0.322 ± 0.47	0.406 ± 0.50	0.237 ± 0.43
SS	Old	83	0.086 ± 0.28	0.272 ± 0.45	0.074 ± 0.26	0.530 ± 0.50	0.123 ± 0.33
	Adult	119	0.178 ± 0.39	0.151 ± 0.36	0.055 ± 0.23	0.671 ± 0.47	0.137 ± 0.35
	X^2		14.49	34.65	18.26	2.04	4.66
	P value		0.00055	< 0.00000	< 0.000	0.153	0.03407

Table 5. Impact of socio-demographical factors and climatic zones on the current state of *C. toka* through GLMs analyses.

CZ: Climatic zones, n: number of individuals surveyed, S: Sudanian, SS: Sudano – Sahelian, and X²: chi-square.



Figure 7. Threat factors by climate zone in Burkina Faso. F. of C. toka: failure of C. toka.

(FL: 32%), ageing (FL: 28%) and debarking (FL: 26%) were reported at a heightened level by the Dafing culture in the SSCZ (Table 4). These disturbances affect the survival of the critically endangered species *C. toka*.

The results globally suggested that pruning, climate change, deforestation, ageing, debarking, farming expansion and bushfires are the major factors that threaten the survival of C. toka in the study areas. Pruning, climate change, and deforestation ranked first, second and third, respectively, signifying that they are the most proximate threatening factors (Fig. 7). In the SCZ, ageing was perceived by Bobo culture; climate change by Bozo culture; debarking, pruning, farming and failure of C. toka by Dioula culture; and deforestation and settlement of infrastructures were mentioned by other ethnolinguistic groups as the main predictors of the decline of the species. However, in the SSCZ, deforestation, farming and pruning were the main causes of the extinction of C. toka identified by the Mossi. The Dafing culture believed that C. toka is threatened due to climate change and human activities (bushfires). According to the Bwaba culture, overgrazing and debarking are the key reasons for the threat in the study area (Table 6). In the BCZ, older people emphasized that the species has declined due to climate change, farming, ageing, deforestation, settlement of infrastructure, bushfires, failure of C. toka, pruning and other causes (Table 6). The overall threat factors of C. toka varied only across ethnolinguistic groups for ageing, pruning, climate change, farming, settlement of infrastructure, and lack of regeneration (p-value < 0.05). In addition, the threat drivers were statistically similar (p-value > 0.05) between both sexes and generations for identifying deforestation, settlement of infrastructures, lack of regeneration, failure of *C. toka* and bushfire as causes (Table 6).

Based on our observations in the field, the threat factor in the BCZ could be natural (hole, wind, drought, crown gall, fungi, and epiphyte) as well as anthropogenic. However, old *C. toka* trees contained very large hollows (Fig. 8) which weakens the species during wind (Fig. 9), or fire action (Fig. 10).

In addition to the hole, *C. toka* has a fasciculate root (Fig. 11) which accentuates the effect of wind. *Ficus thonningii* Blume (Fig. 12), fungi (Fig. 13), and crown gall and holes (Fig. 14) infest *C. toka* tree species.

In the study areas, some livelihoods result in the overharvesting of the leaves of this species either for food or fodder uses (Fig. 15), and the bark by traditional healers (Fig. 16). Furthermore, some individuals died, probably due to drought, ageing, or diseases (Fig. 17).

According to our field observations, the assessment of the threat by climatic zones showed that the SCZ was more exposed than the SSCZ (even though *C. toka* is less abundant in the SSCZ) due to anthropogenic activities such as artisanal activities (Fig. 18), and industrial mining (Fig. 19), and bushfire (see Fig. 10 above).

However, the exploitation of granite negatively affects seed germination and sometimes sapling growth. Some local people often cut *C. toka* (Fig. 20) for charcoal, firewood, or farm settlement purposes.

Epiphyte (Fig. 21), grazing by goats and cattle (Fig. 22), and biological invasion by *Azadirachta indica* A. Juss. (see Figs 11, 21, 22 above) of *C. toka* organs or saplings were noticed in some livelihoods of the study areas. Epiphytes (Fig. 21) infested *C. toka* trees to the point that they lost their leaves and remained apparently dead.

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Tab	le 6. Mea	n values (± St	d) and result	ts of GLM fa	ctors conside	red threats t	o <i>C. toka</i> in	Burkina Fa	so.				
CZ	Variables	n Climate change	Farming	Deforestation	Settle of infrastructures	Ageing	Bushfire	Overgrazing	Lack of regeneration	Failure of <i>C. toka</i>	Debarking	Pruning	Others
Ethn	olinguistic g	sdno											
s	Bobo 1	$45 0.131 \pm 0.34$	0.048 ± 0.22	0.131 ± 0.34	0.055 ± 0.23	0.172 ± 0.38	0.137 ± 0.35	0.041 ± 0.20	0.062 ± 0.24	0.028 ± 0.16	0.180 ± 0.74	0.329 ± 0.47	0.062 ± 0.24
	Bozo	$17 0.222 \pm 0.43$	0.111 ± 0.32	0.056 ± 0.24	0 ± 0	0.111 ± 0.32	0 ± 0	0.056 ± 0.24	0 ± 0	0.056 ± 0.24	0.222 ± 0.43	0.222 ± 0.43	0 ± 0
	Dioula	$21 0.190 \pm 0.40$	0.238 ± 0.44	0.143 ± 0.36	0.048 ± 0.22	0.095 ± 0.30	0.095 ± 0.3	0 ± 0	0 ± 0	0.143 ± 0.36	0.381 ± 1.53	0.381 ± 1.53	0 ± 0
	Others	20 0.142 ± 0.36	0.190 ± 0.40	0.190 ± 0.40	0.190 ± 0.40	0.048 ± 0.22	0.095 ± 0.30	0 ± 0	0.095 ± 0.30	0 ± 0	0.333 ± 0.97	0.367 ± 0.98	0.048 ± 0.22
SS	Bobo	28 0.071 ± 0.26	0.036 ± 0.19	0.071 ± 0.26	0 ± 0	0 ± 0	0.036 ± 0.19	0 ± 0	0.071 ± 0.26	0 ± 0	0.038 ± 0.19	0.077 ± 0.29	0 ± 0
	Bwaba	$64 0.219 \pm 0.42$	0.265 ± 0.45	0.234 ± 0.43	0.188 ± 0.39	0.359 ± 0.48	0.031 ± 0.18	0.234 ± 0.43	0.109 ± 0.31	0.016 ± 0.13	0.188 ± 1.11	0.234 ± 0.43	0 ± 0
	Dafing	$47 0.319 \pm 0.47$	0.085 ± 0.28	0.106 ± 0.31	0.043 ± 0.20	0.276 ± 0.45	0.148 ± 0.36	0.063 ± 0.25	0.085 ± 0.28	0 ± 0	0.26 ± 1.29	0.106 ± 0.31	0.043 ± 0.20
	Mossi	26 0.133 ± 0.35	0.267 ± 0.46	0.267 ± 0.45	0.067 ± 0.25	0.467 ± 0.52	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.56	0.267 ± 0.45	0 ± 0
	Others	26 0.133 ± 0.35	0.267 ± 0.46	0.237 ± 0.41	0.056 ± 0.21	0.237 ± 0.24	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.2 ± 0.56	0.241 ± 0.36	0 ± 0
	X^2	6.41	17.7	2.39	3.7	7.03	2.14	0.44	0.896	0.37	1.15	7.65	1.06
	P value	0.00986	<0.000	0.117	0.0479	0.00699	0.152	0.499	0.01	0.548	0.301	0.00412	0.304
Sexes													
s	Female	$91 0.144 \pm 0.35$	0.022 ± 0.15	0.067 ± 0.25	0.022 ± 0.15	0.167 ± 0.37	0.044 ± 0.21	0.022 ± 0.15	0.033 ± 0.18	0.044 ± 0.20	0.178 ± 0.44	0.158 ± 0.37	0.040 ± 0.17
	Male 1	$12 \ 0.147 \pm 0.36$	0.139 ± 0.35	0.183 ± 0.39	0.095 ± 0.30	0.130 ± 0.34	0.174 ± 0.38	0.043 ± 0.20	0.070 ± 0.26	0.035 ± 0.18	0.252 ± 1.07	0.167 ± 0.39	0 ± 0
SS	Female	$55 0.175 \pm 0.38$	0.125 ± 0.33	0.1 ± 0.30	0.025 ± 0.16	0.35 ± 0.48	0.025 ± 0.16	0.025 ± 0.16	0.075 ± 0.27	0 ± 0	0.075 ± 0.35	0.185 ± 0.37	0 ± 0
	Male 1	$47 \ 0.228 \pm 0.42$	0.184 ± 0.39	0.193 ± 0.40	0.123 ± 0.33	0.254 ± 0.44	0.079 ± 0.27	0.149 ± 0.36	0.088 ± 0.28	0.00 ± 0.09	0.219 ± 1.17	0.221 ± 0.40	0.009 ± 0.09
	\mathbf{X}^2	1.64	5.27	2.12	2.53	2.9	0.05	6.67	1.13	2.12	7.52	0.38	1.26
	P value	0.195	0.0184	0.139	0.103	0.0844	0.832	0.00707	0.279	0.169	0.0159	0.3056	0.1504
Age													
s	Old 1	$08 \ 0.164 \pm 0.37$	0.103 ± 0.30	0.144 ± 0.35	0.068 ± 0.25	0.171 ± 0.37	0.089 ± 0.29	0.006 ± 0.08	0.048 ± 0.21	0.034 ± 0.18	0.171 ± 0.68	0.164 ± 0.37	0.034 ± 0.18
	Adult	$95 0.101 \pm 0.30$	0.051 ± 0.22	0.102 ± 0.30	0.051 ± 0.22	0.085 ± 0.28	0.19 ± 0.39	0.102 ± 0.30	0.068 ± 0.25	0.05 ± 0.22	0.340 ± 1.18	0.101 ± 0.30	0.05 ± 0.22
SS	Old	$83 0.284 \pm 0.45$	0.148 ± 0.36	0.136 ± 0.34	0.086 ± 0.28	0.309 ± 0.46	0.062 ± 0.24	0.136 ± 0.35	0.111 ± 0.32	0 ± 0	0.222 ± 1.25	0.284 ± 0.45	0.061 ± 0.20
	Adult 1	$19 \ 0.137 \pm 0.34$	0.192 ± 0.40	0.205 ± 0.41	0.110 ± 0.31	0.247 ± 0.43	0.068 ± 0.25	0.096 ± 0.30	0.055 ± 0.23	0.014 ± 0.117	0.137 ± 0.69	0.137 ± 0.34	0.014 ± 0.117
	X^2	3.89	0.77	0	0.14	4.2	1.71	1.4	0.95	1.71	0.17	4.51	2.65
	P value	0.0445	0.373	0.938	0.699	0.0372	0.2	0.227	0.321	0.215	0.681	0.0299	0.027
CZ: C	Climatic zone	s, n: number of in	dividuals survey	ved, S: Sudanian,	, SS: Sudano – Si	ahelian, and X ² :	chi-square.						



Figure 8. Risk factor: hollow in most of C. toka natural stands in the BZC. Pictures: Z. Dabré, 2020.



Figure 9. Risk factor: The effect of wind on *C. toka* in BCZ. Pictures: Z. Dabré, 2021.



Figure 10. Risk factor: ageing and fire effect on hollowed out C. toka in SCZ. Pictures: Z. Dabré, 2021.



Figure 11. Risk factor: ageing, action of the wind and *Azadirachta indica* A. Juss. invasion in SCZ. Pictures: Z. Dabré, 2021.



Figure 12. Risk factor: attack by Ficus thonningii on C. toka species in SCZ. Pictures: Z. Dabré, 2021.



Figure 13. Risk factor: attack of fungi on *C. toka*. Pictures: Z. Dabré, 2021.



Figure 14. Risk factor: holes (yellow round), ageing, crown gall (red round) attacks coupled with debarking in the BCZ. Pictures: Z. Dabré, 2022.



Figure 15. Risk factor: heavy pruning of C. toka's leaves in BCZ. Pictures: Z. Dabré, 2021.



Figure 16. Risk factor: debarking for traditional medicinal purposes in the BCZ. Pictures: Z. Dabré, 2020.



Figure 17. Risk factor: natural death due either to drought, diseases, or ageing of the hollowed out *C. toka* in the BCZ. Pictures: Z. Dabré, 2022.



Figure 18. Risk factor: Artisanal mining under C. toka tree in the SCZ. Pictures: Z. Dabré, 2021.



Figure 19. Risk factor: industrial mining in the habitat of C. toka in the SCZ. Pictures: Z. Dabré, 2021.

Traditional potential solutions to the threat

The conservation strategies proposed by the locals included the conservation of *C. toka* and its habitat, the sustainable use of *C. toka*, and the promotion of education and awareness about *C. toka*. However, planting was the most important solution expressed by all ethnolinguistic groups, as confirmed by the high FL (SCZ: 43%, SSCZ: 73%)



Figure 20. Risk factor: cutting of *C. toka* sapling in the SCZ. Pictures: Z. Dabré, 2020.



Figure 21. Risk factor: ageing, epiphytic attack (red circles), holes, crown gall (yellow circles), and *Azadirachta indica* A. Juss. invasion in SSCZ. Pictures: Z. Dabré, 2022.



Figure 22. Risk factor: ageing, rotting, *Azadirachta indica* A. Juss. invasion and animal grazing in the SSCZ. Pictures: Z. Dabré, 2022.

value in the BCZ (Table 3). Planting (45%), conservation of *C. toka* and its habitat (27%), sustainable use of *C. toka* (14%), promoting education and awareness about *C. toka* (10%), and tree/crop association (5%) were the future potential solutions proposed to solve the threat posed to *C. toka* in the study areas. Moreover, potential solutions in the SCZ were planting (34%), conservation of *C. toka* and its habitat (33%) to protect it from human pressures such as cutting, fire, animal grazing and sustainable use of the species (16%) by avoiding overharvesting (pruning, debarking, and overusing the roots). In the SSCZ, planting (58%), conservation (19%), and sustainable use (10%) were the key solutions proposed to address the threats posed to the species (Fig. 23).

In the Sudanian climatic zone, conservation, sustainable use of the species, and planting were cited by the Bobo, Bozo, and Dioula cultures, respectively, as potential solutions to the threats posed to the species. According to the Sudano-Sahelian climatic zone, the conservation of the species and its habitat was mostly perceived by the Dafing and planting by the Bwaba as key solutions to address the threat. Most of the solutions were proposed by males in the BCZ. In the SCZ, older people proposed more solutions than younger adults. A contradiction was found in the SSCZ (Table 8).

The chi-square test results of the different responses of informants who were involved in answering whether to plant *C. toka* show that there is a significant relationship among the respondents in the study sites (Table 7).

The GLM analyses of respondents' perceptions of potential solutions to the threat posed to *C. toka* revealed that local perception varied significantly according to ethnolinguistic groups, sex, and ages for the solution of planting and ethnolinguistic groups for the solution of conservation of *C. toka* and its habitat (p-value < 0.05, Table 8). However, no differences in promoting education and awareness about *C. toka*, associating *C. toka* with crops, and sustainable use of the species were found among all the sociodemographic parameters (p-value > 0.05, Table 8).

Discussion

Local perception of the current state of the agroforest tree *C. toka* in Burkina Faso

The spatial dynamics of *C. toka* declined in the study area. This could be explained by the fact that the species was once rare. For instance, Gonzalez et al. (2012) emphasized



Figure 23. Traditional potential solutions to the threat according to climatic zones in Burkina Faso.

Table 7. Chi-square test showing whether to plant or not *C. toka* in Burkina Faso.

Climatic zones	% of res	ondents		Chi-squar	e
=	Yes	No	DF	X ²	P value
Sudanian	77.02	23.91	1	27.94	< 0.0001
Sudano-Sahelian	99.61	0.39	1	98.43	< 0.0001

DF: degree of freedom, X2: chi-square.

	Variables	n	Planting	Conservationof	Promoting education, and	Tree/crop	Sustainable use of
				C. toka and its habitat	awareness about C. toka	association	C. toka
Eth	nolinguistic	group	os				
S	Bobo	145	0.262 ± 0.44	0.152 ± 0.36	0.090 ± 0.29	0.062 ± 0.24	0.014 ± 0.11
	Bozo	17	0.235 ± 0.44	0.118 ± 0.33	0.059 ± 0.24	0.176 ± 0.39	0.059 ± 0.24
	Dioula	21	0.429 ± 0.51	0.048 ± 0.22	0 ± 0	0.048 ± 0.22	0 ± 0
	Others	20	0.2 ± 0.41	0 ± 0	0.05 ± 0.22	0.05 ± 0.22	$0.\ 05\pm 0.22$
SS	Bobo	28	0.179 ± 0.39	0.107 ± 0.31	0.036 ± 0.19	0 ± 0	0 ± 0
	Bwaba	64	0.563 ± 0.5	0.094 ± 0.29	0.172 ± 0.38	0.031 ± 0.18	0.031 ± 0.18
	Dafing	47	0.489 ± 0.51	0.064 ± 0.25	0.021 ± 0.15	0 ± 0	0.021 ± 0.15
	Mossi	26	0.7 ± 0.51	0 ± 0	0.087 ± 0.26	0 ± 0	0 ± 0
	Others	26	0.6 ± 0.51	0 ± 0	0.067 ± 0.26	0 ± 0	0 ± 0
	X^2		19.55	15.56	1.41	4.21	0.48
	P value		< 0.000	< 0.00	0.244	0.051	0.477
Sexe	:s						
S	Female	91	0.189 ± 0.39	0.111 ± 0.31	0.033 ± 0.18	0.044 ± 0.21	0.033 ± 0.18
	Male	112	0.336 ± 0.47	0.133 ± 0.34	0.106 ± 0.30	0.088 ± 0.29	0.009 ± 0.09
SS	Female	55	0.5 ± 0.51	0.1 ± 0.30	0.15 ± 0.36	0.025 ± 0.16	0 ± 0
	Male	147	0.464 ± 0.50	0.070 ± 0.26	0.070 ± 0.26	0.009 ± 0.09	0.026 ± 0.16
	X^2		6.09	0.29	0.29	0.27	0.05
	P value		0.013	0.596	0.587	0.613	0.831
Age							
S	Old	108	0.285 ± 0.45	0.118 ± 0.32	0.056 ± 0.23	0.063 ± 0.24	0.021 ± 0.14
	Adult	95	0.24 ± 0.43	0.136 ± 0.35	0.119 ± 0.33	0.087 ± 0.28	0.017 ± 0.13
SS	Old	83	0.469 ± 0.50	0.49 ± 0.21	0.135 ± 0.34	0.025 ± 0.16	0.012 ± 0.11
	Adult	119	0.479 ± 0.50	0.109 ± 0.31	0.041 ± 0.20	0 ± 0	0.027 ± 0.16
	X^2		5.53	1.31	0.2	2.24	0.01
	P value		0.018	0.260	0.650	0.151	0.913

Table 8. Mean values (\pm Std) and results of GLMs of potential solutions to the existence of *C. toka* in Burkina Faso.

CZ: climatic zones, n: number of individuals surveyed, S: Sudanian, SS: Sudano – Sahelian, X²: chi-square.

that the density of C. toka has declined in the Guinea ecological zone of the African Sahel due to the climate. Moreover, human action could be the reason for the decrease in C. toka in Burkina Faso. Most youths were unaware of the value of this species; therefore, they cut saplings as well as adult trees. In addition, to establish agricultural land, some residents cut down C. toka individuals. Moreover, habitat loss could be the factor responsible for the changes in the species' population dynamics. However, the transformation of the species' habitat (gallery forests) by market gardening or exotic plants could explain its decline. For instance, in the Sudanian climatic zone, the habitat of C. toka was converted into vegetable crops (such as tomatoes, cucumbers, strawberries, eggplants, sorrels, carrots, papaya and others) and exotic tree plantations (Mangifera indica L., Tectona grandis L.f., Anacardium occidentale L., Eucalyptus cama-Idulensis Dehnh., Annona muricata L., Annona squamosa L., Delonix regia (Boj.) Raf., Citrus lemon (L.) Burm.f.). Dansi et al. (2013) demonstrated that the threat posed to C. toka may be due to forest destruction, bushfires, destructive harvesting methods and a lack of knowledge about the species. In the Sudano-Sahelian zone, C. toka was reported to be rare and even extinct. This extinction could be due to a lack of regeneration to replace the ageing population of the species. Research has shown that the threat

posed to *C. toka* in northern Cameroon is due to the lack of regeneration. For instance, the regeneration was 0% in an unprotected area and 0.14% in protected areas (Moksia et al. 2019). According to the Mossi and other cultures from the SSCZ, the species was extinct due to overexploitation, the change in the environment and the failure of *C. toka* in the area. Hence, *C. toka* has extremely small population sizes and therefore is in extreme danger of extinction. For instance, it was difficult to obtain two or three individuals in the same area, and most of them were old and isolated in their communities. Additionally, the use of the species for traditional medicine may have contributed to its extinction in some study sites, mostly in the Sudano-Sahelian communal zone. Climate has a greater impact on the species abundance and distribution in the Sudano-Sahelian climatic zone than in the Sudanian climatic zone because C. toka is denser in the Sudanian than in the Sudano-Sahelian region. Deforestation caused by agricultural development, infrastructure installation, and climatic variability has an impact on the spatial dynamics of C. toka. Similar findings were reported by Hahn-hadjali and Thiombiano (2000); Garzuglia (2006); Vodouhe et al. (2007); Thiombiano et al. (2010); Bayala et al. (2011); Savadogo et al. (2017) in Burkina Faso. Moreover, C. toka is either extinct or endangered in Chad (Tchobsala et al. 2022), rare in Yemen (Al-Khulaidi 2018), and rare and threatened in Saudi Arabia (Alfaifi et al. 2021).

Hence, different ethnolinguistic groups, sexes and generations have diverse views on the status of *C. toka* due to cultural differences. Traditional knowledge of the dynamics of *C. toka* is influenced by ethnolinguistic groups over time. The scarcity and decline of *C. toka* have been perceived by most sociocultural groups. For instance, the declining factor of *C. toka* was perceived more strongly by the Bobo, Bozo, Dioula Dafing, and Mossi cultures. This is because those cultures are autochthonous and know the status of the species over time. Traditional healers (Bobo, Bwaba, Dioula, Mossi), hunters (Bwaba) and fishermen/women (Bozo) who interact with the habitat of *C. toka* have a better knowledge of the species' status. Knowledge of the declining characteristics of *C. toka* within a hamlet is similar from one generation to another. This could be explained by the fact that both younger adults and the elderly were aware of the species' status and thus noticed the decline and/or extinction of *C. toka*.

Threat to the sustainability of the sacred tree C. toka in Burkina Faso

Even though *C. toka* is a tree associated with mysticism, it faces diverse threats to its continued existence from various anthropogenic activities and natural factors. Extinction and decline of *C. toka* are due to a range of factors, including overharvesting (pruning, debarking, and rooting), climate change, deforestation, ageing of the population, and farming expansion. According to rural residents, overexploitation is the most serious threat because, in conjunction with the scarcity of *C. toka*, organs were harvested in an anarchic way. These findings were similar to those of Moksia et al. (2019). Respondents were confident that roots, leaves, and bark are a critical part of *C. toka*; therefore, its overharvesting may affect its reproduction.

Overharvesting of bark and leaves has been reported to have reduced fruit production in Burkina Faso (Nacoulma et al. 2017). Furthermore, heavy pruning could affect tree development and photosynthesis (Suchocka et al. 2021). Three percent (3%) of the rural community thought C. toka was endangered due to a lack of regeneration. Regeneration could be influenced by either seed availability or seed quality. C. toka has been overpruned to the point where it no longer produces fruits in some areas. The lack of seeds may be the primary cause of the lack of regeneration. Gaoue et al. (2011) demonstrated that the overharvesting of fruits and seeds exposes natural stands vulnerable to population ageing due to the threat to natural regeneration. Overgrazing may also contribute to a lack of regeneration. Four percent (4%) of locals believed that the scarcity of species was caused by overgrazing of seedlings and saplings. Harvesting roots, bark, and even leaves may expose C. toka to diseases such as fungal pathogens, galls, epiphytes, termite attacks, and others. These pathogens could drive the species to extinction by interfering with C. toka reproduction, increasing competition for nutrients, and most likely causing mortality. However, Boussim et al. (2004) indicated that C. toka is parasitized by a pest called Tapinanthus globiferus (A.Rich.) Tiegh. However, Otry and Laflamme (2009) confirmed that various fungi are pathogens of specific tree species and cause tree mortality in vulnerable trees. Approximately 12% of the respondents stressed that climate change is one of the reasons for the extinction of the taboo species. They noticed that during the past 30 years, there have been drastic changes in the frequency and volume of precipitation patterns, rising temperature and wind. Sop and Oldeland (2011) stated that drought is one of the primary causes of vegetation change in the Sub-Sahel of Burkina Faso. However, strong winds in the savannah and rocky zones cause the ageing population of the species to easily be uprooted because the species lacks a taproot, while drought causes the species to dry out. Furthermore, because the ideal temperature for C. toka is between 26 and 30 °C, an increase in temperature could inhibit regeneration (Watrin et al. 2007). According to respondents, floods have an impact on C. toka habitat by destroying habitat and uprooting any C. toka found on the banks because some of them had their roots hanging in the rivers in some way. In addition, ageing (13%) leads to the death of C. toka. Thus, drought and pathogens can hasten this death. Franklin et al. (1987) demonstrated that tree death is a natural ecological process involving one or more pathogens and other microbes. However, the proportion of farming expansion (7%) and urbanization (4%) was less of a contribution to the extinction of the sacred tree C. toka. These factors are still a major challenge today because the human population is growing, and forests are being destroyed to make way for infrastructure and farmlands. According to locals, previously, the C. toka tree was not widespread (1%) in different villages of Burkina Faso, and it was not accessible even for local food, fodder, firewood, and medical purposes. Most of the fishermen, hunters and traditional healers had to travel to other villages to look for the organs of C. toka. According to the respondents, fire (6%) may impact the seeds, seedlings, saplings and even the age population of the species. However, bushfires could reduce the abundance of seedlings by killing seedlings and decreasing the seed bank of C. toka in the soil. Furthermore, bushfires may influence the trunk and hollows of adult individuals, which could potentially lead to the death of the species. Fire triggers plant mortality throughout the crown, stem and root (Miller
and Findley 2014). The severity and impacts, at the regional and global scales, of the burned area resulting from wildfires, have increased in recent decades (Doerr and Santin 2016). Wildfires have an impact on soil properties (Agbeshie et al. 2022), making plant species vulnerable to a decline (Miller et al. 2019). Local knowledge of the threat factors of *C. toka* was dissimilar among the ethnolinguistic groups, generations, and sexes due to the diversity of cultures.

Potential traditional solution to the threat

Three of the five concepts of the Global Strategy for Plant Conservation are the conservation of the species and its habitat, sustainable use, and promotion of education and awareness (CBD 2002). Planting remains the main solution to combat the threat in the BCZ. Although the sacred species C. toka was rare and extinct in some areas, no individuals were found planted near houses, such as in northern Cameroon, where C. toka was planted for agro-silvopastoral purposes (Neba 2009). Additionally, C. toka was planted in farmland in Ethiopia (Rolkier and Abebe 2015). Most rural residents agreed to plant C. toka on their farmlands and in public places (markets, administrations, and schools) because they believed it was the best way to save the species. Some rural residents have decided not to plant it in their compounds because they know that C. toka is a massive plant. Furthermore, the mystical nature of C. toka may have discouraged locals from domesticating it. The second possible solution was the in-situ conservation of C. toka and its habitat (13%) to protect it from all human activities, including animal grazing, bushfires, and cutting of saplings and adult trees. Farmers must respect the 100-metre limit on riverbanks for the conservation of natural habitat. Respondents believed that farming within 100 meters of the riverbanks could cause siltation of water bodies and affect species regeneration. Agricultural activities such as tillage and ploughing cause the siltation of streambeds, resulting in the loss of the original water source (Kumar et al. 2021) and shortening its useful life (Poleto and Beier 2012). However, the conservation of habitats may be the best solution to the threat because seeds could be obtained from the remains of individuals and could be used to produce seedlings in nurseries. Seedlings have the potential to aid in reforestation and soil restoration. For instance, C. toka could be used as a keystone plant to restore deeply destroyed ecosystems. Furthermore, reintroducing C. toka into a restoration framework can aid in the prevention of extinction. The main solution to extinction is in situ and ex-situ conservation of a species (Oldfield 2003). Furthermore, invasive species should be removed from the habitat to reduce competition. According to Barney et al. (2013), invasive alien species alter nutrient pools and fire regimes. However, rural people stated that C. toka is scarcer and more threatened than Vitellaria paradoxa C.F.Gaertn., Lannea microcarpa Engl. & K. Krause, Parkia biglobosa (Jacq.) R. Br. ex G. Don, Bombax costatum Pellegr. & Vuillet, Adansonia digitata L., and yet it is not protected. To reduce the rate of decline of C. toka, local people suggested associating crops with C. toka. We recommend in situ, ex-situ, and circa-situ conservation of C. toka. Circa-situ conservation is a successful conservation strategy in traditional

agroforestry systems and backyard gardens (Sanchez et al. 2010; Lokonon et al. 2021). As such, we recommend that local communities increase their capacity to cultivate the species and replant it in traditional agricultural landscapes and home gardens, as well as promote youth education about good practices and harvesting techniques for plant parts used in food, fodder, medicine, or other specific uses.

Conclusion

This study has shown that the local people of the Sudanian and Sudano-Sahelian areas are aware of the ecological status of C. toka, as well as the potential driving factors influencing species dynamics. Celtis toka was described to be in a state of decline and extinction (in the Sudano-Sahelian zone) due to anthropogenic activities combined with climate change, lack of regeneration and species failure. Moreover, efforts should perhaps be concentrated on the domestication of C. toka to enhance regeneration and increase production. Conservation efforts should perhaps focus on C. toka and its habitat. However, most of the local future potential solutions included planting; conservation of the species, its seeds, regeneration, and its habitat; avoidance of the overuse of C. toka; fire protection; association of C. toka in farmland; and promoting education and awareness of youth about C. toka. The incorporation of local people's perceptions into policymaking is of critical importance in C. toka management and for its sustainable conservation strategies. The findings of this study will aid the conservation of the critically endangered species C. toka at the national level by informing future environmental and biodiversity conservation efforts. Moreover, C. toka could be used to rehabilitate and restore degraded ecosystems to promote the recovery of the species.

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