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Front cover: The Critically Endangered Annam leaf turtle *Mauremys annamensis* resident in the reptile house of London Zoo. See article on page 1. (© Benjamin Tapley ZSL)

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Contents

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Global and regional patterns in distribution and threat status of zoo collections of turtles and tortoises

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Turtles are a globally threatened group of reptiles. Zoo populations may contribute to the conservation of species, including turtles, but collection composition may not align with conservation needs. We combined data from the Zoological Information Management System (ZIMS), EDGE of Existence, the IUCN Red List and the Reptile Database to investigate zoo turtle holdings on global and regional scales. Globally, zoo collections were representative of turtle diversity, regional species distributions and threat statuses, indicating no bias towards threatened species and no taxonomic or distribution blind spots. Species kept in zoos had significantly lower EDGE scores than those not represented, and threatened species were no more likely to have been bred in the year prior to data collection (before March 2022) or have non-viable populations, but were more likely to have a larger population size. Although Africa, Asia and South America have the smallest turtle holdings in terms of species, allowing for regional capacity, these regions hold more, while Europe holds fewer than expected turtle species – North American and Asian holdings do not differ from expected. African, Asian, North and South American regions significantly bias their collections towards native species. We found evidence for significant increases in turtle populations at the genus level following the EAZA Shellshock campaign in Europe. ZIMS data are limited by taxonomy, membership and accuracy of records but provide the best window into patterns of zoo turtle collections. While holding a species in a zoo does not equate to conservation value, based on these data, we recommend that conservation prioritisation exercises are developed for all turtle species, holding institutions or regional taxonomic advisory address population viability and support for institutions working with significant turtle populations in captivity to join ZIMS is provided.

Keywords: chelonia, ex-situ, zoos, conservation, ZIMS

INTRODUCTION

Turtles and tortoises (order Testudines; henceforth 'turtles') have an almost global distribution outside of the poles. Representatives of the group occur in most habitats, from desert to rainforest to coral reef (Ihlow et al., 2012). The group is also deeply connected to human cultures across the globe (Lovich et al., 2018) and performs important ecosystem services (e.g. Falcón & Hansen, 2018).

However, turtles are in the midst of a global conservation crisis; nearly half of the > 350 recognised species of Testudines are threatened with extinction (Ersnt & Lovich, 2009; Stanford et al., 2020). Despite this, and the relatively small size of the group as a whole, approximately one third of all turtle species are yet to be assessed for the IUCN Red List, and many existing assessments are out of date, meaning that threat levels may be higher than current data suggest (Böhm et al., 2013; Rhodin et al., 2018).

Population declines and extirpation are being driven by illegal and legal trade, habitat destruction and degradation, emerging infectious diseases and climate change (reviewed by Stanford et al., 2020). Over-exploitation for pet, meat and traditional medicine trades are probably the greatest specific threat to freshwater turtles and tortoises, which represent all but seven species of Testudines (Schlaepfer et al., 2005), with millions of animals traded both legally and illegally on a global scale (IUCN TFTSG, 2011; Mărginean et al., 2018; Cheung et al., 2006; Chow et al., 2014; Shamsur et al., 2013; Sihombing et al., 2021). The Chinese market for turtles is particularly large (Gong et al., 2009) and demand there has placed particular collection pressure on turtle populations, primarily across Asia, but also beginning to reach into North America (Lau & Shi, 2000).

Finally, at the level of species, turtles represent a significant amount of phylogenetic diversity relative to other tetrapod groups (Gumbs et al., 2020) and many turtle species have been identified as global priorities for conservation by the EDGE of Existence programme by using a combined score of evolutionary distinctiveness and extinction risk taken from the IUCN Red List.

Zoos, aquariums and similar organisations holding captive populations of wild animals (henceforth 'zoos', for convenience, with the explicit acknowledgement that

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zoological gardens sensu stricto may differ importantly from other animal holding organisations included in ZIMS) can form pivotal components of conservation initiatives, especially as part of the IUCN One Plan Approach (Traylor-Holzer et al., 2019) and have had positive impacts on conservation in both ex- and in-situ contexts (Robovský et al., 2020). Zoos are almost unique in their capacity to use ex-situ approaches to contribute to conservation goals, through engaging with the public to raise funds and awareness, holding so-called Ark populations, breeding for conservation translocation, acting as rehabilitation centres for injured wild animals, using captive population research species biology and to trial field methods under controlled conditions. However, zoos can struggle to strike the (albeit difficult) balance between the animal husbandry requirements, impacts on visitor appeal, distribution relative to the location of a given zoo, and threat status of focal groups of ectothermic vertebrates to align collection plans with global conservation needs (Tapley et al., 2015; Dawson et al., 2016; Harding et al., 2016; Biega et al., 2017; Biega & Martin, 2018; Jacken et al., 2020; Wahle et al., 2021).

These competing factors may lead zoo collections to misalign with the conservation needs of particular groups. Information concerning global patterns of collection holdings is useful to inform the zoo community's allocation of resources and collection plan design to more effectively support conservation. However, there is currently a poor understanding of global patterns in turtle species holdings in zoos, and how well they address conservation needs (Horne et al., 2012). Despite this, turtles have been the focus of specific zoo campaigns designed to improve conservation impact. The European Association of Zoos and Aquarium (EAZA) Shellshock campaign of 2004–5 was the largest of these, and raised awareness and funds aimed at supporting turtle conservation through the creation of 'turtle Arks' and collaboration with field programmes. The campaign was specifically associated with a push to increase holdings of threatened turtle taxa in European zoos, with a particular focus on Asian species, where conservation needs were deemed highest due to human consumption of turtles (Buley, 2005; Shellshock, EAZA.net, 2022).

In the present work, we aimed to appraise global patterns in turtle species holdings in zoos via the utilisation of the Zoological Information Management Software (ZIMS) database (Species360, 2022). More specifically, we aimed to investigate phylogenetic representation, the distribution of individuals, breeding success and the relative distribution of individuals and species holdings based on their IUCN Red List status, as well as to identify regional trends in collection composition. We also investigated how the Shellshock campaign affected European zoo holdings of freshwater turtles and tortoises.

METHODS

Zoo holding and geographic data

A list of all currently recognised turtle species (i.e. all taxa within order Testudines) was downloaded from the Reptile Database (Uetz et al., 2022), on 18 March 2022. The available data for turtle holdings in the Zoological Information Management Software (Species360 Zoological Information Management Software (ZIMS), 2022) were then downloaded via the Species Holdings tool as of 19 March 2022. This dataset included, for each species, the number of individuals kept (male, female, other), number of holding institutions, the continental regions (as defined by Species360) in which these institutions resided (Africa, Asia, Europe, North America, South America, Oceania), the number of individuals held in each of these regions and the number of births globally in the last 12 months. Species were allocated to global captive population size categories (N < 10, 11–50, 51–100, 101–200, 201–1000, > 1000), following Wahle et al. (2021). The native presence of each species in each of the continental regions was recorded by comparison with the range data according to the IUCN Red List (IUCN, 2022) and the Reptile Database for each species. Numbers of ZIMS registered animal collection holding institutions by region were provided by Species360 as Europe (586), Asia (114), North America (367), South America (32), Oceania [=Australia in older versions] (83) and Africa (24), as of 6 April 2022. Additional ZIMS registered institutions exist, but these do not hold animal collections and were excluded from analysis.

Taxonomy was aligned with the Reptile Database. Subspecies and obsolete taxa were collapsed into their respective species and recognised senior synonyms by additively combining holding data. Species that appeared on the Reptile Database but not in ZIMS Species holdings records were assigned as 'not in zoos' and all above records recorded as '0' or 'NA'. The Galapagos giant tortoises presented a unique problem in that multiple species are recognised by some authorities (see Kehlmaier et al., 2021), with some (including *Chelonoidis nigra* sensu stricto, under which the other species were considered subspecies) being assessed as Extinct by the IUCN. Many ZIMS records, however, are still assigned to *C. nigra* as subspecific status is not recorded, and consequently numerous individuals of an Extinct species are apparently extant in ZIMS. In order to address this problem, we collapsed all Galapagos giant tortoise species into *C. nigra* sensu lato. This is aligned with recent genetic work indicating shallow divergence and subspecific status of evolutionarily distinct units in this group (Kehlmaier et al., 2021).

The total number of individuals held in European zoos for ten years (1 January 1994–1 January 2004) prior to and ten years following (1 January 2006–1 January 2016) the EAZA Shellshock campaign (2004–5 inclusive) was counted for each turtle genus from the ZIMS database. These large time windows were selected to allow for the time taken for collection planning, species acquisition and breeding to respond to the Shellshock campaign, and to account for annual fluctuations due to breeding or death events. *Trachemys*, *Centrochelys* and *Testudo* were excluded from this analysis due to the huge number of individuals involved and inaccuracy and lack of clarity of records resulting from large numbers of rescued and customs-seized animals moving through collections. Genera with a population count of zero in both time windows were also excluded.

Table 1. Hypotheses, null hypotheses (where relevant) addressed, with datasets and statistical approaches used to test them

H_1	H_0	Variables	Test
Global species holdings do not differ in proportion from described diversity at the family level.	NA	Number of species represented in each family in zoos; numbers of species in each family of Testudines.	Chi-squared analysis for goodness of fit, with p-value simulation (2,000 iterations).
Species holdings are evenly distributed across continental regions.	NA	Number of species held by region; number of institutions per region; total number of Testudines species globally.	Chi-squared analysis for goodness of fit, with p-value simulation (2,000 iterations).
Zoo collections reflect global distribution of species between regions. There is no difference in distribution between global holdings of species by native region and the number of species native to each region.	NA	Global species holding counts, split by species-native region; proportions of number of all turtle species native to each region.	Chi-squared analysis for goodness of fit, with p-value simulation (2,000 iterations).
Regional zoo collections prioritise local faunas, in line with IUCN guidelines for ex-situ conservation.	Species holdings for zoos within each region, split by native region, do not differ from global distribution of species by region.	Regional species holding counts split between species native regions; proportions of number of all Testudines species native to each region.	Chi-squared analysis for goodness of fit with p-value simulation (2,000 iterations), with pairwise proportion post-hoc test with Bonferroni correction.
Global zoo species holdings are biased towards higher IUCN Red List categories.	Species holdings do not differ in distribution from species numbers across IUCN Red List categories.	Counts of species in captivity, split by IUCN Red List category of species; total numbers of species in each IUCN Red List status category.	Chi-squared analysis for goodness of fit, with p-value simulation (2,000 iterations), with post-hoc test. Post-hoc two proportions test with continuity correction.
European zoo species holdings are biased towards higher IUCN Red List categories.	Species holdings do not differ in distribution from species numbers across IUCN Red List categories.	Counts of species in captivity, split by IUCN Red List category of species; total numbers of species in each IUCN Red List status category.	Chi-squared analysis for goodness of fit, with p-value simulation (2,000 iterations), with post-hoc test. Post-hoc two proportions test with continuity correction.
Species in zoos are more likely to be threatened than not threatened.	Threat status of species in zoos, in terms of numbers present in each category, do not differ in distribution from threat status of species not in zoos.	Counts of species in and not in zoos, split by IUCN Red List category of species.	Chi-squared analysis for independence with p-value simulation (2,000 iterations), with pairwise proportion post-hoc test with Bonferroni correction.
Species held in zoos have higher average EDGE scores than species not held in zoos	There is no difference in EDGE scores between species held in zoos.	EDGE scores of species in and not in zoos.	Mann-Whitney U Test (following Shapiro Wilks test showing that data did not conform to a normal distribution).
Among species held in zoos, population sizes are larger for threatened species.	There is no difference in population size distribution between threatened and not threatened species.	Counts of species with global populations in each population size category, split by threatened and not threatened categories.	Chi-squared analysis for independence with p-value simulation (2,000 iterations).
Threatened species are bred more than not threatened species.	There is no difference in proportions bred between threatened and not threatened species.	Numbers of species where breeding was recorded in the last twelve months, split by threatened or not threatened categories.	Two proportions test with continuity correction.
The proportion of threatened and not-threatened species where only a single sex is present in holding institutions differs.	There is no difference in the proportions of threatened and not threatened species represented by a single sex.	Numbers of threatened and not threatened species, numbers of species with only single sex reported in each category.	Two proportions test with continuity correction.
European zoos increased the size of turtle collections after the EAZA Shellshock campaign.	There is no difference in turtle population sizes in European zoos before and after the Shellshock campaign.	Total numbers of individuals in European zoos before and after the campaign, split by genus.	Paired samples Wilcoxon test.

Conservation status

The IUCN Red List of Threatened Species (IUCN, 2022) was accessed on 20 March 2022 and the threat status of all species was recorded; those with no Red List assessment were recorded as 'Not Assessed'. For some analyses, those assessed as Critically Endangered, Endangered or Vulnerable were classed as 'threatened', those assessed as Near Threatened or Least Concern were classed as 'not threatened', and Not Assessed and Data Deficient taxa were assigned to the threat group 'Unknown'. The EDGE score for each species was also accessed from the EDGE of Existence list for reptiles (Gross, 2018; Gumbs et al., 2018). Where EDGE scores did not exist for a given taxa, the record was recorded as NA.

Statistical analysis

Alternative and Null hypotheses and analyses used to test them are presented in Table 1. We sought to test whether zoos, at both global and regional levels, prioritise threatened and region-native turtle taxa in terms of species presence, institutional species holding numbers, population sizes and reproductive output. Analyses were conducted in R 4.2.0 (R Core Team, 2021), using the stats (R Core Team, 2021) and chisquare.posthoc.test packages (Ebert, 2019). Where appropriate, expected values were calculated under the null hypothesis of random distribution of counts. P values were corrected to account for false discovery rate, using the Benjamini-Hochberg method (Benjamini & Hochberg, 1995). P value simulation via Monte Carlo test with 2,000 iterations was used for chi-squared tests where expected values fell below 5 for at least one cell (Hope, 1968). Post-hoc tests, where appropriate, were conducted following the method of Beasley & Schumacker (1995) with Bonferroni corrections.

RESULTS

Full raw data are available at https://github.com/CJMichaels/Turtle-zoo-holdings.git. Reported p values are Benjamini-Hochberg adjusted p values corrected for false discovery rate (see Methods).

Phylogenetic representation

Cross-referencing of the Reptile Database, IUCN Red List and ZIMS yielded a total number of 357 Testudines species in 96 genera and 14 families, of which 248 species in 87 genera were present (captive population > 0) in zoos globally. All families of turtle were represented in zoos, and the family-level composition of the global captive population in terms of species numbers did not differ significantly from that of all recognised Testudines species (X^2 = 10.365, p = 0.6727; Fig. 1), although Geomydidae and Testudinidae were markedly over-represented, and Trionychidae, Kinosternidae and Pelomedusidae were markedly under-represented.

Geographic distribution of captive Testudines

Regional species holdings by region were North America (188), Europe (174), Asia (119), South America (45), Oceania (46) and Africa (32). Total populations by species

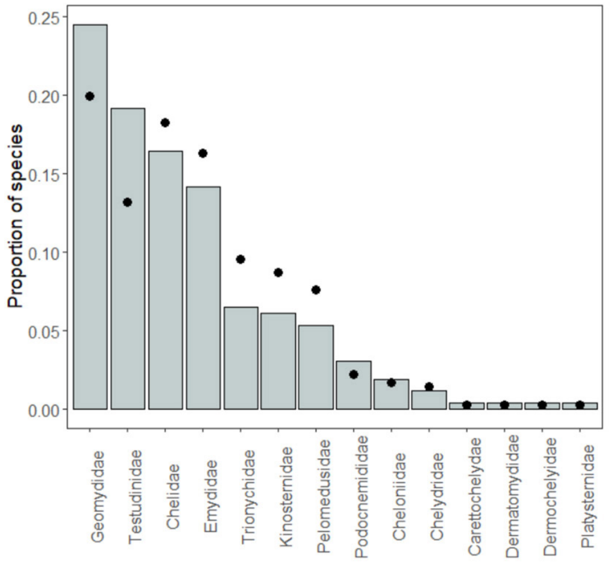

Figure 1. Proportions of species in global zoo holdings split by family (grey bars) against expected proportions derived from known diversity of turtles (black points)

Table 2. Post-hoc p values for comparisons of distributions of zoo holdings in each global zoo region of turtles native to each global zoo region. Significant p values are in bold.

Species Native Region	Zoo Region: Africa	Asia	Europe	N. America	Oceania	S. America
Africa	**0.000**	>0.999	>0.999	>0.999	>0.999	>0.999
Asia	0.071	**0.000**	>0.999	>0.999	>0.999	**0.002**
Europe	0.551	>0.999	>0.999	>0.999	>0.999	>0.999
N. America	>0.999	0.118	>0.999	**0.017**	>0.999	>0.999
Oceania	>0.999	>0.999	>0.999	>0.999	**<0.001**	>0.999
S. America	>0.999	0.872	>0.999	>0.999	>0.999	**<0.001**

ranged from 1 (fifteen species) to 7,390 (*Trachemys scripta*) with a median population size of 41 individuals and an interquartile range of 9–174.75 individuals. The number of species native to each zoo region was North America (101), Europe (15), Asia (107), South America (63), Oceania (49) and Africa (68).

Global proportions of holdings of species native to each region did not differ from the proportions of all species native to each region (X^2_5 = 3.4052, p = 0.6869, Fig. 2a). Species holdings by zoo regions were not proportionate to the number of institutions within each region. Zoo holdings of each region were not distributed proportionately to the number of zoos within them (X^2_5 = 201.93, p < 0.0001; Fig. 2b); Europe held significantly fewer species than proportionate to the number of institutions within the region (post-hoc p < 0.001), while South America, Africa and Asia (all post-hoc p values < 0.001) held significantly more; other regions were proportionately represented (all post-hoc p values > 0.05). At a regional level, South American (X^2_5 = 66.242, p = 0.0023), Asian (X^2_5 = 39.824, p = 0.00175), African (X^2_5 = 47.681, p = 0.0014), North

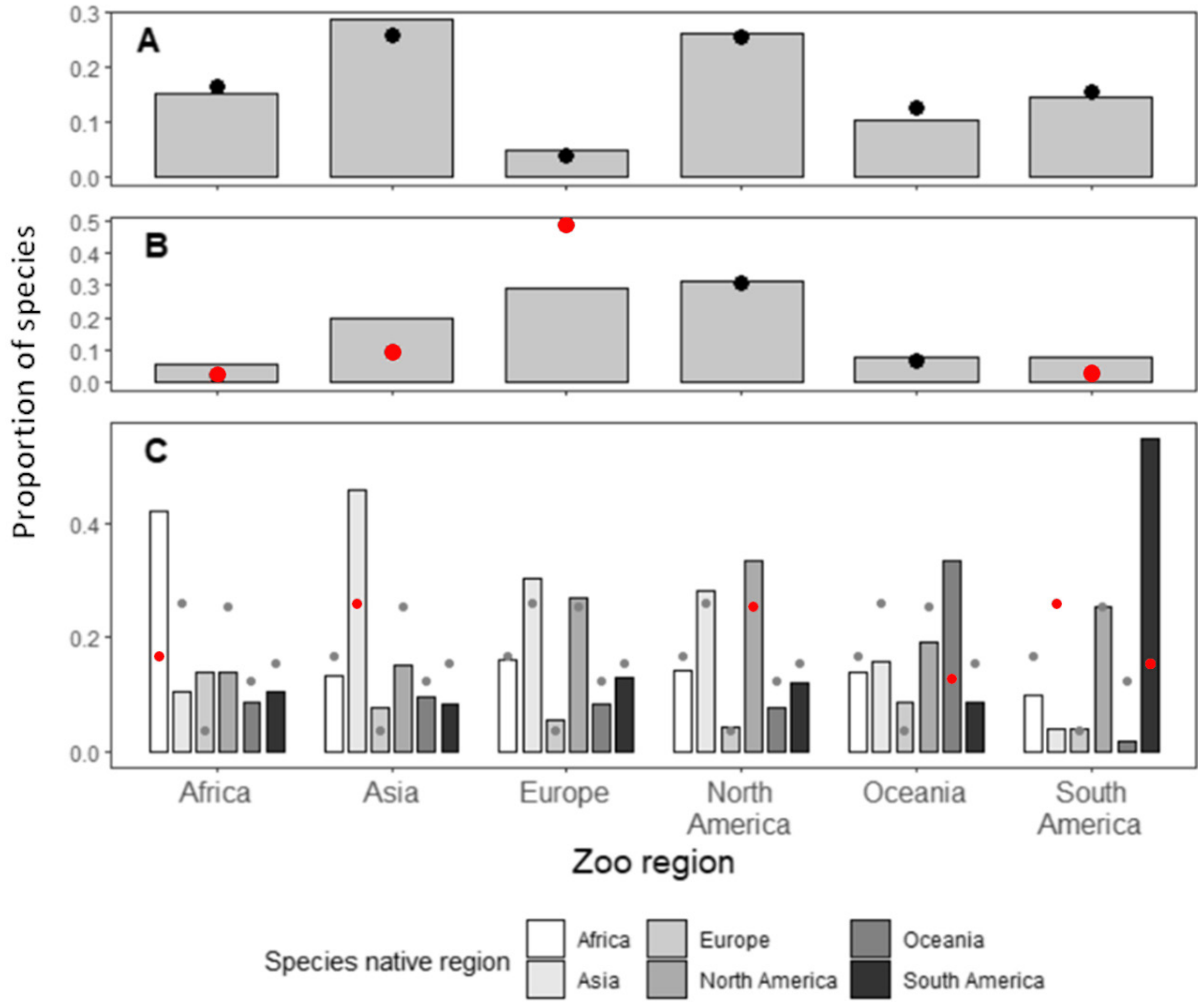

Figure 2. **A**. Proportions of globally zoo-held species split by species native region (grey bars) against expected numbers derived from distributions of total global turtle diversity (black points), which do not differ significantly. **B**. Proportionate species holdings by zoo region (grey bars) vs. expected numbers derived from total global species and numbers of ZIMS registered collections per region (points), which differ significantly (red points) such that Europe holds fewer species than predicted by numbers of institutions (post-hoc p < 0.001), while South America (post-hoc p = 0.003) and Asia (post-hoc p = 0.001) held significantly more. **C**. Proportionate zoo holdings of turtle species in each zoo region (x axis) split by species native range of held taxa (bars), against expected proportions (points) derived from global turtle diversity. Distributions of holdings differ from expected distributions for all regions other than Europe, with red points indicating those categories which differed significantly from expected. See Table 2 for post-hoc p values. The legend pertains only to panel C.

American (X^2_5 = 12.028, p = 0.0448) and Oceanian (X^2_5 = 28.501, p = 0.0017) zoo species holdings were primarily skewed towards native regional faunas (Fig. 2c), while European (X^2_5 = 7.0296, p = 0.3044) collections were not significantly different in composition to global proportions (see Table 2).

Threat status, species holdings and breeding success

Of the 248 turtle species represented in zoos, 125 were listed as 'threatened', 62 as 'not threatened' and 61 as 'Unknown'. Distribution across IUCN Red List categories was Not Assessed (57), Data Deficient (4), Least Concern (38), Near Threatened (24), Vulnerable (40), Endangered (35), Critically Endangered (50). Proportions of species in captivity in IUCN Red List Category did not differ significantly from proportions of all recognised species in Red List category (X^2 = 5.7872, p = 0.5133; Fig. 3a). In European collections specifically, which were subject to the Shellshock campaign, there was a significant difference (X^2 = 13.801, p = 0.03148), which was caused by lower-than-expected holdings of Not Assessed species (post-hoc p = 0.002438).

Comparing species numbers in each IUCN Red List category between species in and species not in zoos, distributions were not equal (X^2 = 54.42, p = 0.0007; Fig. 3b), such that the former were significantly more likely to be Least Concern (post-hoc p = 0.008) and less likely to be Not Assessed (NOA) (post-hoc p < 0.0001). Median (range) EDGE scores were 25.16 (6.54–149.70) for species in zoos, and 41.20 (9.63–52.63) for species not represented in zoos. Species in zoos had lower EDGE scores on average than species not represented in zoos (W = 12545, p = 0.0007).

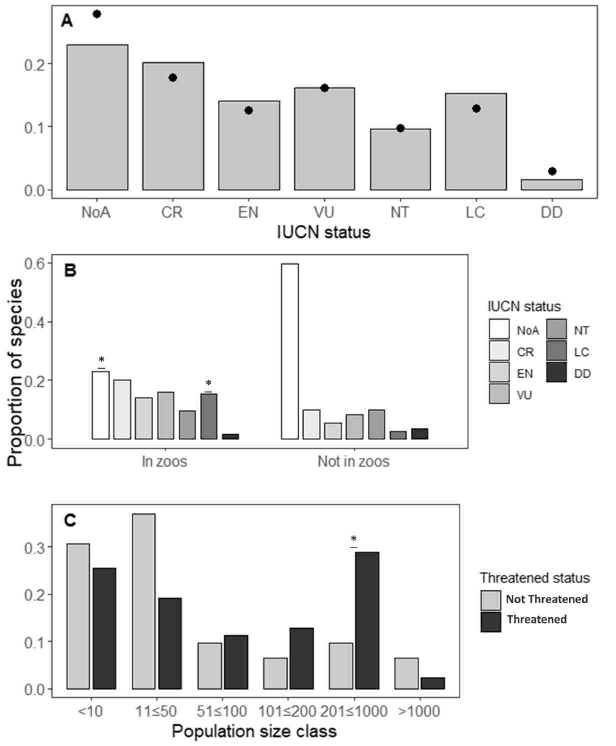

Figure 3. **A**. Proportions of global zoo turtle species holdings split by IUCN Red List threat category (bars) against expected proportions of species derived from all turtle Red List assessments (black points), which do not differ significantly. **B**. Proportions of species split by IUCN Red List threat category in zoo holdings (left) and not in zoos (right). Asterisks indicate categories where proportions of species in zoos differ significantly from their corresponding categories not in zoos. **C**. Proportions of species held in zoos globally split by populations size class and threatened/not threatened status. The asterisk indicates the category where there is a significant difference between threat categories.

Populations of threatened species were disproportionately more likely to comprise population sizes of 201–1000 individuals than were not threatened species, but there were no other differences in distributions between threatened and not threatened taxa (X^2 = 15.885, p = 0.001, post-hoc p value = 0.038; Fig. 3c). Three not threatened and six threatened species were represented by only a single animal in zoos globally, hence representing non-viable populations; these proportions did not differ significantly between threatened and not threatened groups (z = 0.0116, p = 0.99202); i.e. threatened species were no more or less likely to comprise non-viable breeding populations. All species with at least two individuals reported either at least one male and one female, or reported individuals of unknown sex, and are therefore here considered at least potentially viable in the loosest sense of being able to produce offspring. 48,091 individuals were held in ZIMS institutions, of which 10,231 were recorded as being male, 11,700 as female and 26,160 recorded as 'Other' (meaning unknown sex). Threatened (46 %) and not threatened (34 %) species did not significantly differ in whether breeding occurred in the last twelve months (X^2_1 = 1.89, p = 0.2644).

Shellshock campaign

Seventy-two genera were present in zoos in at least one time period. There was a significant increase in turtle populations by genus in European zoos between the ten years immediately preceding and the decade immediately following the Shellshock campaign (V_{71} = 1797.5.5, p = 0.01). The median (Q1, Q3) percentage change in population size by genus was 29.65(-17.74, 108.58) % with a range of -100 to 1350 % change. No genera were lost from zoos between time periods, and only one genus (*Dogania*) was gained with representation of a single animal.

DISCUSSION

Our analysis of the ZIMS database and subsequent analyses show that global zoo holdings of turtles proportionately represent family-level diversity and regional distribution, but that at a regional level institutions (with the exception of Europe) tend to bias turtle collections towards region-native faunas. Globally, species held did not differ proportionately in IUCN Red List threat status from listings of all turtle species. These data suggest that, at a global level, zoo collections represent a random cross-section through turtle species with no evidence for selectivity towards threatened species, regions of origin or particular taxonomic groups (although the Geomydidae and Testudinidae are somewhat over-represented, this is not significantly different from expected). Conversely, Dawson et al. (2016) found that North American, European and Oceanian threatened species of amphibian were proportionately better represented in zoos globally, partly as a result of the sheer numbers of threatened taxa found in other regions.

The tendency to bias collections towards native faunas may align with IUCN (McGowan et al., 2017) and the Convention of Biological Diversity (Glowka et al., 1994) guidance to focus ex-situ activities on local species. However, it is likely that this may be incidental, and the trend actually reflect local availability of species, especially given that a large proportion of turtle taxa are included on an appendix of CITES (CITES, 2022), which increases the complexities in the international move of animals. This may also be the reason for the absence of some genera from zoos; no capacity for, or focus on ex-situ turtle conservation may exist in range, with no logistic or legal ability to move animals outside of range. Collaboration with range states to build capacity in range could address this and provide an avenue to ex-situ conservation without requiring export of animals from range states. Questionnaire-based research might determine whether this is the case. These results align with those of Wahle et al. (2021) for Australian zoos, which typically held regionally native skink species, and for European zoos, which held cosmopolitan collections, but not for North American or Asian collections, which were more cosmopolitan for skinks than we found to be the case for turtles.

As well as assessing turtle holdings in zoos, we also performed analyses comparing species held in zoos with those not held in zoos. This approach can provide additional insight into the selection of species by zoos

globally, and the potential conservation value thereof. Species in zoos were more likely to be assessed as Least Concern and less likely to be Not Assessed than those not held in zoos, and had a lower average EDGE score. These findings reinforce the fact that zoos, at a global level, do not bias collections towards threatened or phylogenetically important turtle species. Indeed, the few genera with no species representation in zoos at all (*Cyclanorbis*, *Natator*, *Palea*, *Psammobates*, *Rafetus*, *Rheodytes*, *Rhinemys*, *Vijayachelys*) are mostly small or monotypic, threatened groups. The under-representation of Not Assessed taxa in zoos, compared with those not in zoos, is probably an artefact of lag between species description and IUCN Red List assessment. Tapley et al. (2018) showed that, for amphibians, recently described species are likely to remain unassessed for some time, and this is likely the same for turtles. Zoos are less likely to have access to recently described species due to the length of time involved in sustainably procuring species that are not currently in captivity already, and lack of IUCN Red List assessment may also de-prioritise these taxa in institutional and regional collection planning processes, as well as for funding organisations that may be necessary to initiate ex-situ projects. Additionally, in the case of taxonomic splits, ZIMS records may be slow to be updated accordingly. In amphibians, as a comparison, Biega et al. (2017) showed in a paired approach that there was no difference in threat status between amphibians kept in zoos and closely related species not held in zoos. This indicates a similar situation with regard to threatened species representation, but without bias towards non threatened taxa.

Despite holding one of the highest numbers of turtle species once regional institution numbers are accounted for, European collections hold fewer than expected turtle species, and collections in South America, Asia and Africa hold more than expected species. In their study on skink holdings in zoos, Wahle et al. (2021) also reported the lowest absolute species numbers in African and South American institutions, which they linked to available resources and infrastructure as well as historic circumstances, but do not present an analysis allowing for regional numbers of institutions. Similarly, Ziegler et al. (2016) and Jacken et al. (2020) showed that European and North American collections held large proportions of varanid lizards and amphibian species, respectively, which they attribute to greater resources and historical factors, but also did not correct for numbers of collections in these regions.

For skinks, varanid lizards, amphibians, and turtles alike (where substantial technical knowledge is required for successful husbandry), European zoos are in the unusual position of having a large expertise and resource capacity for maintaining the animals in question, but a relatively small (or in the case of varanids, a total absence of) native fauna on which to practice this expertise. This may contribute to the under-representation of turtles in European collections, but may also be linked to a number of other factors, including public preferences and relative costs of maintenance.

As well as holding fewer species of turtle than predicted by numbers of institutions, Europe specifically did not show bias towards more threatened species (only a tendency to under-represent Not Assessed species, likely for the same reasons as outlined above on the global scale), despite the Shellshock campaign of 2004–5. European collections may over-represent Asian species as a factor of the Shellshock campaign, which had a strong focus on the Asian turtle crisis, the international pet trade of the 1980s and 1990s – which created conditions of ready availability of many Asian turtle species in Europe – and the relatively small number (15 species) of native European species available to provide local focus.

A comparison of turtle populations in European zoos before and after the Shellshock campaign suggests that the campaign is associated with an increase in numbers of turtles held, but no meaningful increase in representation of genera. Data from ZIMS were from the European region, which imperfectly overlaps with EAZA members, as some EAZA members fall outside the ZIMS European region, and some European zoos are not EAZA members. Additionally, the identified link is circumstantial and further research, outside the scope of this study, would be needed to better understand the impact of the Shellshock campaign. If the Shellshock campaign did cause this population increase in zoos, then this would bolster its established success in raising funds for turtle conservation, although the lack of focus on threatened species may dilute the intended impact. The failure to increase diversity of holdings at the genus level may also limit the success of Shellshock in that aspects of phylogenetic diversity still lack captive populations in zoos. In-depth analysis of conservation impact would be needed to understand how the campaign addressed its ultimate goal of addressing the global turtle conservation crisis.

Within those species that are held in zoos globally, we did find evidence of a tendency to afford larger captive population sizes to threatened turtle species. These data align with those presented by Dawson et al. (2016) and Jacken et al. (2020), who showed that threatened amphibian species were afforded increasingly large captive population sizes in zoos. Although the few species that fall into the highest population size category of > 1000 are not threatened, these are represented by taxa that are very common within the pet trade. *Trachemys scripta* (> 7,000 individuals) and *Centrochelys sulcata* (> 2,000 individuals) represent the species with the two highest population sizes and both are commonly rescued or abandoned pet species, as are five others of the nine species in this population size category (Petrozzi et al., 2018; Valdez, 2021).These species are often housed in great numbers by zoos as part of responses to pet welfare and alien species crises. It is unclear if the tendency to afford threatened species with a higher captive population size is related to conservation breeding programmes, but several threatened species with large population sizes include substantial subpopulations registered in range-country institutions that are part of direct conservation breeding initiatives. For example, more than 90 % of ZIMS-registered *Astrochelys yniphora* (Critically Endangered) are housed in a breeding centre in Madagascar. Despite larger populations sizes for threatened species, we found no evidence of higher breeding success, with no difference in proportions of species bred in the last twelve months between threatened and not threatened species. Indeed, across both categories, only about half of all held species had been recently bred. It is possible that this reflects the long lifespan of turtles and finite holding capacity, which necessitates little recruitment in captive populations, but alternatively could reflect the difficulty of successfully breeding many turtle species in captivity.

When considering the trends described here, as well as those presented for other taxa such as skinks (Wahle et al., 2021), monitor lizards (Ziegler et al., 2016) and amphibians (Dawson et al., 2016; Biega et al., 2017; Biega & Martin, 2018; Jacken et al., 2020), it is important to critically appraise several assumptions. These are that a) holding a threatened species in zoos is reflective of conservation value, and b) that, conversely, holding not threatened taxa has lesser, nil or even negative conservation value. Put in another way, does it matter if zoos are not biasing collections towards threatened species? Zoos and similar captive institutions have played key roles in the conservation of a number of species globally (Robovský et al., 2020), and specifically for turtle species (Raghavan et al., 2015; Murphy, 2016a; 2016b). The Ark concept of keeping threatened species safe in captivity only brings long-term conservation value if such populations are eventually able to provide animals to return to the wild. This requires both successful husbandry (and reproduction in most cases) and the mitigation of threats in the field. The vast majority of threatened turtles in captivity are not part of formal or active conservation projects and, beyond safeguarding individuals in captivity, little direct conservation benefit is gained from holding them. For example, Vyas (2006) surveyed holdings of the threatened Indian star tortoise *Geochelone elegans* in Indian zoos and questioned the conservation impact of the sector in that the substantial captive population resulted in no reproduction and that, in many cases, holding institutions had little knowledge about their animals. From our dataset, this may still be the case, as from a population of nearly 750 animals globally, a third are unsexed, and from 260 females only 13 offspring were born in the last 12 months from a species capable of producing multiple clutches of up to ten eggs each annually (Vyas, 2005).

Beyond the Ark, zoo conservation contribution comes not only in the provision of animals for translocation, but includes research to inform on species biology, husbandry requirements and to test field techniques, and engagement with the public to raise awareness and funds. The latter remits are not dependent on holding threatened taxa as they can be conducted using representative not threatened taxa. Recently, there have been some encouraging developments aligned with the one plan approach to conservation (Byers et al., 2013) with the aim of greater collaboration between zoos, aquariums and range state counterparts to further both direct and indirect conservation roles for threatened turtles (e.g. Goetz et al., 2019). However, the approach of looking at zoos as a collective entity, either at global and regional levels, implicitly assumes aligned goals and working practices causing institutions to collaborate – for example, treating captive turtles of a given species as a metapopulation. In reality, varying standards of care, institutional goals, resources and collection plans, and international -, institutional- and individual- level politics prevent this from being a reality and, indeed, zoos may actually act more as competitors than collaborators (Maynard et al., 2020). All this means that species holding data are not a perfect proxy for the conservation value of collections, and the data presented here should be considered just one dimension in estimating the impact of zoos on turtle conservation.

ZIMS is the best available database to understand global and regional trends in holdings of captive turtles, as well as other exotic taxa. However, limitations exist in terms of its comprehensiveness and accuracy. Not all institutions maintaining living collections are registered on ZIMS; among other reasons, the subscription is not free and many institutions may be unable or unwilling to provide the necessary funds. For turtles specifically, there are notable exceptions – for example, the Turtle Conservation Centre in Cuc Phuong National Park is a singularly important turtle collection for conservation in Vietnam, involved in ongoing conservation projects for threatened turtles (e.g. Hoang et al., 2021), but is not registered on ZIMS. Similarly, the Charles Darwin Research Station, which has undertaken decades of captive breeding and conservation work to restore Galapagos Giant Tortoises, is also absent from ZIMS. Moreover, for collections that are registered on ZIMS, taxonomic confusion, difficulty in identification of individuals and incomplete or outdated records are a virtual certainty, although impossible to detect without a fine scale survey of individual zoos at a global level. ZIMS, although rich in information content, is also difficult to extract data from and there are limited options to filter results. This makes finer scale investigations of holding trends logistically impossible. These factors must be taken into consideration while interpreting the data presented here. However, despite these limitations, the sheer scale of the ZIMS database provides an insight into global turtle holdings that would otherwise be impossible to gain.

We suggest the following set of actions that could be adopted by the zoo community in order to have a greater impact from their turtle holdings:

i) conservation prioritisation exercises such as regional collection plans are developed for all turtle species in the near future, so that institutions have more direction with regard to which species they should hold;
ii) holding institutions or regional taxonomic advisory groups should phase out the species with non-viable populations or work with other institutions or regional associations to acquire additional stock to make populations viable;
iii) support for non ZIMS member organisations that maintain turtles for conservation purposes, particularly in low to middle income countries, should be offered, so that the conservation community has a more detailed overview of global turtle holdings;

iv) international collaboration both between zoos and between in-situ conservation organisations to align collection plans with conservation needs and to maximise conservation impact.

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Living on the EDGE: From the evolutionary uniqueness to the conservation status of the Colombian elapids and viperids

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Phylogenetics applied to conservation provides a comprehensive and alternative approach that contributes to prioritising species and areas for conservation, even if the species have significant information gaps concerning their ecology. Using a distribution of 10,000 phylogenetic trees of the 30 elapid and 21 viperid snakes in Colombia, we calculated the species evolutionary distinctiveness (ED) scores. Then, based on the ED median values reported from previously fully-sampled phylogenies of squamates, we quantified evolutionarily distinct and globally endangered (EDGE) scores and, with updated distribution maps of the species, we computed and plotted biogeographically weighted evolutionary distinctiveness (BED) scores. Among threatened species, *Bothrocophias campbelli* reached the highest ED score. This species, together with *Micrurus medemi*, are the top EDGE species, and with *Micrurus renjifoi* achieved the highest BED scores. The spatial patterns of richness and BED values highlight the Andean, Amazonian and Pacific regions as biodiversity hotspots. Although some areas are under some protection status, anthropic pressures, such as deforestation, along with the lack of knowledge about these snakes, exhibit a worrisome panorama. Thus, it is imperative to implement conservation measures focused on areas where species with both ecological and evolutionary value are concentrated.

Keywords: Coral snakes, Elapidae, evolutionary distinctiveness, global endangerment, phylogenetic diversity, Viperidae, vipers

INTRODUCTION

Diverse strategies have been developed to adequately allocate limited resources for conservation and reduce the impact of global biodiversity loss (Possingham & Wilson, 2005). The main conservation strategies have focused on prioritising species based on their degree of endemism and threat status, as well as on species that fall within the concept of charismatic, flagship, indicator, keystone, rare and umbrella, among others (Joseph et al., 2009; Li & Pimm, 2016; IUCN, 2022). However, these approaches may leave out important aspects, like the evolutionary history of species (Mace et al., 2003; Pellens & Grandcolas, 2016), leading to gaps concerning the protection of ecological, genetic and morphological diversity (Mace & Purvis, 2008; Magnuson et al., 2010; Collen et al., 2011). Therefore, it is necessary to implement methodological perspectives conducting more effective conservation, taking into account aspects such as phylogenetic diversity (Redding et al., 2010; Safi et al., 2013; Winter et al., 2013; Redding & Moers, 2015; Mazel et al., 2018). In this sense, the 'EDGE of Existence programme' is an initiative focused on the conservation of species that represent a high level of phylogenetic uniqueness or evolutionary distinctiveness (ED) that measures the contribution of each species to the phylogenetic diversity (PD) of a given clade (sensu Faith, 1992), and that are also threatened and globally endangered (GE) (Isaac et al., 2007).

Phylogenetic metrics applied to conservation are a particularly helpful approach when applied to clades with significant information gaps, like reptiles (Fenker et al., 2014; Forest et al., 2015; Pellens & Grandcolas, 2016; Tingley et al., 2016). These vertebrates are being explored with the ED and EDGE scores at several geographical scales (Boland & Burwell, 2020; Colston et al., 2020;

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Chan & Grismer, 2021). Because many conservation actions are implemented at local or regional scale, and threats and vulnerability of species tends to vary from one country to another (Moilanen & Arponen, 2011; Dallimer & Strange, 2015), it is necessary to integrate conservation and evolutionary history in the context of political administrative boundaries. Analyses at this scale can contribute to overcoming the lack of knowledge that often restricts the scope of large-scale conservation actions. Assessing how much of the evolutionary history of a clade is represented by the species subset found in each area, such as a country, could be a useful tool to lead a comprehensive prioritisation of species and areas for conservation.

In recent years, indexes have emerged that combine aspects of the evolutionary history and the distribution pattern of species (Murali et al., 2021; Gumbs et al., 2020; Jetz et al., 2014). One of these indexes is the biogeographically weighted evolutionary distinctiveness (BED) index (Cadotte & Davies, 2010) which focuses on the evolutionary uniqueness and the extent of a species' distribution range within a given area. The distribution of a species may cover a significantly large proportion of one region and may cover a tiny portion of another one; the BED allows a detailed assessment of the spatial patterns of phylogenetic diversity, given that it allows a higher value to be assigned to species that are evolutionarily distinctive and spatially restricted. However, the calculation of biogeographically BED has been scarcely applied in vertebrates, including reptiles (Quan et al., 2018), and still no studies have focused on any country in the neotropics, one of the most diverse regions in the planet (Rosenzweig, 1995).

Among reptiles, snakes have been exhibiting dramatic declines in their population size because of anthropogenic activities, mainly deforestation for agriculture and cattle-raising (Reading et al., 2010; Morales-Betancourt et al., 2015). Many snakes are wrongly considered to have a low aesthetic value and are the source of a widespread phobia and innumerable myths, which reduces willingness to protect them (Prokop & Randler, 2018). Despite this, snakes are particularly important for human well-being, in as much as they act as biological control agents of species that otherwise could be potential pests and reservoirs of infectious diseases (Cortés-Gomez et al., 2015; Valencia-Aguilar et al., 2013; Beaupre & Douglas, 2011). Further, venomous snakes are also a valuable resource for medicine, to the extent that some active substances of their toxins are used in the development of drugs for the treatment of several diseases (Galvis, 2007). This is the particular case of venomous snakes of the Elapidae (coral snakes, mambas) and Viperidae (rattlesnakes, vipers) families (McDiarmid, 2012; Vitt & Caldwell, 2014).

About 8 % (272 spp.) of the world's snake species are found in Colombia, where they are threatened by deforestation, expansion of the agricultural frontier, mining, leaf litter removal and human aversion (Campbell & Lamar, 2004; Morales-Betancourt et al., 2015); 19 % (53 spp.) of these species belong to Elapidae and Viperidae families. Elapids comprise 30 species of fossorial snakes that tend to exhibit low population densities. They are distributed from sea level, as is the case of *Hydrophis platurus* that inhabits the Pacific Ocean, to the Andean ecosystems at 2,000 meters of elevation (Lynch, 2012; Lynch et al., 2014). Viperids are represented in Colombia by about 23 species ranging from sea level to 2,600 meters of elevation (Angarita-Sierra et al., 2022). They are restricted to humid forests with little intervention, although there are species, such as *Bothrops asper* and *Bothrops atrox*, that quickly adapt to disturbed habitats and transformed landscapes (Lynch et al., 2014).

Regrettably, the study of snakes in Colombia has not been a priority (Lynch, 2012) and few initiatives have been undertaken to protect them (Lynch et al., 2014). In the case of elapids and viperids, none of the species have population studies and only 6 % of them are ranked under some degree of local threat (Morales-Betancourt et al., 2015). Studies of these snake groups focus on the description of new species, diet, distribution and snake bite cases (Silva, 1989; Renjifo & Lundberg, 2003; Castro et al., 2005; Ayerbe & López, 2005; Folleco Fernández, 2010; Pitalua-L et al., 2018; Pérez et al., 2019; Peláez & Perlaza, 2020; Pereañez et al., 2020; Cañas et al., 2021; Angarita-Sierra et al., 2022), but compared to other animal groups, the information available about them is scarce and isolated.

Despite the remarkable diversity of Colombian elapids and viperids and their socio-ecological importance, the widespread lack of knowledge about aspects of their natural history negatively affects our ability to prioritise and protect them. To shed light on the knowledge gaps and contribute to the conservation of elapids and viperids in Colombia, our aims in this study were:

i) to review the conservation status of Colombian elapids and viperids;
ii) to rank the species according to the ED, EDGE, and BED scores;
iii) to identify the distribution patterns of the richness and the BED scores of these snakes in the country.

We hope to offer baseline information to identify snake species and lineages, and geographic areas in Colombia to be prioritised in future conservation and management plans.

METHODS

Species lists

We compiled an updated list of elapid and viperid species occurring in Colombia from bibliographic sources (Ayerbe & López, 2005; Folleco Fernández, 2010; Lynch et al., 2014; Morales-Betancourt et al., 2015; Ospina-L, 2017; Díaz-Ricaurte et al., 2017; 2018; Quiñones-Betancourt et al., 2018; Pitalua-L et al., 2018; Díaz-Ricaurte & Ferreto Fiorillo, 2019a; 2019b; Angarita-Sierra et al., 2022).

ED and EDGE scores

For a given community, the sum of the ED of species, measured by "fair proportions" (Isaac , 2007), will equal phylogenetic diversity (Faith, 1992). Thus, this study considers both the contribution of each species of elapids and viperids to the local community's phylogenetic diversity (i.e. to the subset of species occurring in Colombia; hereinafter ED_{local}), and the contribution of each species to the global phylogenetic diversity (i.e. to the overall squamate clade; hereinafter ED_{global}). Because most species do not have a threat category for Colombia, we do not have a national equivalent to the GE that can be weighted with the ED of the species at the local level. Therefore, EDGE scores were calculated with the ED_{global} and GE, following International Union of Conservation of Nature (IUCN) categories.

For the ED_{global} scores approach, we used median ED values of target species calculated by Tonini et al. (2016) over fully sampled phylogenies of Squamata. For the ED_{local} scores approach, we used the median ED values of target species calculated over 10,000 phylogenetic trees pruned to just the Colombia species from the distribution provided by Tonini et al. (2016) and available at VertLife project (http://vertlife.org). For this, we calculated the ED scores with the evol.distinct function implemented in the picante R package (Kembel et al., 2010). Then, we calculated their respective median value with an R base function. The median and variance of these ED scores were visualised as a boxplot chart made in the ggplot2 R package. It should be noted that the subsequent analyses were performed on the basis of the ED_{global} scores.

In order to get EDGE scores, we compiled the species conservation status from the IUCN Red List of Threatened Species (www.iucnredlist.org) as a measure of Global Endangerment (GE), and assigned numerical scores to these categories in the following manner: Least Concern (LC) = 0, Near Threatened (NT) and Conservation Dependent (LR/cd) = 1, Vulnerable (VU) = 2, Endangered (EN) = 3 and Critically Endangered (CR) = 4. Thereafter, we compiled and applied the formula EDGE= ln(1+ED)+GE*ln(2) in a custom R function edge.species (Supplementary material 1) to obtain the EDGE score of each species. For the above, we followed both the numerical equivalent of the IUCN Red List categories, as well as the EDGE formula proposed by Isaac et al. (2007).

Distribution maps and BED scores

We used the distribution map polygons for 47 of the 51 species (94 %) from Roll et al. (2017) available as supplementary material in the Data Dryad repository (https://datadryad.org/stash/dataset/doi:10.5061/dryad.83s7k). For the remaining four species (*Hydrophis platurus*, *Micrurus multifasciatus*, *Bothrocophias campbelli* and *Bothrops venezuelensis*), we used the distribution map polygons from the IUCN Red List of Threatened Species database (https://www.iucnredlist.org/). All polygons were cut to define the distribution area of the species in Colombia.

To quantify the proportion of the Colombian territory that overlaps with the distribution of each elapid and viperid species, we set a map of Colombian geopolitical land boundaries with a 1 x 1 degree grid (totaling 129 cells covered by the Colombian land territory). Then, we counted the number of cells occupied by the distribution of each species. For the marine species *H. platurus* we set up a map of Colombian geopolitical maritime boundaries grid with the same parameters (totaling 95 cells covered by the Colombian maritime territory); thereafter, we performed the same procedure as mentioned above. Subsequently, to calculate the BED scores of each species, we used the formula proposed by Cadotte & Davies (2010), where the ED score of each species was divided by the proportion of Colombian territory the species occupies.

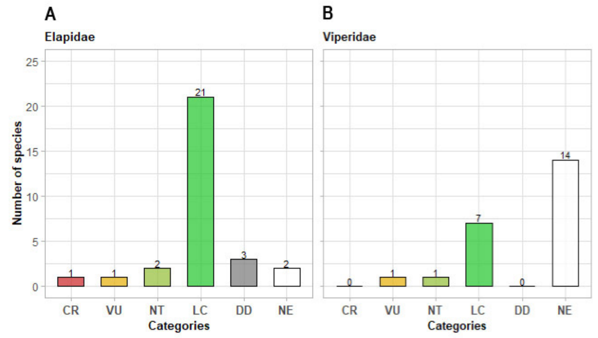

Figure 1. Number of Colombian elapid and viperid species in each category of the IUCN Red List

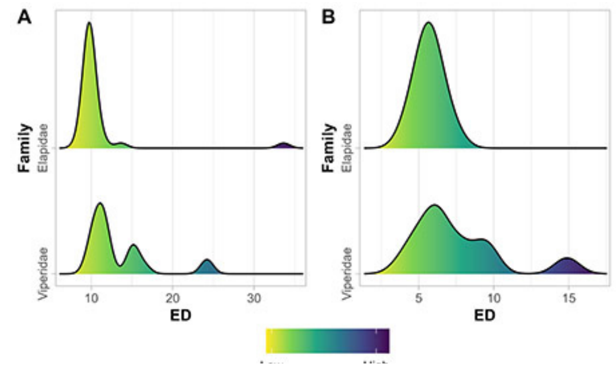

Figure 2. Distribution of **(A)** the median ED_{local} scores and **(B)** ED_{global} scores of Colombian elapids and viperids

Species richness and BED score maps

We elaborated maps of species richness and BED scores for elapids and viperids using QGIS 3.28 (QGIS.org, 2022). We rasterised values of species richness and BED scores for each species in the target groups. We used the r.series function to summarise the values. Then, we performed a neighbourhood analysis (r.neighboor function) with a window size of 10 km and a sigma value for a gaussian filter of 2 to avoid a discrete border effect (Jackisch, 2007).

RESULTS

Species lists

In Colombia, the family Elapidae is represented by 29 species of the genus *Micrurus* and one species of *Hydrophis*, while Viperidae is represented by ten species of the genus *Bothrops*, seven of *Bothrocophias*, two of *Porthidium*, two of *Lachesis*, one of *Bothriechis* and one of *Crotalus*. 64.2 % of elapids and viperids in Colombia have been categorised by the IUCN in some extinction

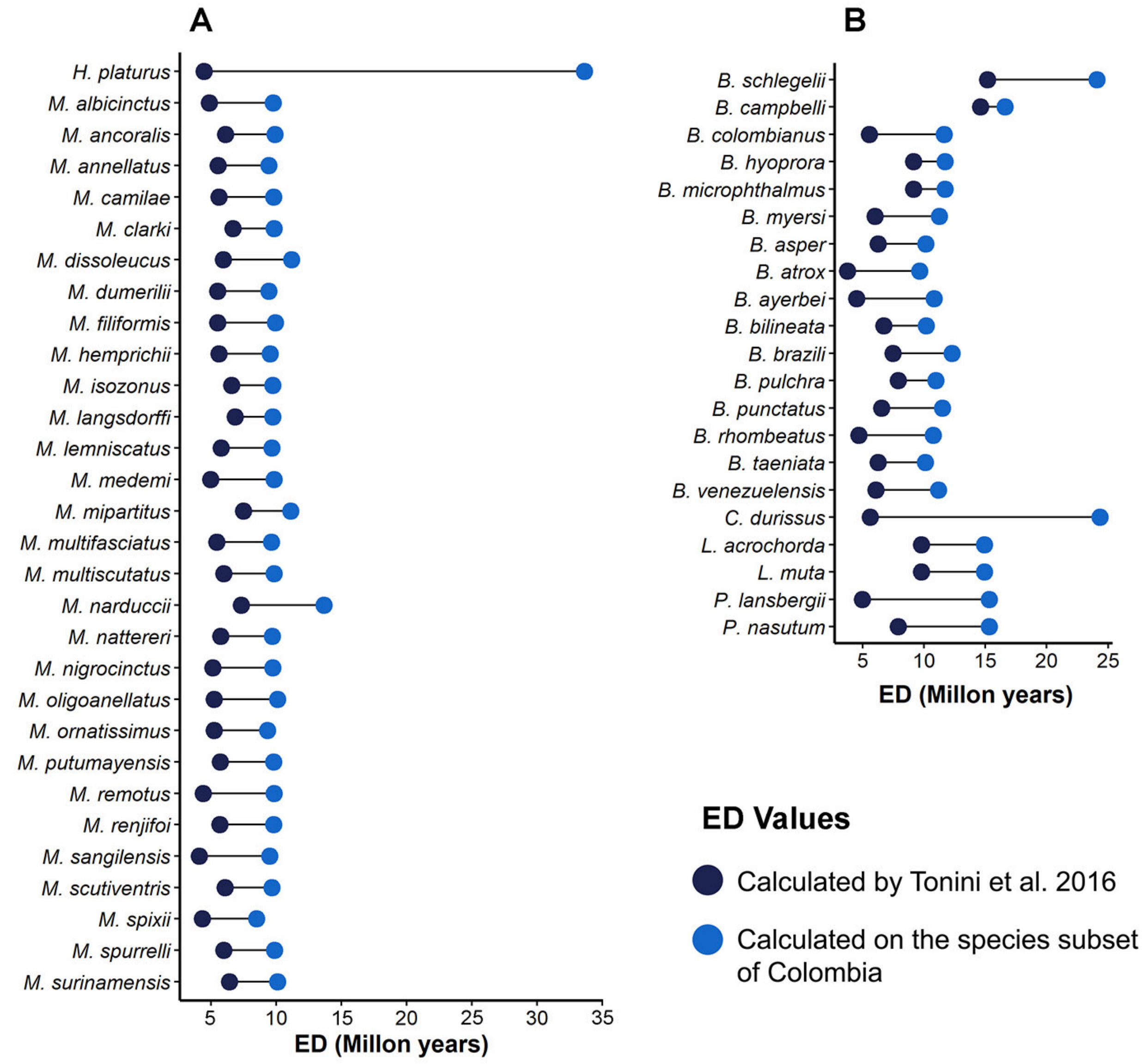

Figure 3. Comparison of ED_{local} scores and ED_{global} scores of Colombian elapids **(A)** and viperids **(B)**

risk category; 52.8 % of the species are classified as Least Concern (LC), 3.8 % as Vulnerable (VU), 5.7 % as Near Threatened (NT), and one species (*Micrurus medemi*) as Critically Endangered (CR). In all, 35.8 % of species (9.4 % of elapids and 26.4 % of viperids) have not been evaluated (NE) or are Data Deficient (DD) (Fig. 1), so we did not include them in the quantification of EDGE scores.

ED and EDGE scores

Regarding elapids, the mean ED_{local} score was 10.72 ma (Fig. 2a), with *H. platurus* (ED_{local} = 33.62 ma) reaching the highest ED_{local} scores (Fig. 3a and Fig. S1a). Whereas, accounting for the ED_{global} scores, the mean ED_{global} value was 5.68 ma (Fig. 2b), and the species with the highest ED_{global} scores were *Micrurus mipartitus* (ED_{global} = 7.48 ma) and *Micrurus narduccii* (ED_{global} = 7.32 ma) (Fig. 3a). According to the IUCN, the species with the highest risk of extinction are *M. medemi* (CR) and *Micrurus sangilensis* (VU); consequently, they exhibit the highest EDGE scores among elapids (EDGE = 4.56 and 3.01 respectively) (Fig. 4a).

For viperids, the mean ED_{local} score value was 13.30 (Fig. 2a). The species *Crotalus durissus* (ED_{local} = 24.33 ma) and *Bothriechis schlegelii* (ED_{local} = 24.09 ma) (Fig. 3b and Fig. S1b) reached the highest scores. Regarding the ED_{global} scores, the mean ED value was 7.51 ma (Fig. 2b); the highest scores were for *B. schlegelii* (ED_{global} = 15.16 ma) and *B. campbelli* (ED_{global} = 14.60 ma) (Fig. 3b). When linking the ED scores of species with their extinction risk, *B. campbelli* (VU) and *B. schlegelii* (LC) show the highest EDGE scores among the viperids (EDGE = 4.13 and 2.78 respectively) (Fig. 4b).

When looking at the species classified into threat categories, the viperid *B. campbelli* (VU) obtained the highest scores (ED_{global} = 14.60; ED_{local} = 16.59). While the elapids *M. medemi* (CR) (ED_{global} = 4.97; ED_{local} = 9.81) and *M. sangilensis* (VU) (ED_{global} = 4.09; ED_{local} = 9.5) obtained lower scores, but very similar to each other. Notably, *B. campbelli* (VU) together with *Bothrocophias myersi* (NT) are the only viperid species evaluated that are not at low risk (LC) (Supplementary material 2).

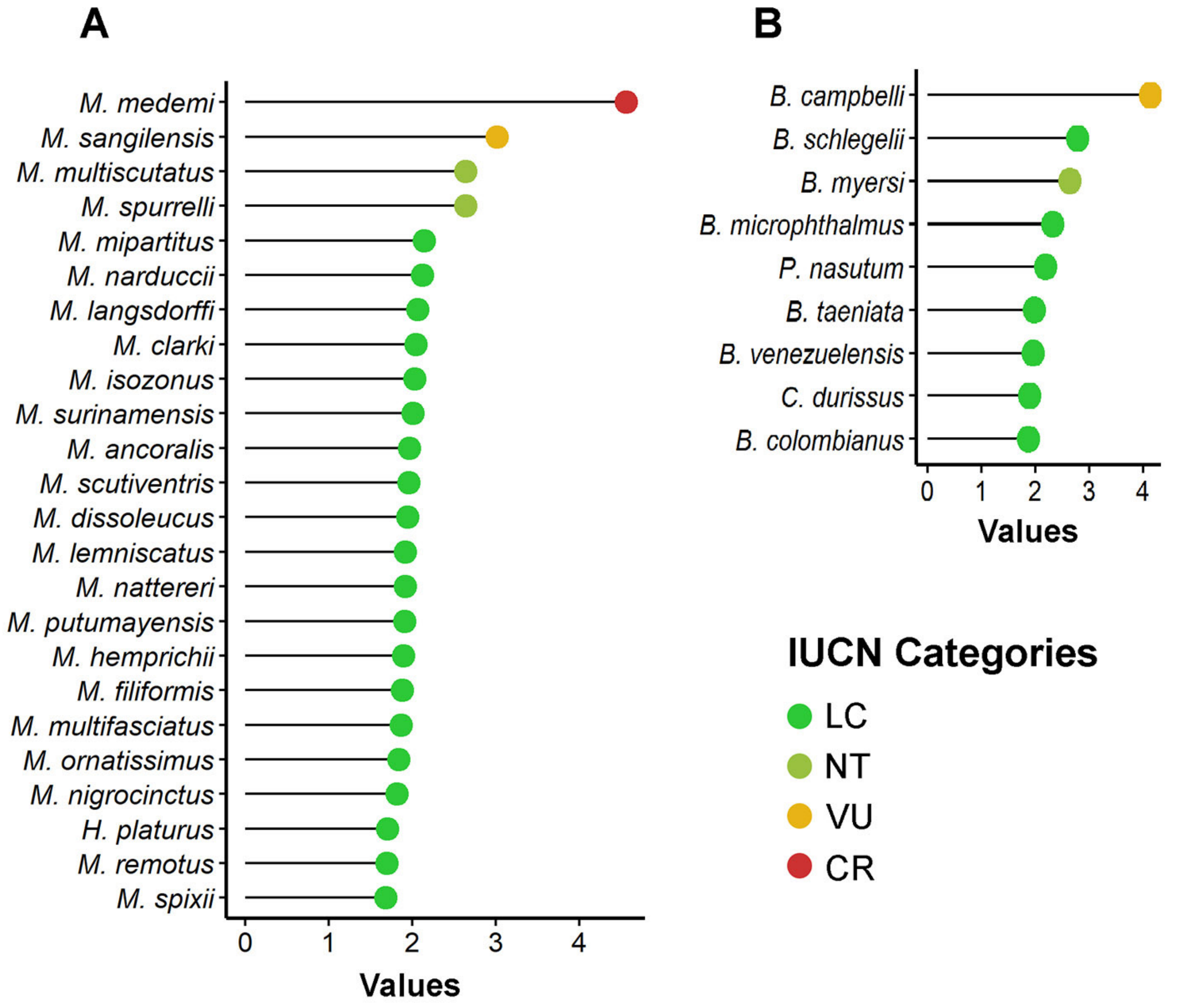

Figure 4. EDGE scores of Colombian elapids **(A)** and viperids **(B)**

Distribution maps and BED scores

Among the elapids, *Micrurus lemniscatus* was the species present in the largest number of cells, 88 out of 129 (BED = 0.065), while *Micrurus renjifoi* and *M. multifasciatus* were the most restricted, each occurring in only one cell, and those with the highest BED scores (5.688 and 5.446 respectively) (Fig. S2a). In viperids, *B. atrox* was the species present in the largest number of cells, 119 out of 129 (BED = 0.031), whereas the species *Bothrops pulchra* was present in the lowest number of cells, only 2 of 129, achieving the highest BED score (3.936) (Fig. S2b) (Supplementary material 2).

Species richness and BED score maps

Spatial patterns of species richness and BED scores showed considerable variation within and between Elapidae and Viperidae. The Amazon was the region where species richness was commonly high for both families; however, viperids were more represented than elapids in the Andean, Caribbean and Pacific regions. Regarding the pattern of BED scores, there are several specific areas within Colombian regions that exhibit the highest values; for elapids, the highest BED values were in some portions of the Caribbean region including the area of the Darién strains towards the Caribbean Sea, the Pacific region and the Amazon region. In contrast, the highest BED scores for viperids are found in the south and middle part of the Pacific region, and in the north-eastern Andes, on the border with Venezuela. A more detailed description of localities and areas where highest species richness and BED scores were found is provided in Supplementary material 3.

DISCUSSION

A widespread lack of knowledge exists about the conservation status of elapids and viperids in Colombia, which is reflected by five species of elapids and 12 species of viperids without adequate data (DD and NE) (Fig. 1). Among them, species like *Micrurus camilae*, *Micrurus oligoanellatus* and *M. renjifoi* were described over 15 years ago but have been poorly studied (in DD according to IUCN); therefore, their extinction risk is unknown and likely remains underestimated (Lamar, 2003; Renjifo & Lundberg, 2003; Ayerbe & López, 2005; Peláez & Perlaza-Berrío, 2020). This is concerning given that reptiles

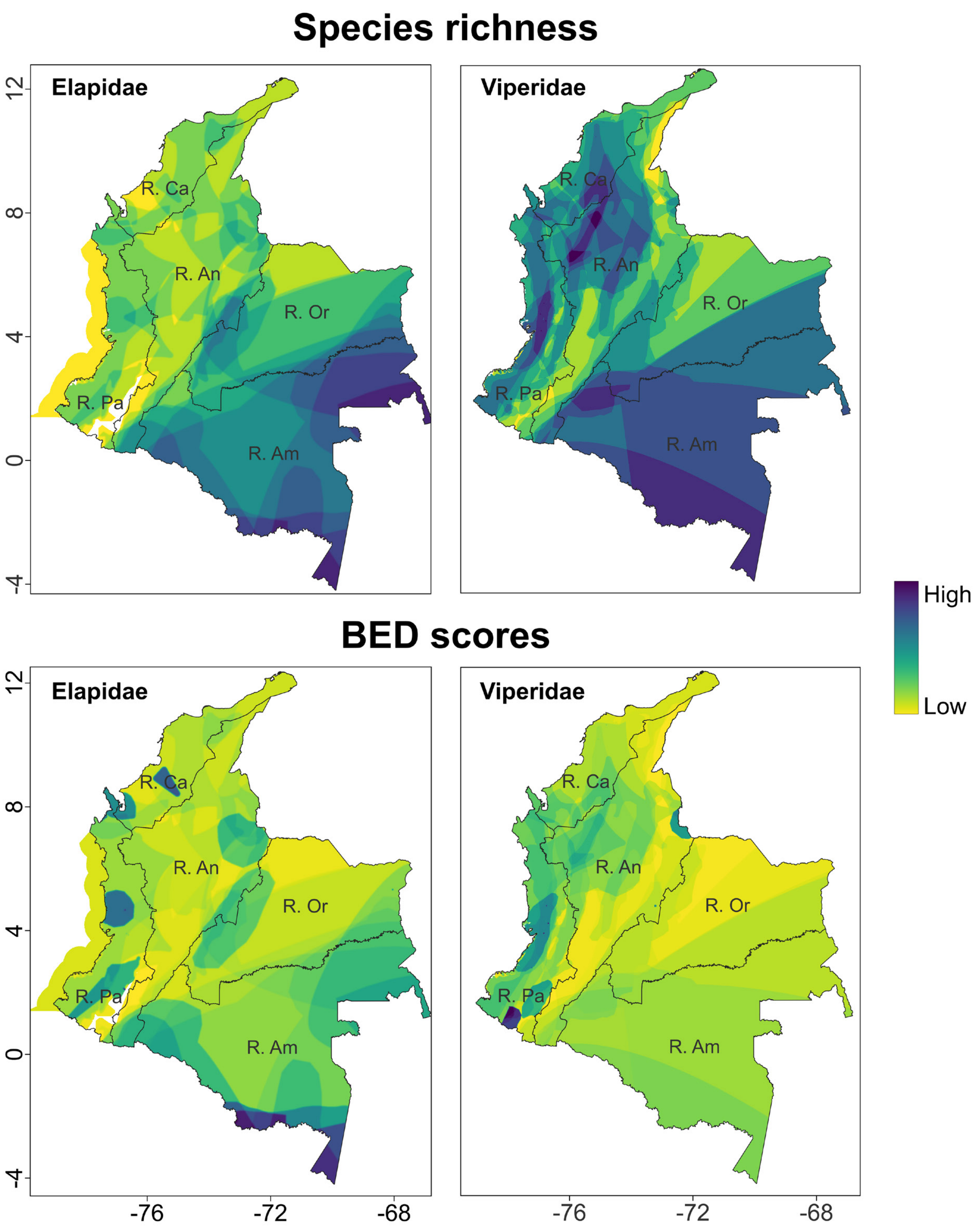

Figure 5. Spatial patterns of species richness (upper maps) and BED scores (bottom maps) of Colombian elapids (left maps) and viperids (right maps). The map shows the boundaries of the main regions of Colombia as follows: R. Am: Amazonian region; R. An; Andean region, R. Ca: Caribbean region; R. Or: Orinoco region; and R. Pa: Pacific region. Note that maps for elapids include a sea species (*H. platurus*) in the Pacific Ocean.

comprise about 30 % of vertebrate species on the planet (Böhm et al., 2013; Bland & Böhm, 2016; Maritz et al., 2016; Tingley et al., 2016), and in the last decades global average declines in reptile populations of more than 50 % have been estimated using approaches such as the Living Planet Index (LPI) (Saha et al., 2018). Other neotropical countries also have made efforts to locally assess the conservation status of their elapids and viperids. In Ecuador, for example, a relatively large number of species have been locally categorised, including *Lachesis acrochorda* and *Lachesis muta* as VU; *B. pulchra* and *Bothrops punctatus* as NT; *Bothrocophias hyoprora*, *B. asper*, *B. atrox*, *Bothrops bilineata* and *Bothrops brazilias* as LC; and *Micrurus annellatus* as DD (Carrillo et al., 2005).

According to these authors, most of the species under LC category are distributed in the Ecuadorian Amazon. However, at the time they were evaluated, this region was not so fragmented, a situation that has undoubtedly changed in recent years (Castro, 2022; Campos & de Melo Faria, 2022). In Brazil, some categorisation schemes have classified the species *B. bilineatus* and *L. muta* as VU. However, such a large country has both national and state classifications, making it possible for a species to be in more than one category. For example, in the state of Rio de Janeiro *L. muta* is endangered (EN) while *B. bilineatus* is probably extinct (Decreto 1499-R/2005; Decreto 51.797/2014; PORTARIA 37/2017; Resolução SEMAS 1/2017; SEMA, 2018).

The ED_{local} scores distribution of Elapidae and Viperidae seems to have their most distinctive species with typically high values (Fig. 3); Elapidae: *H. platurus* (ED = 33.62), Viperidae: *C. durissus* (ED = 24.33) and *B. schlegelii* (ED = 24.09). Although *Hydrophis* is one of the clades with the highest rates of diversification within Elapidae (Lee et al., 2016), *H. platurus* reached the highest value of local ED due to it being the only species of the genus distributed in Colombia. Moreover, *Hydrophis* diverged >30 Mya from *Micrurus*, the genus that includes all other Colombian elapid species (Tonini et al., 2016). Regarding viperids, *B. schlegelii* is the only species of a clade of Central American origin with a distribution that comprises areas in north-western South America (Fenker et al., 2014; Mason et al., 2019). Similarly, *C. durissus* is the only species of a well-diversified clade that arrived in South America via the Panama isthmus and achieved widespread disjunct distribution (Wüster et al., 2002; Alencar et al., 2016). Both species belong to clades that diverged >20 Mya from the remaining Colombian viperid species (Tonini et al., 2016) (Fig. 3b). When analysed within a geographical delimitation, distinctive species constitute rare or unique samples of lineages that, even if highly diversified or widely distributed, display reduced richness and have few or no related taxa in a given area (e.g. country).

As expected, higher EDGE scores correspond to species included in threatened categories. This is the case of the Critically Endangered *M. medemi* and the Vulnerable species *B. campbelli* and *M. sangilensis* (Fig. 4). At a global scale, the EDGE species list comprises 607 species of reptiles, including two elapids: *Bungarus slowinskii* (EN) distributed in south-east Asia, *Hemiaspis damelii* (VU) distributed in Australia, and four viperids: *Protobothrops mangshanensis* (EN) distributed in China, *Mixcoatlus barbouri* (EN), *Mixcoatlus melanurus* (EN) and *Ophryacus undulatus* (VU) distributed in Mexico. It should be noted that none of the South American species of Elapidae and Viperidae are included in this global EDGE list. In this sense, it is essential to take into account the spatial scale in which the prioritisations are made, to not underestimate the risk of extinction of species that may not be considered threatened or have a low risk of extinction at the global level but may be the most critical at the local level. For this reason, it is essential to update local red lists, such as Morales-Betancourt et al. (2015) that, despite being less than a decade old, has a very limited species coverage, resulting in large gaps in information on groups like viperids and elapids.

In Colombia, some areas with a high diversity of elapids and viperids overlap with protected areas. In the case of Elapidae, the areas within the Amazon region where the greatest number of species is concentrated includes the Amacayacu National Natural Park and the Puinawai National Natural Reserve. The Amacayacu National Natural Park also would play an important conservation role from the BED perspective, as well as Los Katios and Munchique National Natural Park in the Pacific region. However, there are other areas without any special protection, such as those in the south-central Amazon, and the Caribbean and Pacific Regions. With respect to Viperidae, the areas identified as richness hotspots include the Natural Parks of Amacayacu, Río Puré and Cahuinarí in the Amazon region, and the Farallones National Park in the Pacific region. Meanwhile, the areas with the highest BED values for Viperidae suggest that the Farallones National Park is also highly important, as well as the Natural Parks Doña Juana-Cascabel Volcanic Complex (between the Andean and Pacific regions) and Tamá (Andean region), the Galeras Flora and Fauna Sanctuary in the Andean region. There are areas of equal importance that have no protection status, such as south of the Amazonian region, eastern Orinoco region, central west and south of the Pacific region, central Antioquia, and south-central Pasto (Andean Region) (Fig. 5c). Here, it can be noted that the most important protected areas for the conservation of elapids and viperids are the National Natural Parks of Amacayácu and Farallones respectively. Therefore, these Natural Parks and their areas of influence should be recognised as priority areas to implement strategies that involve research projects and work with local communities to increase the protection of those vertebrates (Caten et al., 2020).

Metrics such as ED, EDGE, and BED, which consider the evolutionary history and geographic patterns of species, can be useful tools to identify areas, in a local or regional context, as priority for conservation. This approach can harbour species that are both distinct and rare and are not considered in traditional diversity analyses based on species richness (Polasky et al., 2001; Forest et al., 2015; Tucker et al., 2012). For example, the present study identified areas, such as north-eastern Colombia, on the border with Venezuela, and the centre of the Pacific region harbouring elapid and viperid species that are evolutionarily distinctive and geographically restricted (Figs. 5, S5 & S6). In other words, areas like these, with high BED scores, should be considered when planning the establishment of natural reserves or educational plans when seeking to optimise the conservation of elapids and viperids and their evolutionary history in Colombia. It should be clarified that not all species with high BED scores in this study are endemic to Colombia; actually, some of them have a wide distribution spanning two or more countries, but within Colombia they are distributed in a restricted area (Fig. S2b). For instance, elapids *Micrurus albicinctus* and *M. annellatus* are widely distributed in the Amazon rainforest of several countries but are only found within a small area in the Colombian Amazon region. Then, when calculating the BED scores of these species, considering only their distribution area in Colombia, these scores increase considerably, and consequently, the BED scores of those areas in Colombia increase as well (Fig. 5b). If conservation of elapids and viperids is planned at regional (international) scale, said aspect should be considered with priority assigned to areas with species that are equally important, according to ED, EDGE, and BED scores; for example, *B. campbelli*. Determining priority areas for conservation using an evolutionary history perspective at regional scale is beyond the scope of this study, but studies in this regard are necessary (see examples by Isaac et al., 2007; Fenker et al., 2014).

Overall, when testing spatial diversity patterns through species richness and BED scores approach, there is an important mismatch, but there are some localities in the Andes, Amazon and Pacific regions that are priority from both perspectives (Figs. 5, S3–S6). The situation in the Andean and Pacific regions, home of ten and seven endemic species respectively, is particularly worrisome, as they contain some of the least protected in the country (Forero-Medina & Joppa, 2010) and their protected areas are threatened by a critical increase in deforestation levels in the post-conflict years (Clerici et al., 2020). This scenario is even more concerning under climate change scenarios performed by Velásquez-Tibatá (2014) who highlight that the Andean region has the most alarming panorama for diversity. Taking into account the previous problematic and the results in this study, we suggest incorporating within the conservation plans the prioritisation of areas with a high BED scores. This perspective makes it possible to include aspects related with the evolutionary history of lineages and their restricted range of distribution in a specific country or region and, therefore, their level of risk and eventual adaptability to local and global changes in the environment.

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Spatial ecology of the Turks and Caicos Boa *Chilabothrus c. chrysogaster* Cope, 1871 (Serpentes: Boidae)

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Obtaining ecological and natural history data from cryptic squamates can be challenging, but is crucial to understanding species' biology, particularly in the context of conservation. In the Greater Antilles, this challenge is especially apparent, particularly among the West Indian boas (genus *Chilabothrus*). Most species have had only minimal natural history study, with a few exceptions. The Turks and Caicos boa (*C. chrysogaster*) has been studied intensively for over 16 years on the small privately owned island of Big Ambergris Cay, Turks and Caicos Islands. We conducted a multi-year radio-tracking study on the species to generate information relevant to spatial habitat use and movement that will inform conservation decision-making in the face of increasing development pressure. We tracked a total of 19 female snakes using surgically implanted transmitters, enabling us to obtain between 16 and 40 location observations per boa over the lifetime of each transmitter. We found that females have an average home range of 1.83 ha and a core space use area of 0.39 ha. We also estimated occurrence distributions, the use of space between specific time intervals, finding an average occurrence area of 0.76 ha. Several females overlapped in their spatial habitat use, with an average overlap proportion of 28 %. During this study we observed female boas using two novel habitats for the species (iron shore wrack and red mangrove). This study provides valuable information on the spatial ecology of an endangered boa and will serve to inform conservation work that is currently underway.

Keywords: conservation, movement, home range, snake, spatial ecology

INTRODUCTION

Understanding how animals such as snakes use physical space in their habitats can provide a tremendous amount of valuable natural history information, including characterisation of habitat use, population health, mortality, density, dispersion, and/or demographic dynamics, among others (Gregory et al., 2001; Collinge, 2010). Further, common techniques for measuring spatial use in terrestrial animals, such as snakes, require repeated serial observations of the same individual at different times, which can yield many important behavioural and ecological insights into how they interact with their environments (e.g. Huey et al., 1989; Madsen & Shine, 1996; Bruton, 2013). Collectively, these data can better characterise a species' natural history, but in the case of threatened species these data might also be crucial to designing conservation interventions (e.g. Newman et al., 2019; Nordberg et al., 2021). For example, human alterations to habitats can produce negative impacts on the fitness and survival rates of species that depend on specific aspects of their environment; aspects which might have been unknown prior to spatial habitat use studies (Harrison et al., 1991; Webb & Shine, 1997; Roe et al., 2004).

Snakes can be especially challenging to study, as many species are cryptic, nocturnal, and exist at relatively low densities compared to some other small squamate reptiles (Macartney et al., 1988). Despite their generally low densities, snakes are important members of local ecological communities, assuming the role of both predator and prey (Greene, 1997). As a result, the spatial ecology of a given species has strong effects in structuring the trophic ecology of a community. This means that even the largest snakes can exhibit a huge variety of spatial use patterns, ranging from nearly sedentary (Smaniotto et al., 2020) to having home ranges similar to large predators such as jaguar (8–87 km^2; Hart et al., 2015; Marshall et al., 2019).

Radiotelemetry, or radio tracking, generally uses implantable Very High Frequency (VHF) transmitter packages and has revolutionised ecological research on snakes (Ciofic & Chelazzi, 1991; McDiarmid et al., 2012). Transmitters are surgically implanted intraperitoneally, or subcutaneously in larger species, while the 5–10 cm antenna is most often threaded subcutaneously. Snakes are subsequently located using an antenna and receiver tuned to individual frequencies. Such techniques have resulted in a wealth of natural history information, including seasonal variation in movement, location of basking and hibernaculum sites, movement rates, home ranges, habitat use, site fidelity, and many, many more (Webb & Shine, 1997; Gregory et al., 2001; Brito, 2003; Pearson et al., 2005; Gerald et al., 2006; Zappalorti et al., 2015; Smaniotto et al., 2020).

On Caribbean Islands, snakes, particularly boas (*Chilabothrus* and *Boa*) can be the largest terrestrial predators and occupy tertiary and quaternary food chain roles (Reynolds et al., 2023). Hence, it is important to understand the amount of space and habitat they require to persist. One of the more common uses of radiotelemetry is to obtain a series of locations of an individual snake, using these data to then calculate measurements of home range and spatial use. Such studies have revealed a tremendous amount of information for the Caribbean boa species that have been studied in this way, although only three other members of the genus have been the subject of published spatial ecological research, and each of these used few focal animals.

Jamaican Boas *C. subflavus* studied near caves show sex differences in movement, homing ability over 1 km, philopatry, and a lack of strong overlap in spatial use among individuals (Miersma, 2010; Koenig, 2019). A radio tracking study of eight females and six male Jamaican Boas *C. subflavus* was conducted between 2008 and 2012 near the Windsor Research Centre in Cockpit Country, Trelawny Parish, Jamaica (Miersma, 2010; Koenig, 2019). Boas moved a mean distance of 20 m per day, with no difference in mean daily movement distance between males and females. Cave-associated boas (two males and one female) had smaller home ranges (95 % Minimum Convex Polygons [MCPs] 0.75 to 2.51 ha) than free-ranging boas; the latter ranged from 16.28 ha to 70.11 ha in males (50 % MCP 0.20 ha to 0.34 ha) and from 2.16 ha to 19.56 ha in females (50 % MCP 0.64 ha to 6.63 ha). Boas were also found to move as far away as 1 km and return to the exact point of previous capture, suggesting excellent navigation skills and some evidence for philopatry and territoriality (Miersma, 2010). Newman et al. (2019) studied short-distance translocation (SDT) in *C. subflavus* as a potential conservation tool to move animals away from dangerous situations. Translocation distances for seven females ranged from 693–3,545 m. Two boas returned to their original point of capture in the first two months following translocation; others remained 500 m to > 1,000 m from their points of capture. Thus, translocation in *C. subflavus* might be an effective conservation practice, but only at distances over several hundred metres.

The Puerto Rican Boa *Chilabothrus inornatus* has been studied using radio telemetry at four localities including intact forest and fragmented habitat, showing seasonal shifts in movement as well as home range sizes, and difference in home range size depending on habitat (Puente-Rolón, 1999; Puente-Rolón & Bird-Picó, 2004; Wunderle et al., 2004). Like the Jamaican Boa, cave-associated populations of boas tend to have smaller home ranges than "surface" populations (Puente-Rolón & Bird-Picó, 2004; Wunderle et al., 2004). In a study focused on a cave-associated population near Arecibo, Puente-Rolón & Bird-Picó (2004) found that male and female home range sizes (based on 95 % MCPs) did not differ significantly, although females did have a slightly larger mean home range size of 0.79 ha vs. male mean home range size of 0.50 ha (95 % MCP, n = 11; range 0.01–1.8 ha). In a surface population, home ranges (95 % MCPs) ranged between 0.7–44.7 ha in females and 2.6–68.1 ha in males (Wunderle et al., 2004).

A third species, the Bahamas Boa *C. strigilatus*, has been studied on Andros Island, albeit incidentally, and were found to consume novel prey and move up to 248 m in a single day (Knapp & Owens, 2004). The authors found that, while radio tracking hatchling *Cyclura cychlura*, boas consumed some of the hatchlings and so they were able to obtain spatial data on six adult snakes. Mean daily movement ranged from nine to 198.5 m over tracking periods between five and 28 days. The authors estimated a 100 % MCP range between 0.03 and 2.0 ha. A study on the same species on the island of South Bimini has been conducted (R. Potts, pers. comm. to RGR) but those data are not yet available. Similarly, a study is currently ongoing on *C. angulifer* at the Naval Station Guantanamo Bay (P. Tolson, pers. comm.).

Although the Turks and Caicos boa *C. chrysogaster* has been extensively and intensively studied for over 16 years (Reynolds, 2011; Reynolds & Gerber, 2012; Reynolds et al., 2011; 2020), nothing is known about how much they move, how often they move, how much space they use and what their home ranges are. These data are not only meaningful for understanding boa biology and ecology, but also are especially relevant to potential conservation measures. To understand the spatial ecology of boas on Big Ambergris Cay, we conducted a two-cohort, multi-season, multi-year study. We wanted to determine 1) the distance snakes move on the island during a given time period, 2) what size home ranges they have, and 3) does their use of space differ from their use of a core home range. We examined these questions using both traditional approaches (minimum convex polygons) as well as spatial interpolation analyses, including kernel density estimates and Brownian bridge movement models. We compare these data to what is known of congeneric spatial ecology and demonstrate the utility of using multiple spatial analysis methods to inform snake spatial ecology and conservation.

MATERIALS & METHODS

Study area and specimens

This study was conducted on the privately owned island of Big Ambergris Cay, Turks and Caicos Islands located near the south-eastern edge of the Caicos Bank (Fig. 1; latitude: 21.299, longitude: -71.633, maximum elevation 32 m), a mostly submerged carbonate platform with several larger and many smaller emergent islands. The island is 6.4 km in length, a maximum of 1.6 km in width and ~400 ha in total area. The island consists of a reduced representation of salt-tolerant coastal vegetation varieties found in the Turks

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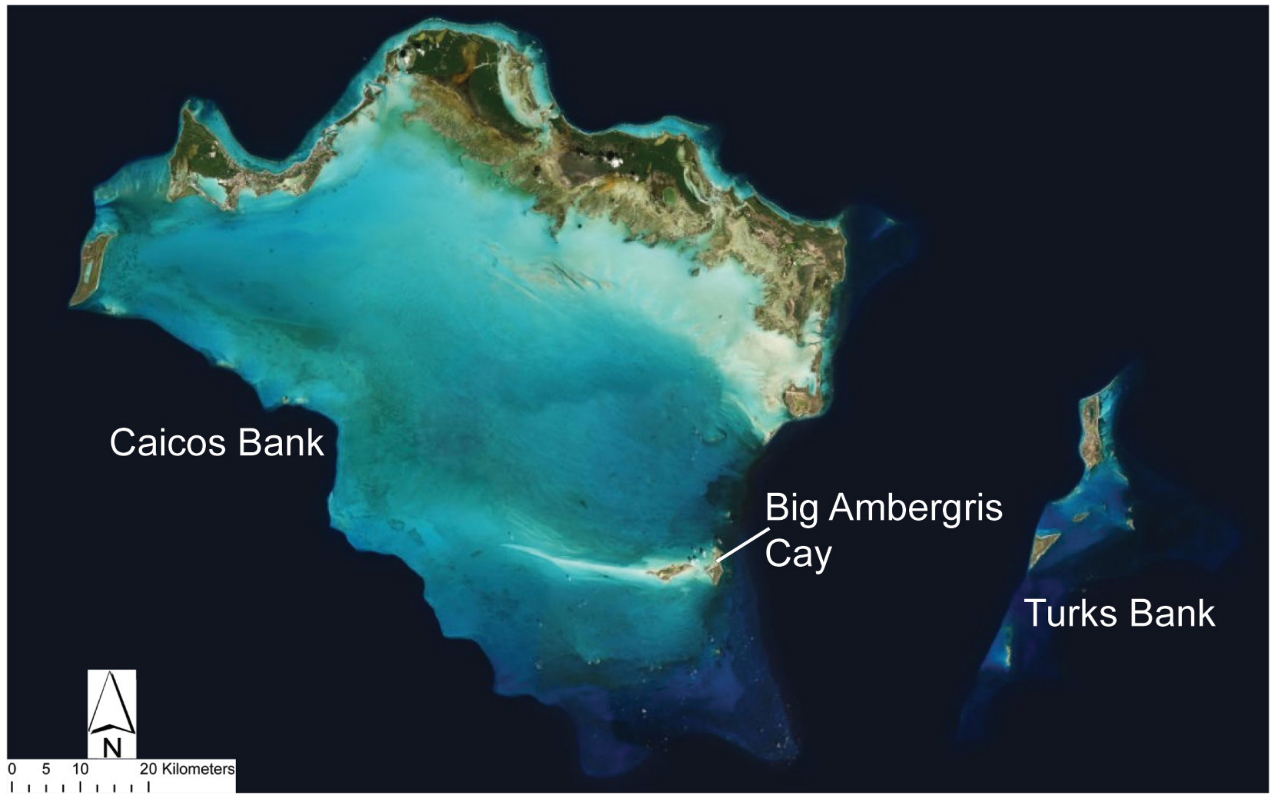

Figure 1. The Caicos and Turks banks as seen in satellite imagery (ArcGIS 2021, Redlands, CA). The Turks and Caicos are a series of seven larger islands and several hundred smaller cays and rocky islets distributed across two shallow carbonate banks at the south-eastern terminus of the Lucayan Archipelago. These islands are home to several endangered terrestrial reptile species including the Turks and Caicos boa *Chilabothrus chrysogaster* and the Turks and Caicos rock iguana *Cyclura carinata*. The study was conducted on Big Ambergris Cay, near the south-eastern margin of the Caicos Bank.

and Caicos —supporting sandy soil and palm *Cocothrinax* forest in low-lying areas, rocky cactus scrub, mangrove stands on the leeward side of the island and whiteland coppice developed over loose limestone elsewhere. This island is one of the last strongholds of Turks and Caicos boas and is an ideal place to study them for several reasons. First, no introduced predatory species, such as cats or rats, have been allowed to persist on the island. Second, the island has the highest known density of boas, with over 12 snakes per hectare (Reynolds, 2011; Reynolds & Gerber, 2012; Reynolds et al., 2020). Big Ambergris Cay has been intensively developed since 2004, now hosting the largest private air strip in the greater Caribbean, two restaurants, tennis courts and a marina that has been built into formerly natural salina salt flats (Fig. 2). The island is also criss-crossed with a network of over 20 km of unpaved roads.

Two cohorts of boas were studied over a period of 20 months between August 2018 and March 2020. The first cohort, Cohort A, was initiated between 23 July and 10 August 2018. For this cohort, 13 adult female boas were captured, and only large females that were ≧ 150 g in body mass were selected. Cohort B was initiated between 4–7 August 2019 and consisted of six adult females > 200 g in mass. All boas were collected by hand between 1800 h and 0100 h and maintained overnight at ambient temperature prior to transmitter implantation (described below).

Two types of temperature-sensitive radio transmitters were custom ordered from Holohil Systems, Inc. (Ontario, Canada): 'button-type' transmitters (model PD-2T), and 'barrel-type' transmitters (model SB-2T) in two batches, one in June 2018 and one in November 2018. These transmitters had frequencies in the range of 172.024–172.965 MHz and a battery life of six months (button-type) or one year (barrel-type) once activated. The transmitters were sterilised by individually sealing the entire unit, including the antenna, in gas sterilisation bags and then subjecting them to human surgical-grade peroxide gas sterilisation conducted by a hospital sterile processing department. Sterilisation took place between 8–15 days prior to implantation, and transmitters were stored in a protective and cushioned case during transport to the field site.

Transmitter types were matched to female body sizes, with the smaller females receiving 3.5 g button type transmitters and the larger females receiving 5.5 g barrel type transmitters. These transmitters were matched such that they weighed ≤ 3 % of snakes' body mass (Table 1).

Surgical procedures

A brief pre-surgical health exam was conducted on all snakes the morning following their capture. The agents alfaxalone or dexmedetomidine plus ketamine were administered per standard techniques. When dexmedetomidine was administered, the antagonist (atipamezole) was administered at the conclusion of the procedure. The agents used were chosen based on the temperament of the snake and surgical approach. Local analgesia was provided in all cases by injection of lidocaine in a line along the intended surgical incisions. After completion of each procedure,

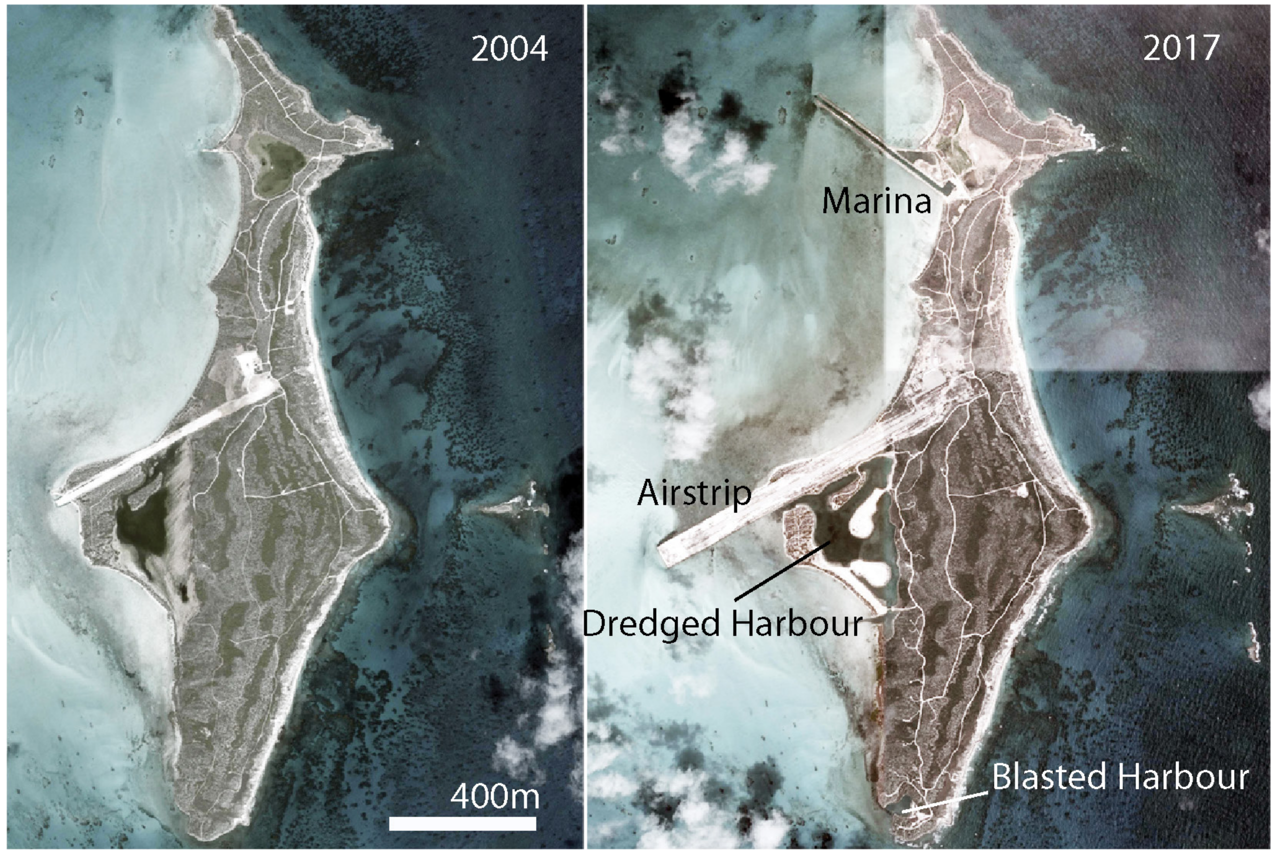

Figure 2. Development has increased dramatically on Big Ambergris Cay since 2004 (left) and 2017 (right) including the addition of private homes, a private airstrip, marina, tennis courts and restaurants. Images are from Google Earth.

each animal was given a nonsteroidal anti-inflammatory (meloxicam) as well as the antibiotic ceftiofur crystalline free acid (Excede, Zoetis, Parsippany, New Jersey).

Animals were maintained at ambient shade temperature for the duration of the procedure. Times for first effect (characterised by visual slowness), loss of righting reflex, and start and end time of surgery were recorded for each procedure. All animals underwent surgical preparation by cleansing their bodies caudal to the heart with a dilute chlorhexidine solution left in contact with the skin for 2–3 minutes. Incision areas were swabbed with alcohol and animals were placed in right lateral recumbency while the snake was held manually or taped into position with paper tape. The eyes were covered with cloth and sterile surgical drapes were placed on and around the body to establish a sterile field. Standard surgical techniques were used including sterile equipment and supplies (i.e. gloves, surgical mask, instruments, gauzes, sutures). Radio transmitters were surgically implanted using one of the two following techniques, generally following the methods of Weatherhead & Anderka (1984).

For subcutaneous implantation (n = 13), a small incision was made 2/3 of the way down the length of the snake on the dorsal-lateral left side. The transmitter was placed subcutaneously, caudal to the incision and affixed to the underlying muscle with a single absorbable suture around the neck of the transmitter. The Teflon® insulated antenna was passed subcutaneously towards the head (cranially) through a rigid polypropylene catheter or blunt surgical stylet guide.

For transmitters placed intracoelomically (n = 6), the initial skin incision was made 2–3 rows of scales above the intersection of the ventral and lateral scales. A small incision was made through the muscle below the ribs at that site. The transmitter was placed into the coelom caudal to the stomach and a suture surrounding the transmitter through the musculature was used to attach the transmitter to the body wall. The antenna was brought out through the musculature and tunnelled subcutaneously anteriorly using a polypropylene catheter or blunt surgical stylet guide. The muscle was opposed using absorbable sutures. Skin incisions were closed using skin adhesive (Nexaband®) and monofilament suture of appropriate size (3-0 to 5-0 USP).

Passive integrated transponders (PIT tags, Trovan®) were inserted subcutaneously approximately 5 cm anterior to the cloaca on the left side of each boa pre-recovery for subsequent identification. A 5 mm section of the tail tip was removed for future genetic analysis. Post-surgery, snakes were kept in 25–28 °C individual enclosures until they were fully recovered from anaesthesia and handling.

During the week of 4–10 January 2019 each snake with a functioning transmitter was collected for a health exam or surgical repair (if needed). Due to the shorter lifespan of the button transmitters, one healthy animal with a button transmitter underwent a second surgical procedure to remove the subcutaneous button transmitter and replace it with a new barrel transmitter intracoelomically (snake T13; Table 1). Health exams consisted of a weight comparison, brief overview of site of incision and

Table 1. Transmitter type, mass, and snout-vent lengths (SVL in mm) of 19 female *Chilabothrus chrysogaster* individuals that underwent implantation for tracking via radiotelemetry (mean SVL = 1040.2 ± 60.8 mm, mean mass = 438.7 g ± 98.2 g). Prop. Mass represents the proportional mass of the transmitter relative to the snake capture weight. Cohorts are described in the text.

ID	Cohort	Transmitter Type	Mass (g)	SVL (mm)	Prop. Mass	Notes
T1	A	button	162	820	2.1%	Removed from study
T2	A	barrel	182	815	3.0%	—
T3	A	button	166	830	2.1%	—
T4	A	barrel	900	1,355	0.6%	—
T5	A	barrel	855	1,315	0.6%	—
T6	A	button	227	900	1.5%	—
T7	A	barrel	320	1,030	1.7%	—
T8	A	button	259	949	1.3%	—
T9	A	button	276	953	1.3%	—
T10	A	barrel	505	1,170	1.1%	—
T11	A	button	271	920	1.3%	—
T12	A	barrel	330	990	1.7%	—
T13	A	button, then barrel	1,675	1,475	0.2%; 0.3%	Transmitter replaced
B23	B	barrel	1,135	1,500	0.5%	—
C42	B	barrel	234	1,001	2.3%	—
D45	B	barrel	580	1,190	0.9%	—
D51	B	barrel	280	1,025	1.9%	—
E9	B	barrel	256	940	2.1%	—
E40	B	barrel	278	1,005	2.0%	—

Table 2. Initiation dates and tracking dates for each cohort of boas

Cohort	Tracking Dates
A	25 July–10 August 2018
A	17–20 December 2018
A	04–10 January 2019
B	04–15 August 2019
B	06–12 March 2020

ID	Cohort	Initiated	Last Day Located	Total # Tracking Days	Total # Locations
T1	A	25/07/18	31/07/18	7	7
T2	A	27/07/18	08/08/19	25	40
T3	A	25/07/18	06/08/18	11	16
T4	A	28/07/18	06/01/19	20	35
T5	A	29/07/18	10/01/19	21	34
T6	A	29/07/18	08/09/18	12	20
T7	A	29/07/18	01/06/19	17	28
T8	A	30/07/18	07/08/19	17	19
T9	A	30/07/18	09/08/19	10	18
T10	A	30/07/18	10/01/19	21	36
T11	A	31/07/18	09/08/18	10	18
T12	A	31/07/18	10/01/19	20	36
T13	A	02/08/18	15/03/19	18	32
B23	B	04/08/19	13/03/20	16	27
C42	B	05/08/19	12/03/20	16	27
D45	B	06/08/19	12/03/20	15	26
D51	B	06/08/19	13/03/20	16	27
E9	B	07/08/19	09/03/20	11	19
E40	B	07/08/19	13/03/20	14	25

transmitter placement, and overall behaviour (whether the animal actively resisted restraint and had good muscle and skin tone or was listless with loose skin tone).

Telemetry Data Collection

Animals were released at the site of original capture a minimum of six hours after completion of the surgery once total recovery from anaesthesia was confirmed, but no animal required observation longer than 48 h to recover. A series of tracking sessions commenced (Table 2), during which we attempted to locate every boa twice per day, once at mid-day and once after dark with a minimum interval of five hours between consecutive locations. Animals were located using R1000 VHF telemetry receivers (Telonics Inc., Mesa, Arizona) in conjunction with a hand-held Yagi three-element antenna connected via a coaxial cable. Once the animal was located (either visually or within < 2 m if underground), the latitudinal and longitudinal co-ordinates of the site were recorded in decimal degrees (WGS 84, 3 m accuracy) using a Garmin eTrex10® handheld GPS (Olathe, Kansas).

Spatial Analyses

All data were analysed using R v 4.2.3 (R Core Team, 2023) implemented in RStudio v 2023.3.0 (RStudio Team, 2023). Data for most analyses consisted of .csv formatted data matrices which included columns of snake identity as well as the latitude and longitude for each telemetric location and the time/date when the location was obtained. Decimal degree WGS 84 spatial points were converted to a Universal Transverse Mercator (UTM) projection (zone = 19N, datum = NAD83) using the SpatialPoints(), CRS(), and spTransform() functions in the package sp (Bivand et al., 2013). Total distances travelled were then calculated for each boa using the SpatialLines() function in the package sp, which allowed for the comparison of the total movement distances of all females in the study, but does not allow for estimates of distances travelled per day.

When calculating spatial characteristics, it is important to differentiate between space use, or the use of all space documented during the study, and occurrence area, the movement of an animal over a period of time (Crane et al., 2021). We chose to calculate space use as 1) the home range, constituting the 95 % contour of locations the animal used, and 2) the core area, the 50 % contour of space use. Occurrence area was calculated using the 50 % contour of the movement path over the sampling period. Two types of space use estimates were used (sensu Crane et al., 2021), range distributions (RDs) and occurrence distributions (ODs). Range distributions correspond to the traditional

definition of a home range, which is the spatial extent of an animal's use of area throughout its lifetime (Burt, 1943; Crane et al., 2021), although we note that home range has been defined numerous ways, including the use of space during a sampling period (Gregory et al., 2001). Methods to estimate RDs use known location data to extrapolate areas-of-use (including future use) and are best suited for calculating traditional lifetime spatial use models; but we note that RDs are less ideally suited (although still commonly used) to estimate sampling period bounded home ranges (Crane et al., 2021). Occurrence distribution models are interpolations developed specifically for investigating the movement of an animal during the period of a study, and hence do not explicitly refer to a home range (although, in practice they might closely represent a home range depending on study design and species). Occurrence distribution models use known spatial points paired with time between sampling intervals to estimate the occurrence area between sampling points. Further, it is important to note that most RDs and ODs in practice represent spatial use in two dimensions, rarely incorporating elevational data to calculate three-dimensional space use. Hence, landscape topography would be a separate factor to consider (although it is not relevant to our study given the low topography of the island).

RDs were calculated using autocorrelated kernel density estimates (AKDEs; Fleming et al., 2015; Fleming & Calabrese, 2017). Kernel density estimates represent the utilisation distribution, or the probability of 2-dimensional space the individual uses, indicating a home range in the context of an RD (although many studies have improperly interpreted it in the context of an OD). Until recently, few studies using KDE accounted for any effect of autocorrelation, even though radio telemetry data violate an assumption of data independence (Fleming et al., 2015; Silva et al., 2022). Traditional KDE has been implicated as being strongly influenced by autocorrelated data, which might dramatically underestimate space use and space use uncertainty (Fleming et al., 2015). However, AKDEs are robust to small sample size, heterogeneous sampling intervals and autocorrelation in datasets (Fleming et al., 2019; Silva et al., 2022). Both 50 % and 95 % AKDEs were estimated for each boa in the dataset using a weighted AKDE method (wAKDE; Silva et al., 2022) using the R package ctmm (Calabrese et al., 2016; Fleming & Calabrese, 2017). The perturbative hybrid REML method and AICc were used to select the best fitting model, and resulting 95 % range isopleths were visualised in R, inclusive of confidence intervals. We then calculated range overlap among boas that crossed ranges during the study using the overlap() function in ctmm, which estimates a Bhattacharyya coefficient to quantify proportional overlap on a scale of 0–1 from AKDE ranges (Winner et al., 2018).

Range residency was further estimated using effective sampling size and variograms calculated in the package ctmm. Range residency is established when tracking data suggest that the animal has crossed its range at least once and can be indicated by effective sampling size > 5.0, which is the tracking duration divided by the number of home range crossings (Silva et al., 2022). Further, variograms allow visualisation of home range stability, when an asymptotic curve is apparent in a plot of duration versus range. While range residency is an assumption of AKDE methods, we elected to retain boas that did not fit this assumption for some analyses, identifying them as appropriate. However, summary statistics are not reported from boas that do not fit these assumptions.

Because so many previous studies of snakes have used two traditional estimators: minimum convex polygons (MCPs) and kernel density estimates (KDEs), these were calculated as well, with the caveat that autocorrelation, range residency and small sample size will cause significant bias (e.g. Row & Blouin-Demers, 2006). Minimum convex polygons connect spatial points such that a resulting polygon incorporates all the location fixes and are perhaps the most commonly used way to represent both RDs and ODs (although unfortunately most studies rarely diagnose which models they are using; Crane et al., 2021). The mcp() function was used in the adehabitatHR package (Calenge, 2006) to calculate MCPs for each boa in the study and visualised using ArcGIS Pro v 2.7.1 (Esri Inc., Redlands, California). Estimates for both 50 % and 95 % KDEs were calculated using the ctmm package.

Neither of these traditional approaches (MCP and KDE) to estimating RDs and ODs are without flaws, particularly as they are both technically suited for RD and not OD (despite their frequent use for the latter; Crane et al., 2021). Therefore, an explicit OD approach using Brownian bridge movement models (BBMMs) was selected, which incorporates different weights for spatial habitat use by accounting for time steps between observations (Horne et al., 2007; Silva et al., 2020). Brownian bridge movement models are OD estimations that focus on using movement between location points to build a model that measures the probability of space use between each location (Horne et al., 2007; Silva et al., 2018). Time steps between each data point were calculated to the nearest hour, then the functions in the R package BBMM 3.0 (Nielson et al., 2015) were used to construct 50 % BBMMs for each boa. A 50 % cut-off was chosen, as generating higher cut-offs would have required more spatial points per boa than we had available. Output files with spatial grid information in a polyline format were created that we could then import into ArcGIS Pro. Areas for the BBMMs were calculated by transforming the polyline data into an area polygon using the construct polygon tool in ArcGIS Pro v. 2.7.1 and vertices edited to join lines where needed. Areas of the resulting polygons are then available in the layer attributes table.

RESULTS

Radio Transmitter Implantation

A total of 20 radio transmitters were implanted in 19 female snakes (Table 1). The mean SVL of snakes was 1,107 mm (range 815–1,500 mm), with a mean mass of 524 g (range 160–1,675 g; Table 1). Of the 19 snakes, seven received button transmitters and 12 received barrel transmitters, with one individual (T13) receiving both types following a replacement of one (Table 1). The mass of boas receiving barrel transmitters was 182–1,290 g and 160–1,675 g

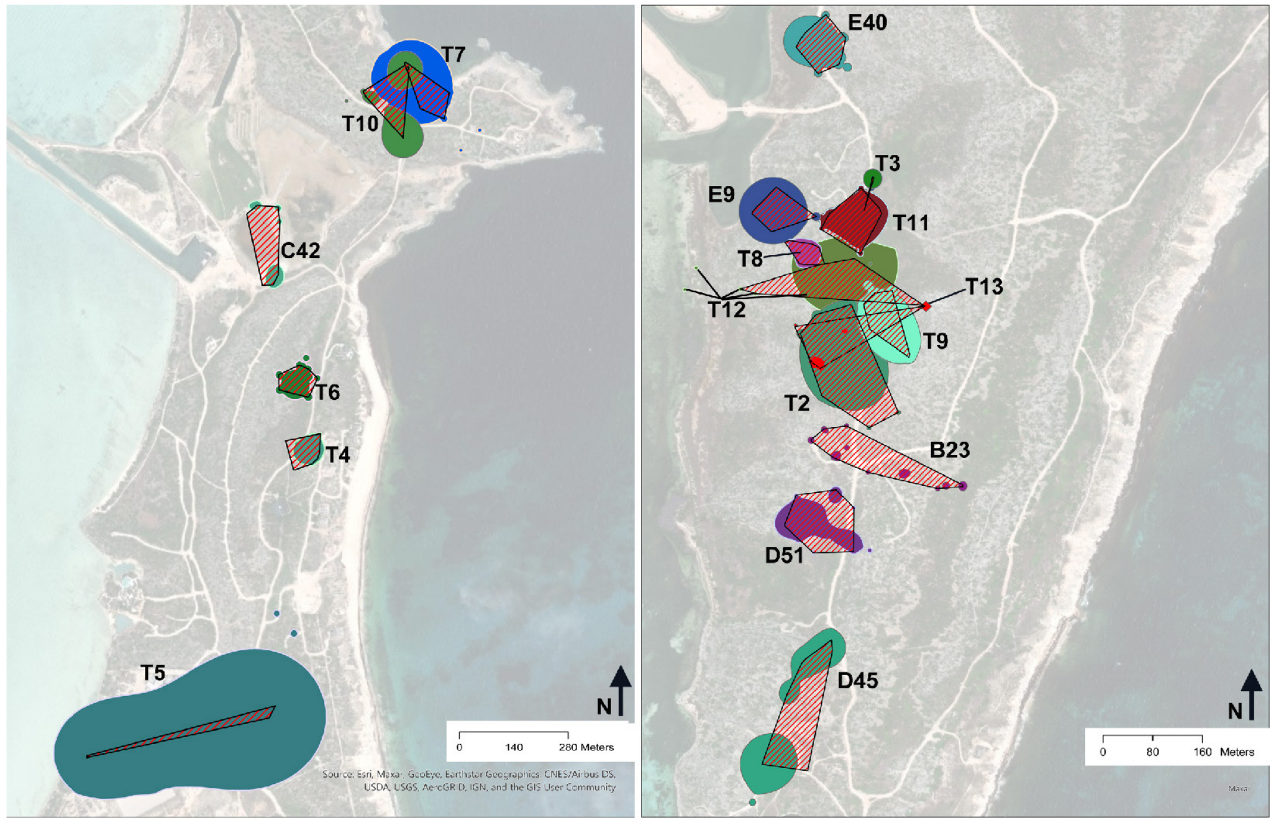

Figure 3. Spatial use models for boas in the study, with boas on the north of the island to the left, and boas from the south of the island to the right. Coloured polygons represent 50 % BBMMs for each boa. Striped polygons represent MCPs for each boa, overlaid on the BBMMs for comparison. Note that BBMMs can have more than one polygon for an individual, and that some MCPs and BBMMs are very different from each other, for example boa T13. Small circles outside the main BBMM polygons are single location observations of an individual.

for those receiving button transmitters, while the mean transmitter to mass ratio proportion was 2.0 % (range 0.2–3.0 %). One boa (T13) was confirmed to be heavily gravid in August 2018 via handheld ultrasound.

Implantation surgeries resulted in zero attributable deaths throughout the experiment, although we suspect (but were unable to verify) that boas T5 and T10 were killed by vehicular traffic during the study. Of the 20 surgeries performed, 15 were without any complications nor health concerns during subsequent observations. Three individuals (T1, T3, and T4) showed signs of minor health concern (details below) that resulted in the transmitter and individual being removed earlier than desired from the study. A further two individuals (T7 and E40) were found to have lost their transmitters, presumably through pressure necrosis, and the wounds had healed when the snakes were relocated by happenstance.

Boa T1 was recaptured 31 July 2018 showing signs of pressure necrosis from its subcutaneous button-type transmitter 16 days post-surgery. Nevertheless, the fascia and muscle underneath the transmitter appeared healthy, so the transmitter was removed, the necrotic tissue was trimmed, and the wound was cleaned and sutured. Boa T3 was recaptured on 8 August 2018 and was also exhibiting signs of pressure necrosis, so the transmitter was similarly removed. Boa T4 appeared healthy throughout August 2018, but when located on 6 January 2019 the antenna was found to be protruding from the skin and the snake had lost 10 % of its mass. The transmitter was removed, the surgical site was lavaged with sterile saline, the necrotic tissue debrided, the site re-sutured, and the animal was given a dose of ceftiofur crystalline free acid intra-muscularly (IM). Boa T5 was initiated on 29 July 2018 and, apparently unaffected by the surgery, was found to have consumed a 350 g female *Cyclura carinata* iguana on 4 August 2018. The distention caused by this meal, constituting 41 % of the mass of the snake, caused suture rupture, which was easily cleaned and repaired.

The button-type and barrel-type transmitters were expected to have battery life of six months or one year respectively. Nevertheless, transmitter signals for boas T6, T8, T9, and T11 could not be located 4–5 months after implantation. As the island is easily traversed in its entirety, the boas were likely not lost, and instead the transmitters most likely failed early.

Spatial Analyses

One boa (T1) was not included in further analyses owing to the short tracking duration (seven locations) prior to transmitter removal. The remaining 18 boas were tracked between 10 and 25 days each (mean = 16 days), with a maximum of 204 days between tracking sessions. Between 16 and 40 location recordings were obtained for each boa (mean = 26.8, total = 483). Boas moved on average a total of 1,361 m while being tracked (median = 732 m; range = 192–9,419 m; Table 3). Eight boas (within the same cohort) were observed to overlap each other's home ranges both spatially and temporally (Fig. 3). Overlap calculations

Figure 4. Many boas were observed actively foraging or resting in the open during tracking. Clockwise from top left: a large female rests at night underneath a *Cocothrinax* palm; boas were observed using coastal ironshore wrack habitat for the first time, a female observed crawling just before sunset.

ranged from 0.07–0.74 (on a scale of 0–1), with a mean overlap proportion of 0.28 (Table 4).

Data from eight of the 18 boas suggested that range residency had been observed, as evidenced by effective sample sizes (Table 3) and variograms (Supplementary material Appendix I). RD calculation using wAKDEs for boas that had established range residency (Table 3, Supplementary material Appendix II) resulted in an average 50 % RD of 0.39 ha per boa (range 0.18–0.82 ha) and an average 95 % RD of 1.83 ha per boa (range 0.88–3.99 ha). For our BBMMs, we found an average 50 % OD of 0.76 ha per boa (range 0.41–1.97 ha).

Our calculation using MCPs (Supplementary material Appendix III) yielded an average RD of 0.70 ha per boa (range 0.01–1.74 ha). We found similar values for our KDE calculations (Supplementary material Appendix III), with an average 50 % RD of 1.17 ha per boa (range 0.18–2.73 ha) and an average 95 % RD of 5.30 ha per boa (range 0.26–13.11 ha).

No correlation was found between the number of locations obtained per boa and the distance a boa travelled (Spearman rank correlation ρ = -0.15, P = 0.54) nor the OD measure using BBMM (ρ = 0.35, P = 0.15). Similarly, our RD measures were not correlated with the number of locations per boa for 50 % wAKDE (ρ = 0.04, P = 0.88) nor 95 % wAKDE (ρ = 1.88, P = 0.45). But our measures of RD were significantly positively correlated with the number of observations per boa for MCP (Pearson correlation coefficient = 0.71, P = 0.0008), 50 % KDE (ρ = 0.48, P = 0.04), and 95 % KDE (ρ = 0.59, P = 0.01). When restricting these correlations to just the eight range resident boas, none of the RD nor OD estimates were significantly correlated with the number of locations obtained (ρ = 0.12–0.69, P = 0.06–0.78).

DISCUSSION

Over the course of 20 months, space use estimates were generated by radio tracking 19 female *C. chrysogaster*. Taken together, boa home range (RD sensu lato) is likely between 0.7 and 1.2 ha. Our measures of RDs are strongly influenced by the number of locations obtained, suggesting that we could likely improve these estimates with more data. But, reassuringly, our estimates of total distance moved, as well as our ODs, were not correlated with the number of locations obtained. We were not able to generate enough location fixes in both winter and summer to make comparisons between seasons, despite this being an original component of the study design.

Our RD (wAKDE) and OD (BBMM) models yielded complimentary results for space use, with each model

Table 3. Spatial use data obtained for *Chilabothrus chrysogaster* females on Big Ambergris Cay, Turks and Caicos Islands. Data for weighted autocorrelated kernel density estimates (wAKDEs) are shown, which are estimates of home range (RD). Both 95 % (home range) and 50 % (core area) estimates, with confidence intervals (CI) are shown. Brownian bridge movement model (BBMM) is an estimate of OD. One boa (T1) of the original 19 was excluded owing to the small number of tracking observations (n = 7). All areas are in hectares. DOF area is the degrees of freedom area, equivalent to the effective sampling size. Values less than 5 indicate range instability. Bold indicates boas that were found to have range residency (stability). Xbar is the average of values from boas exhibiting range residency.

ID	DOF area	95% wAKDE	95% CI	50% wAKDE	50% CI	BBMM 50%
T2	**34.4**	**3.99**	**2.77, 5.43**	**0.73**	**0.64, 0.81**	**1.97**
T3	0.50	32.47	0.03, 163.79	7.61	0.75, 14.93	0.07
T4	**17.6**	**1.15**	**0.68, 1.75**	**0.23**	**0.19, 0.27**	**0.41**
T5	2.65	166.43	29.74, 418.5	42.33	23.21, 59.9	15.42
T6	**11.8**	**1.23**	**0.63, 2.02**	**0.33**	**0.26, 0.39**	**0.61**
T7	4.37	6.37	1.87, 13.58	1.47	0.96, 1.95	2.55
T8	**18.0**	**0.88**	**0.52, 1.32**	**0.18**	**0.15, 0.21**	**0.19**
T9	2.49	5.71	0.94, 14.68	1.35	0.72, 1.93	0.94
T10	4.32	6.07	1.76, 12.98	1.52	0.98, 2.01	1.51
T11	**17.0**	**1.32**	**0.77, 2.01**	**0.37**	**0.31, 0.43**	**0.71**
T12	**35**	**1.84**	**1.28, 2.49**	**0.19**	**0.17, 0.21**	**0.75**
T13	3.93	6.09	1.63, 13.42	1.62	1.02, 2.17	0.06
B23	2.27	5.13	0.75, 13.66	1.28	0.66, 1.86	0.11
C42	1.99	9.35	1.28, 26.07	2.38	1.14, 3.52	0.27
D45	2.90	6.20	1.23, 15.12	1.60	0.91, 2.24	1.28
D51	**7.28**	**3.03**	**1.25, 5.60**	**0.82**	**0.60, 1.02**	**0.71**
E9	3.68	2.15	0.54, 4.84	0.58	0.36, 0.78	0.97
E40	**11.94**	**1.22**	**0.63, 2.01**	**0.30**	**0.24, 0.36**	**0.71**
$\bar{x}$	19.13	1.83	—	0.39	—	0.76

producing a mean space use between 0.39 (core area, 50 % wAKDE) and 1.83 (home range, 95 % wAKDE) ha per range-resident boa (Table 3). Boas that were not found to be range resident produced considerably larger estimates of RD and OD, which is expected from the lack of precision given the data for those boas. Our OD estimates (50 % BBMM) tended to be in-between range sizes of the 50 % and 95 % wAKDEs (Table 3).

More traditional measurements of RD, such as MCP, resulted in a similar mean estimate of home range (0.70 ha); albeit with a broader range of estimates among boas (0.01–1.74 ha; Supplementary material Appendix III). Whereas KDE estimates tended to be somewhat larger (mean home range size 0.57–2.51; Supplementary material Appendix III). Instructively, the difference we calculated for our MCP versus KDE estimates captures the sensitivity of traditional geometric (i.e. MCPs) versus probabilistic approaches (KDE) in estimating features of spatial ecology. Though slight in our dataset, this disparity serves as a reminder that these different methods shed light on different biological questions (also see Fig. 3). For example, KDE is the weighted probability distribution of the total space that an animal uses. Hence, when animals move between disjunct locations along a narrow corridor, KDE captures the distance in between these locations as part of the distribution estimation. MCP can yield a similar outcome if there are point locations that widen a corridor between disjunct locations, but if the polygon is narrow, then the total estimate of space use is much lower. Further, it is clear that approaches which are explicit in first estimating range residency as well as correcting for small sample size and autocorrelation (Fleming et al., 2019; Silva et al., 2022) yield very different estimates of RD. Our weighted AKDE using range-resident boas suggested that the actual RD was much smaller than what would be found using traditional KDE (Table 3). This is the opposite of what has been suggested in the literature (Fleming et al., 2015), that autocorrelation might underestimate space use, but it nevertheless is an important result that shows the value of accounting for sample size, range residency and autocorrelation.

Table 4. Proportions of overlap between pairwise AKDE RDs of boas observed to cross ranges during the study period. Overlap is the Bhattacharyya coefficient, which is a measure of the closeness of two samples, on a scale of 0 (no overlap) to 1 (complete overlap). Individuals in bold were found to be range-resident. Dof is the degrees of freedom, with values < 5.0 indicating insufficient sample sizes, which are also shown.

Boa 1	Boa 2	Dof	95 % CI low	Overlap	95 % CI high
2	9	24.8	0.02	0.09	0.28
12	**9**	8.9	0.04	0.23	0.64
13	**2**	5.53	0.48	0.74	0.94
13	9	4.96	0.001	0.07	0.64
$\bar{x}_i$	$\bar{x}_i$	—	—	0.28	—
Insufficient sample					
7	10	0.44	0.40	0.88	1.0
12	**2**	< 0.001	—	—	—
12	**8**	< 0.001	—	—	—
12	**11**	< 0.001	—	—	—
13	**8**	2.52	0.001	0.13	0.9
13	**12**	< 0.001	—	—	—

Novel Habitat Use

Habitats on Big Ambergris Cay vary from marshy salina to mangrove to open cactus scrub to nearly closed-canopy coppiced forest. *Chilabothrus chrysogaster* have been observed using all available habitat types on the island except for mangroves (Reynolds & Gerber, 2012; Reynolds et al., 2020). Most radio-tracked boas stayed exclusively in one type of habitat (coastal scrub), which is the most dominant habitat on the island (Fig. 3). But, during this study some novel natural history insights were observed, including regular discovery of boas in habitats not previously known to be used (Fig. 4). For example, several boas were located foraging out in the wrack line along the rocky limestone coastal shore (Fig. 4), an area we had not previously looked for boas under the assumption they would not use this habitat. While two other members of this genus (*C. exsul* and *C. strigilatus*) have been found resting under wrack debris lying on sand in the Bahamas (Sheplan & Schwartz, 1974; Tolson & Henderson, 1993), no other boa in the Caribbean has been noted to forage nor actively move along rocky wrack lines. Further, some boas were observed heading out into the red mangrove forest for days at a time and were found actively foraging (although no predation events were recorded). One boa (T12; Fig. 3) moved so far out into the red mangroves *Rhizophora mangle* that it was over 20 m from shore, entirely over water. When located, RGR had to avoid a blacktip shark *Carcharhinus limbatus* that was swimming just beneath the boa. Five other members of the genus (*C. angulifer*, *C. gracilis*, *C. striatus*, *C. inornatus*, *C. strigilatus*) are known to regularly use mangrove (*Avicennia* and/or *Rhizophora*) habitats (Sheplan & Schwartz, 1974; Henderson & Powell, 2009; Rodríguez-Cabrera et al., 2020; Reynolds et al., 2023).

Table 5. Comparison of spatial use among species of the genus *Chilabothrus*. Note that these data are not directly comparable, as different methods were used. Data for *C. chrysogaster* are reported only for range-resident individuals. AK = 2-dimensional Adaptive Kernel.

Species	OD (method)	RDM (method)	Reference
C. chrysogaster (n = 8)	0.76 ha (BBMM)	1.83 ha (95% AKDE) 0.39 ha (50% AKDE)	This study
C. inornatus (n = 11)	—	0.01–1.8 ha (95% MCP) 0.0006–0.664 (50% MCP)	Puente-Rolón& Bird-Picó, 2004
C. inornatus (females, n = 9)		0.02–9.7 ha (50% MCP) 0.7–44.7 ha (95% MCP) 0.1–4.7 ha (50% AK) 2.0–66.8 ha (95% AK)	Wunderle et al., 2004
C. inornatus (males, n = 9)		1.2–8.5 ha (50% MCP) 2.6–68.1 ha (95% MCP) 0.3–3.8 ha (50% AK) 3.6–105.5 ha (95% AK)	Wunderle et al., 2004
C. strigilatus (n = 6)	—	0.03–2.0 ha (100% MCP)	Knapp & Owens, 2004
C. subflavus (cave, female, n = 1)	—	1.12 ha (50% MCP) 2.51 ha (95% MCP) 0.81 ha (50% AK) 4.78 ha (95% AK)	Miersma, 2010; Koenig, 2019
C. subflavus (cave, male, n = 2)		0.20–0.34 ha (50% MCP) 0.75–2.25 ha (95% MCP) 0.16–0.63 ha (50% AK) 1.10–5.92 ha (95% AK)	Miersma, 2010; Koenig, 2019
C. subflavus (surface, female, n = 7)	—	0.64–6.63 (50% MCP) 2.16–19.56 ha (95% MCP) 0.57–4.09 ha (50% AK) 3.12–22.04 ha (95% AK)	Miersma, 2010; Koenig, 2019
C. subflavus (surface, male, n = 4)	—	0.20–0.34 (50% MCP) 16.28–70.11 ha (95% MCP) 0.02–2.81 ha (50% AK) 0.59–64.95 ha (95% AK)	Miersma, 2010; Koenig, 2019

Conservation and Comparisons

Only three of the 14 species of the genus *Chilabothrus* have published spatial data using telemetry, yet these provide some valuable comparisons to our data (Table 5). Broadly defined, 95 % RD home ranges of Turks and Caicos boas (0.88–3.99 ha) were similar to cave-associated populations of Puerto Rican boas (0.01–1.8 ha) and Jamaican boas (0.75–5.92 ha), but not to other populations of these species (Puente-Rolón & Bird-Picó, 2004; Wunderle et al., 2004; Miersma, 2010; Koenig, 2019). Non cave-associated Puerto Rican boas had 95 % RD home ranges between 0.70–105.5 ha, while non cave-associated Jamaican boas had 95 % RD home ranges between 0.59–70.11 ha (Table 5). Presumably, cave-associated boas have smaller home ranges owing to a concentration of food resources near caves (bats). Lizard densities on Big Ambergris Cay are exceptionally high (authors' pers. obs.) which could suggest that Turks and Caicos boas needn't have large home ranges to find food. Similarly, Bahamas boas have RD home ranges between 0.03–2.0 ha in areas with high concentrations of iguana hatchlings (Knapp & Owens, 2004).

Radio telemetry and spatial analyses have been used to demonstrate that habitat fragmentation negatively impacts snakes, supporting the need for protected areas and low impact zones of habitat, especially for threatened species. Indigo snakes *Drymarchon couperi* were found to have reduced survival in fragmented landscapes owing to human interaction (Breininger et al., 2012), and timber rattlesnakes *Crotalus horridus* required some protected area in which to overwinter (Nordberg et al., 2021). The habitat on Big Ambergris Cay is certainly being fragmented, particularly by the many roads that now cross the island. Indeed, we suspect that some of our study animals were killed on roads and buried to hide the evidence from us, as we have seen evidence of this for non-study animals (RGR & GPG, pers. observ.). But we found two important conclusions regarding the chances for persistence of the species: 1) the home ranges of large adult females are generally small, less than 2 ha (Table 3); and 2) home ranges of these females can overlap in time and space (Table 4). Overlapping home ranges have been found in Burmese Pythons in Florida (Hart et al., 2015), and this could have important implications if it indicates that the species is not territorial. Territoriality in snakes can limit densities (Webb & Shine, 1997), and given that the island is only 400 ha territoriality would significantly curtail the number of individuals that could coexist, especially as habitat is converted to developed areas. Jamaican boas *C. subflavus* were shown to not strongly overlap in their spatial use (Miersma, 2010; Koenig, 2019), and to-date this is the only example of potential territoriality among boas.

While the use of radiotelemetry involves the assumption that the behaviour of the telemetered individual is unaltered by the technique, and that data retrieved are representative of normal behaviour, previous studies have shown that growth, reproduction, and survival of many snake species are affected by implantation either directly or indirectly. These negative effects could come from the surgical intervention or from constant human disturbance (Webb & Shine, 1997). For example, black rat snakes *Pantherophis obsoleta* with versus without transmitters showed lower annual growth in mass but not length, and females produced lighter clutches of eggs relative to their body size (Weatherhead & Blouin-Demers, 2004). Such negative effects might be explained by impaired behaviour, cost of movement and infection. That is why we attempted to take such care with our surgical techniques, as well as minimise the number of locations of each animal, and allow periods of time with no tracking, particularly during the fall when parturition occurs (Tolson & Henderson, 1993).

There have been older (e.g. Macartney et al., 1988) and more recent (Crane et al., 2021) calls for standardisation of spatial data collection on reptiles, particularly snakes, such that meta-analysis can be conducted. Nevertheless, as Macartney et al. (1988) correctly posit, studying snakes is challenging, and it can be hard to study some species at regular intervals, to study more than a dozen at a time, or to conduct precise and comparable seasonal studies. That is perhaps why most studies of snake spatial ecology have been conducted in only a few geographic regions and a few species, for example in the United States on the genera *Crotalus* and *Pituophis* (Crane et al., 2021). Well-characterised studies, such as that on the cobra *Ophiophagus* in Thailand (Silva et al., 2018) and India (Barve et al., 2013; Rao et al., 2013) used only two individuals, while another on the anaconda *Eunectes murinus* used only eight (Smaniotto et al., 2020). Perhaps acknowledging the challenges of studying snakes, researchers such as Crane et al. (2021) now suggest that as much transparency as possible be included in spatial studies, including making data open, including specific definitions of what is being calculated (e.g. RDs versus ODs), and explicitly specifying influential model parameter values (e.g. smoothing parameters; Silva et al., 2022).

Future Directions

The size of snake home range can be influenced by sex (Breininger et al., 2012; Hyslop et al., 2014) as well as seasonal trends (Brito, 2003; Heard et al., 2004; Diffendorfer et al., 2005). While we attempted to generate enough data for seasonal comparisons, we were unable to do so. Therefore, future work on *C. chrysogaster* might include a seasonal analysis of movement. Importantly, no sex differences were found in RDs for Puerto Rican boas in two separate studies (Puente-Rolón & Bird-Picó, 2004; Wunderle et al., 2004). Free-ranging male Jamaican boas showed a trend of having larger RD home ranges (16.28–70.11 ha) than females (2.16–19.56 ha), but cave-associated populations did not (Table 5).

Female versus male movement has differed on both a per-move basis (mean daily movement per move) and monthly basis (mean daily movement per month) in *C. inornatus*, where males moved farther than females and females were immobile for significantly longer average time periods (Wunderle et al., 2004). Additionally, an increase of male snake movement can be seen throughout the reproductive season (April to June for *C. inornatus* in Puerto Rico, September for *Vipera latastei* in Portugal), suggesting that males actively search for females during specific months of the year (Brito, 2003; Wunderle et al., 2004). Additional data concerning spatial ecology associated with reproductive seasons of male and female *C. chrysogaster* would strengthen the home range and spatial use analyses for this species.

Finally, new technology being developed might allow easier, more efficient, and more robust data collection. Electronic trackers using global positioning satellites are becoming smaller, such that some are suitable for use on larger snakes (Smith et al., 2018). Drone tracking is another promising area of development (Hui et al., 2021), and while we attempted to use drones during August 2019 of our study, high winds made for impossible flight with a platform similar to that used by Hui et al. (2021). We also experimented with the use of Bluetooth trackers (Tile® Pro, paired with an iPhone® 7), but we found the square tags challenging to attach to snakes, and the tags had very poor reception if the snake was under a rock or not directly in line-of-sight. Nevertheless, technologies leveraging Bluetooth or radio frequency identification (RFID) technology might hold promise under certain conditions, and automated detectors are also showing some promise in specific deployment scenarios (DeGregorio et al., 2018).

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Data Accessibility Statement

All R code and raw data are available on Dryad (Doi https://doi.org/10.5061/dryad.crjdfn390).

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Dark future for a black salamander: effects of climate change and conservation implications for an endemic alpine amphibian

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Climate change is threatening several montane species across the world, including a large number of endemics, needing the development of forward-looking conservation strategies to foster their future survival. In this context, Species Distribution Models (SDMs) represent a useful method to forecast changes in species' habitat suitability under different scenarios of global warming, often advising conservation frameworks with credible, defensible and repeatable information. In this paper, we estimate the environmental and bioclimatic suitability for an endemic mountain amphibian (*Salamandra lanzai*) in the western European Alps through an SDM approach, considering both current and future scenarios, to address short- and long-term management and conservation actions, and to update the current IUCN extinction risk assessment. The ensemble model forecasts predict a dramatic decline of the climatically suitable area for the Lanza's alpine salamander in the next 20–40 years, even considering an optimistic CO_2 emissions scenario, leading to a theoretical extinction of this species in 2100 in case the worst global warming prediction will be actualised. This underlines the urgent need for up-to-date conservation and management strategies to ensure the successful mitigation of climate change effects on *S. lanzai*, especially by adapting and improving the network of protected areas, immediately removing additional threats and identifying possible management actions able to increase fine-scale habitat suitability and connectivity among populations. In addition, a significant range contraction in the future has to be considered when assessing the extinction risk for this species, possibly exacerbating the effect of other threatening factors, such as the spread of lethal pathogens.

Keywords: Salamandra lanzai, ensemble models, environmental suitability, bioclimatic suitability, future projections

INTRODUCTION

Climate change is threatening biodiversity worldwide and the number of species under extinction risk is expected to steeply increase with the rise of global temperatures in the near future (IPCC, 2022). In this context, mountain systems represent one of the most vulnerable ecosystems (Chakraborty, 2021; Schmeller et al., 2022), not only because they host a significant proportion of the global biological diversity (Körner & Spehn, 2002; Perrigo et al., 2020), but most importantly because average air temperatures at high elevations proved to increase faster than the overall global warming rate in the last decades (IPCC, 2018). Accordingly, several montane species are requiring attention from a conservation point of view (especially endemics, Manes et al., 2021), needing the development of forward-looking management strategies able to foster their resilience and adaptation to climate change, even anticipating future distribution and habitat suitability shifts (Clark et al., 2001; Tulloch et al., 2020).

Species Distribution Models (SDMs) are a widely used method to project species distributions in space and time under climate change (Araújo et al., 2019), often advising conservation frameworks with credible, defensible and repeatable information (Sofaer et al., 2019). In their most common correlative form, SDMs typically use the known locations of a given taxon and information on the corresponding environmental conditions to produce habitat suitability maps (Peterson et al., 2011), which can be also projected in the future according to the available climatic and environmental scenarios, allowing to detect possible species range shifts/expansions/contractions through time (Yalcin & Leroux, 2017), to inform extinction risk assessments (Syfert et al., 2014), and to address biodiversity conservation efforts (Guisan et al., 2013).

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Since amphibians show the highest proportion of threatened species among the world's vertebrates (Hoffmann et al., 2010), with climate change representing one of major drivers for the decline of many taxa (Blaustein et al., 2010), SDMs are a frequently used approach to predict future distributions within this animal group. In particular, a number of modelling attempts (e.g. Cordier et al., 2020; Feldmeier et al., 2020; Jacobsen et al., 2020; Lyons & Kozak, 2020; Dubos et al., 2023) have been focused on high-elevation species, which are expected to be particularly sensitive to climatic alterations due to their special adaptations to relatively low temperatures, restricted activity periods and intermittent water availability.

The Lanza's alpine salamander *Salamandra lanzai* (Nascetti et al., 1988) is one of the few amphibians that successfully colonise alpine habitats in the European Alps, thanks to several biological, physiological, ecological, and behavioural traits. In particular, this urodele emancipated from water for reproduction, evolving an aplacental viviparous strategy that allows for better offspring thermoregulation, while ensuring food availability through oophagy or adelphophagy (Bovero et al., 2013). In addition, this species is able to continuously track suitable climatic conditions by alternating epigean and hypogean phases, with surface-dwelling individuals becoming visible only on relatively humid nights and on rainy days between April and October (Andreone et al., 1999a). Accordingly, the Lanza's alpine salamander leads a predominantly subterranean life (Andreone, 2006), benefiting from the relatively stable microclimatic conditions of the underground network of cracks and crevices (Ribéron & Miaud, 2000; Mammola et al., 2016), the so-called Milieu Souterrain Superficiel (MSS; Culver & Pipan, 2014), which also provides a suitable habitat for hibernation and for young salamanders' growth until sexual maturity (Andreone, 2006). Above ground, alpine grassland and shrubland habitats surrounded or interspersed by rocks and screes are usually preferred by this species (Abdulhak, 2016, unpublished report), with only some populations living in larch woodlands (Andreone, 2006).

Sedentarity is another important feature of *S. lanzai*'s ecology: radio-tracked adults showed a rather limited dispersal capacity, with a mean home range of about 50 m^2 exploited in a 4-week period (Ribéron & Miaud, 2000), while cumulative movements of a few hundreds of metres (< 400 m) each year can be inferred from capture-mark-recapture observations (Andreone et al., 1999a). Surface activity is thus probably dedicated mainly to feed on ground-dwelling insects (Andreone et al., 1999b), as well as to the search for mating partners (Andreone, 1992). However, despite very limited daily or inter-annual movements, the Lanza's alpine salamander is characterised by an inter-generational dispersal over long distances, as highlighted by its genetic structuring, revealing the progressive colonisation of relatively far new favourable areas among generations, starting from several refugia where populations became isolated during the last glacial maximum (Montgelard et al., under review).

Salamandra lanzai is subjected to a rigorous protection in the European Union (EU Directive 92/43/EEC, Annex IV) and it is classified as Critically Endangered by the International Union for Conservation of Nature (IUCN), in view of its restricted distribution (West-Alpine endemic) and the predicted spread of the lethal fungal pathogen *Batrachochytrium salamandrivorans* (*Bsal*), which has more than 50 % probability to cause the extinction of this species in the next 45 years (IUCN SSC Amphibian Specialist Group, 2022). In addition, populations are inferred to be locally decreasing, owing to the localised decline in habitat extent and quality, and to the severe impact of road mortality in some areas (IUCN SSC Amphibian Specialist Group, 2022). Surprisingly, the IUCN assessment does not mention climatic variations as a possible additional factor that could compromise the survival of this salamander in the future, likely because future projections on the distribution of this endemic urodele are still lacking (but see Dubos et al., 2023), preventing the availability of reliable data to support extinction risk evaluations across different climate change scenarios and time periods.

In order to fill this information gap, this study applies an SDM approach to map the current and future environmental and bioclimatic suitability for the Lanza's alpine salamander throughout its range. The first aim is to identify the main environmental and bioclimatic variables currently correlated with the distribution of *S. lanzai*, then projecting them in space, in order to outline the suitable area where further research can be performed, and where to focus short-term management and conservation actions. Furthermore, this study aims to project the bioclimatic suitability for this urodele in the future, in order to forecast if and how the extent of the suitable area will change in the next century, due to rising temperatures and the expected changes in precipitation regimes, possibly providing useful information to update the current IUCN extinction risk assessment and for long-term conservation planning (e.g. improvement of the current protected areas network).

MATERIALS & METHODS

Calibration area, occurrence data and predictors

Models were calibrated within a rectangular area encompassing all the known range of *S. lanzai* in the western European Alps (3433 km^2), between Italy and France (Fig. 1; Appendix S1.1). In this area, 360 geo-referenced occurrence points were selected for the modelling procedure (Fig. 1), starting from a raw database of 3382 spatially-autocorrelated records (see Appendix S1.2). In particular, data were first sorted temporally (focusing on the 2000–2021 period) and then spatially (one record per ~150 m pixel, in accordance with predictors' resolution).

In a first model arrangement (hereafter defined as "environmental suitability model"), the total solar radiation in June (Srad06), the minimum temperature of the coldest month (Bio06), total annual precipitations (Bio12), the Normalised Difference Vegetation Index (NDVI) values of July (NDVI07) and the mean temperature of the driest quarter of the year (Bio09) were selected among an initial

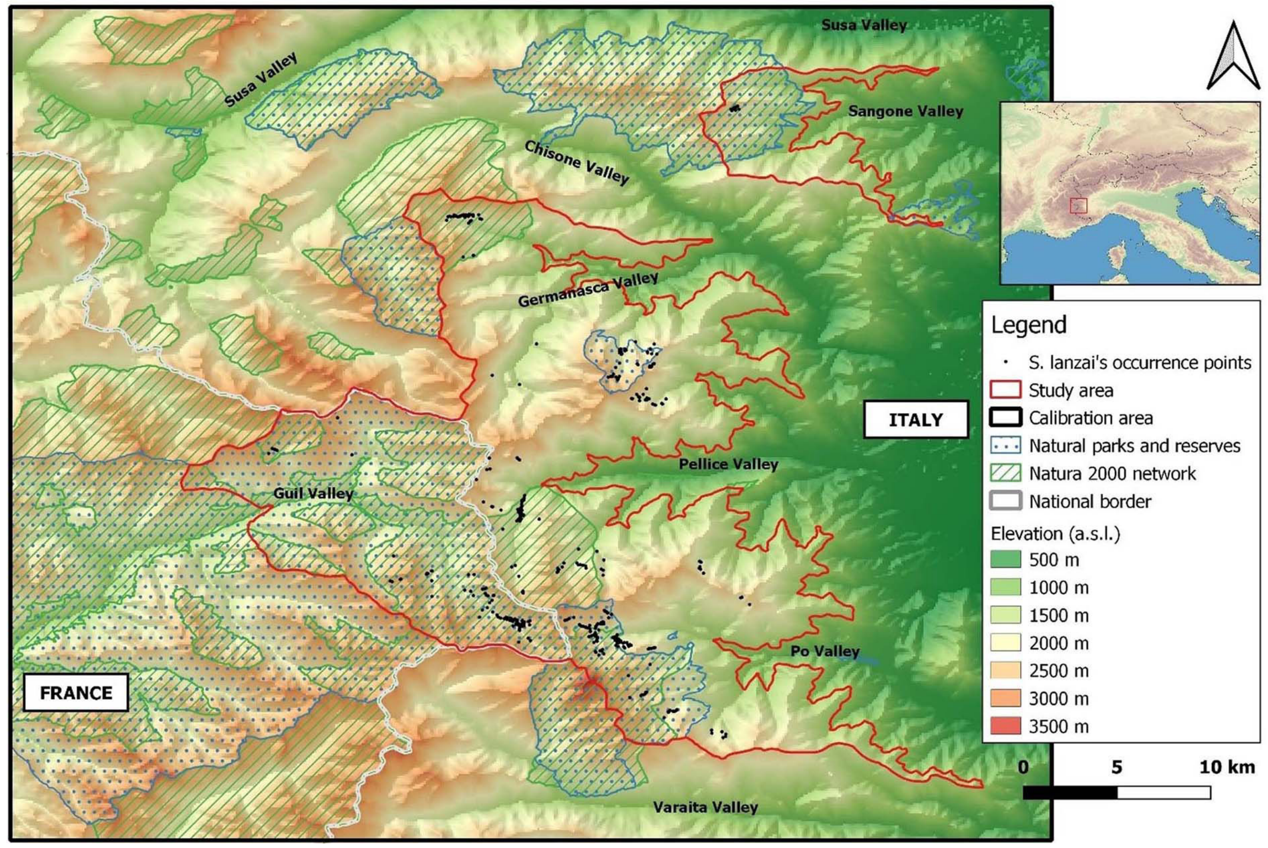

Figure 1. Map of the calibration area (black box), in which the perimeter of the subset study area is highlighted in red. Black dots represent the Lanza's alpine salamander's occurrences used to calibrate the models (N = 360). The extent of Natura 2000 sites (in green) and natural parks and reserves (in blue) is also reported. The Digital Terrain Model (DTM) provides the baseline map.

array of 25 topographic, bioclimatic and environmental predictors (Table 1; resolution: ~150 m, see Appendix S1.4), after evaluating the inter-correlation between parameters and the relative importance of each variable (Appendix S1.3). Secondly, since future projections are available only for temperature and precipitations data, a further variable selection procedure was carried out considering only bioclimatic and topographic predictors (the latter assumed to be constant through time), in order to allow the calibration of an additional "bioclimatic suitability model", required as a preparatory step to run future predictions. In this case, the minimum temperature of the coldest month (Bio06), precipitation seasonality (Bio15), total annual precipitations (Bio12) and mean temperature of the driest quarter of the year (Bio09) were the selected parameters.

Future projections of Bio06, Bio09, Bio12 and Bio15 were obtained from the CMIP6 downscaled future climate projections, according to three Global Circulation Models (GCMs) (ACCESS-ESM1-5; MPI-ESM1-2-HR; MIROC6) and two Shared Socio-economic Pathways (SSPs; Meinshausen et al., 2020) (SSP126, low CO_2 emissions; SSP585, high CO_2 emissions) in two time periods (short-term, 2041–2060; long-term, 2081–2100) (see Appendix S1.3). Accordingly, 12 different projections of the selected variables were considered to predict the future bioclimatic suitability for the Lanza's alpine salamander (i.e. 3 GCMs x 2 SSPs x 2 time periods).

Modelling procedure

An ensemble forecasting method (Araújo & New, 2007) was applied to model the environmental and bioclimatic suitability for *S. lanzai* within the calibration area, involving eight widely-used niche-based algorithms, all available in the biomod2 package (ver. 4.2.2; Thuiller et al., 2023) in R (ver. 4.2.2; R Core Team, 2022): Generalized Boosting Model (GBM; Ridgeway, 1999), Classification Tree Analysis (CTA; Breiman et al., 1984), Flexible Discriminant Analysis (FDA; Hastie et al., 1994), Generalized Additive Models (GAM; Hastie & Tibshirani, 1990), Generalized Linear Models (GLM; McCullagh & Nelder, 1989), MaxEnt (MXT; Phillips et al., 2006), Multiple Adaptive Regression Splines (MARS; Friedman, 1991) and Random Forest (RF; Breiman, 2001). All the algorithms were run 10 times, each one corresponding to a randomly-selected series of 500 pseudo-absence points throughout the calibration area. A spatial block cross-validation strategy (Muscarella et al., 2014) was applied to test models' predictive power, then quantified by means of two evaluation metrics: the true skill statistic (TSS; Allouche et al., 2006) and the area under the relative operating characteristic curve (AUC; Fielding & Bell, 1997). Model robustness was evaluated five times per algorithm in each one of the ten performed runs, in order to obtain an average value of model performances, and the final models were calibrated on 100 % of the data. Then, the response of the Lanza's alpine salamander to each predictor was evaluated by applying the evaluation strip method proposed by Elith et al. (2005).

Table 1. List of the 25 candidate predictors considered in the first phase of the modelling process regarding *S. lanzai*. For each predictor type, all variables are listed reporting their code, a short description and the data source (SRTM = Shuttle Radar Topography Mission). The parameters selected for the final model calibration (ESM = environmental suitability model; BSM = bioclimatic suitability model) are highlighted in bold.

Type	Code	Description	ESM	BSM	Source
Topographic	DTM	Elevation			SRTM (Farr et al., 2007)
	Exp	Exposition			SRTM (Farr et al., 2007)
	Slo	Slope			SRTM (Farr et al., 2007)
	TPI	Topographic Position Index			SRTM (Farr et al., 2007)
Bioclimatic	Bio01	Mean annual temperature			WorldClim 2.1 (Fick & Hijmans, 2017)
	Bio02	Mean diurnal range			WorldClim 2.1 (Fick & Hijmans, 2017)
	Bio03	Isothermality			WorldClim 2.1 (Fick & Hijmans, 2017)
	Bio04	Temperature seasonality			WorldClim 2.1 (Fick & Hijmans, 2017)
	Bio05	Max temperature of warmest month			WorldClim 2.1 (Fick & Hijmans, 2017)
	Bio06	**Min temperature of coldest month**	**X**	**X**	WorldClim 2.1 (Fick & Hijmans, 2017)
	Bio07	Temperature annual range			WorldClim 2.1 (Fick & Hijmans, 2017)
	Bio08	Mean temperature of the wettest quarter			WorldClim 2.1 (Fick & Hijmans, 2017)
	Bio09	**Mean temperature of driest quarter**	**X**	**X**	WorldClim 2.1 (Fick & Hijmans, 2017)
	Bio10	Mean temperature of warmest quarter			WorldClim 2.1 (Fick & Hijmans, 2017)
	Bio11	Mean temperature of coldest quarter			WorldClim 2.1 (Fick & Hijmans, 2017)
	Bio12	**Total annual precipitation**	**X**	**X**	WorldClim 2.1 (Fick & Hijmans, 2017)
	Bio13	Precipitation of wettest month			WorldClim 2.1 (Fick & Hijmans, 2017)
	Bio14	Precipitation of driest month			WorldClim 2.1 (Fick & Hijmans, 2017)
	Bio15	**Precipitation seasonality**		**X**	WorldClim 2.1 (Fick & Hijmans, 2017)
	Bio16	Precipitation of wettest quarter			WorldClim 2.1 (Fick & Hijmans, 2017)
	Bio17	Precipitation of driest quarter			WorldClim 2.1 (Fick & Hijmans, 2017)
	Bio18	Precipitation of warmest quarter			WorldClim 2.1 (Fick & Hijmans, 2017)
	Bio19	Precipitation of coldest quarter			WorldClim 2.1 (Fick & Hijmans, 2017)
	Srad06	**Total solar radiation June**	**X**		WorldClim 2.1 (Fick & Hijmans, 2017)
Environmental	**NDVI07**	**NDVI July**	**X**		MOD13A3.061 (Didan, 2021)

Model runs with TSS values below 0.7 were discarded from the ensemble forecasting procedure, with consensus distributions resulting from the averaging of model predictions, proportionally weighted basing on their TSS evaluation. Final environmental and bioclimatic suitability maps were then obtained by averaging ensemble forecasts from the ten pseudo-absence runs. In addition, the original suitability maps were transformed into maps of suitable vs unsuitable areas (binary maps), by choosing the occurrence probability threshold that maximised the TSS value (Liu et al., 2005; Jiménez-Valverde & Lobo, 2007).

Future predictions were built starting from the current bioclimatic suitability model outputs, but considering the future projections of Bio06, Bio09, Bio12 and Bio15 as predictors. Firstly, three bioclimatic suitability maps and three binary maps were generated per time period and per SSP, according to the future predictions provided by the three GCMs considered. Then, final maps (N = 4, one per time period and per SSP) were obtained by averaging the results from the three GCMs. In the final binary maps, presence was attributed where the majority of GCMs (i.e. two out of three) predicted presence, otherwise attributing absence.

Model validation in the field

In addition to the statistical model performance evaluation, targeted field surveys were carried out within the calibration area by well-trained operators (D.S., P.E.B., R.C., M.F. & D.G.), in order to verify the actual occurrence of *S. lanzai* in the suitable areas predicted by the ensemble models for the current scenario. In particular, previously little-explored areas were selected for surveys, profiting from model outputs to search for possible new populations or to highlight local inconsistencies in predictions.

Between June and early September 2022, nine sectors of the calibration area (Fig. 2) were inspected following a standard monitoring protocol, based on visual encounter surveys in two sampling sessions per area. Each survey was carried out in rainy days and/or by night, searching for surface-dwelling Lanza's alpine salamanders in their typical habitats. The explored area was tracked with a GPS, georeferencing each observation, while if no individuals were found in both sampling sessions, the species was considered as absent from the surveyed sector.

Spatial analyses

In order to obtain quantitative data from the ensemble model outputs, some spatial analyses were performed on the final suitability maps, focusing in particular on binary

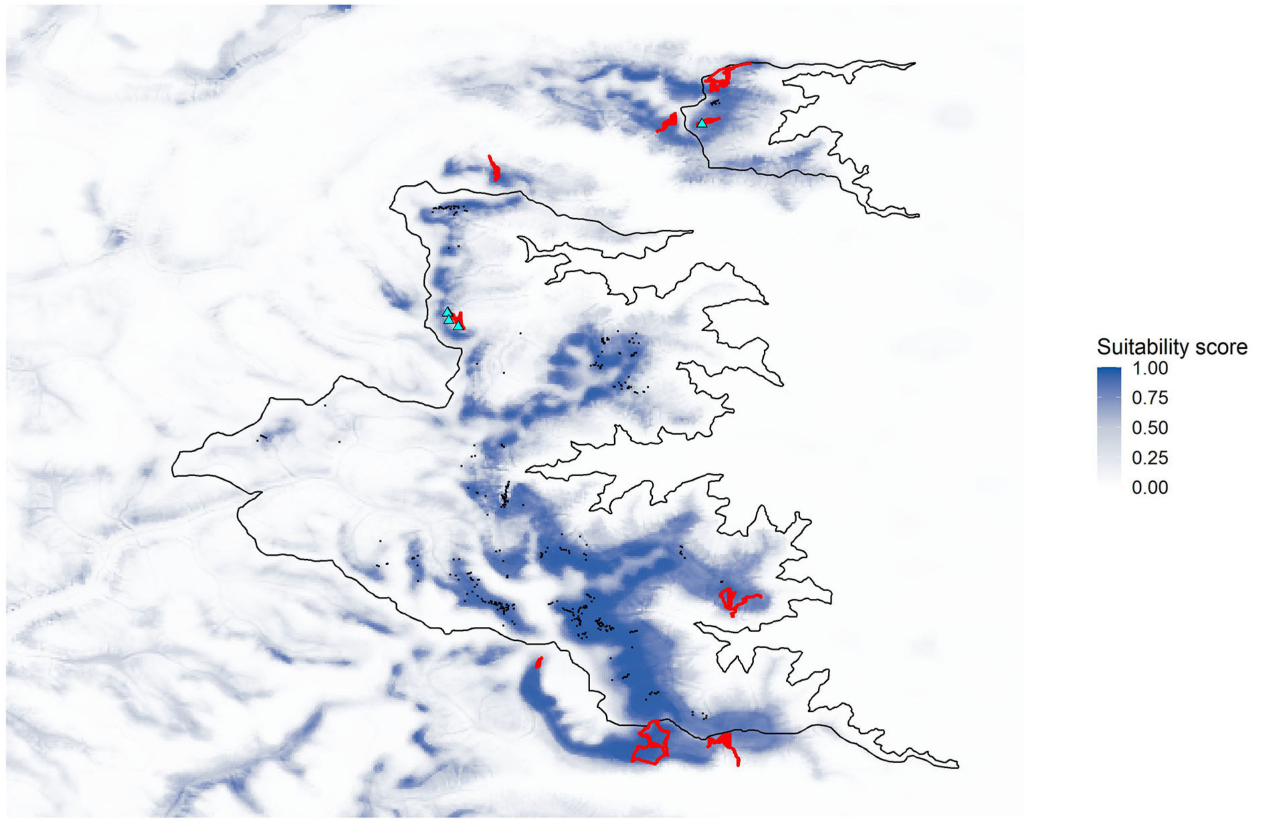

Figure 2. Map reporting the GPS tracks (in red) recorded during the targeted surveys carried out to validate the ensemble model results in the field. The light-blue triangles highlight the new occurrence points of *S. lanzai* resulting from these surveys (N = 14). Black dots represent the Lanza's alpine salamander's occurrences used to calibrate the models (N = 360), while the black line identifies the perimeter of the study area considered in spatial analyses. The baseline map reports the suitable area for *S. lanzai*, as predicted by the environmental suitability model.

maps. Since occurrence data and targeted field surveys proved that the current distribution of *S. lanzai* actually covers only a portion of the overall predicted suitable area, spatial analyses were carried out considering a subset area (assumed to correspond to the G_0 area, sensu Peterson et al., 2011; hereafter "study area"; 778 km², Fig. 1), outlined within the calibration area following an expert-based approach (Appendix S1.1).

The binary maps cropped on the study area were used to calculate the predicted extent of the suitable area for *S. lanzai*, its overlap with protected areas (i.e. natural parks, natural reserves and Natura 2000 sites) and the potential elevation range, also highlighting future variations based on the two SSP and the two time periods considered in the modelling procedure. Moreover, two additional distribution parameters (extent of occurrence, EOO; area of occupancy, AOO) were calculated by means of the red package (ver. 1.5.0; Cardoso, 2020) in R (R Core Team, 2022), in accordance with the guidelines to assess species' extinction risk provided by the International Union for Conservation of Nature (IUCN Standards and Petitions Committee, 2022).

RESULTS

Model performance

Most of the algorithms involved in the environmental suitability model showed a good performance, with an average TSS of 0.832 ± 0.079 and a mean AUC of 0.955 ± 0.034. RF is the modelling technique that performed better, followed by GBM and CTA, while GAM and GLM showed the worst predictive power. Similar results in terms of model performance were obtained by the bioclimatic suitability model (mean TSS: 0.788 ± 0.011; mean AUC: 0.942 ± 0.044), with RF as best performing algorithm, followed by GBM, CTA, and MXT (Table 2).

According to the fitted response curves (Fig. S2.1, S2.2), the probability of occurrence of *S. lanzai* within the calibration area is highest where the total solar radiation in June (Srad06) is about 23000 Kj/m², where the minimum temperature of the coldest month of the year (Bio06) is about -9 °C, where NDVI values in July (NDVI07) fall between 0.40 and 0.65, where the mean temperature of the driest quarter of the year exceeds 11 °C and where precipitations show a relatively low variability throughout the year (Bio15 = 17–20 %). A bimodal response is highlighted concerning the annual precipitations (Bio12), with the highest probability of occurrence at 800 mm and 1200 mm.

Current potential Lanza's alpine salamander distribution

According to the environmental suitability model, the current potential range of *S. lanzai* in the study area covers 226 km² (Fig. 3a–c), with a mean elevation of 2159 ± 250 m a.s.l. The core suitable area is located around the Monviso massif, extending in particular in the upper Po, Pellice and

Table 2. Evaluation metrics (mean ± standard deviation) concerning the environmental and bioclimatic suitability models, calculated for each one of the eight algorithms considered in the ensemble modelling procedure (TSS = true skill statistic; AUC = area under the relative operating characteristic curve). Algorithms are listed in alphabetical order (CTA = Classification Tree Analysis; FDA = Flexible Discriminant Analysis; GAM = Generalized Additive Models; GBM = Generalized Boosting Model; GLM = Generalized Linear Models; MARS = Multiple Adaptive Regression Splines; MXT = MaxEnt; RF = Random Forest).

Algorithm	Environmental suitability model		Bioclimatic suitability model	
	TSS	AUC	TSS	AUC
CTA	0.855 ± 0.036	0.956 ± 0.020	0.847 ± 0.038	0.954 ± 0.021
FDA	0.793 ± 0.029	0.952 ± 0.010	0.727 ± 0.046	0.926 ± 0.013
GAM	0.767 ± 0.072	0.919 ± 0.052	0.670 ± 0.097	0.879 ± 0.069
GBM	0.882 ± 0.017	0.987 ± 0.003	0.865 ± 0.023	0.978 ± 0.006
GLM	0.760 ± 0.038	0.935 ± 0.021	0.700 ± 0.065	0.920 ± 0.020
MARS	0.806 ± 0.029	0.957 ± 0.009	0.748 ± 0.047	0.935 ± 0.013
MXT	0.810 ± 0.032	0.936 ± 0.017	0.766 ± 0.047	0.944 ± 0.020
RF	0.987 ± 0.004	1.000 ± 0.000	0.983 ± 0.006	1.000 ± 0.000

Guil valleys. This area is in connection northwards with other suitable patches located in the upper Germanasca Valley, constituting a continuous suitable range until the Albergian massif. Conversely, the environmental suitability for the Lanza's alpine salamander appears to be more fragmented in the westernmost part of its range, with a single isolated patch identified in the Malrif area. Then, the model also predicts a disjunct suitable area in the upper Sangone Valley.

The 47.8 % (108 km²) of the environmentally suitable area currently overlaps with protected areas, with the Natura 2000 network contributing in *S. lanzai*'s protection on 86 km² (38.0 %), while natural parks and reserves cover 75 km² (33.2 %) of the potential species range within the study area. According to the IUCN criteria and basing on the environmental model outputs, the extent of occurrence (EOO) of the Lanza's alpine salamander covers 1058 km², while the area of occupancy (AOO) extends for 560 km².

Similar results in terms of core range were obtained by running the bioclimatic suitability model, although the outputs proved to be less conservative than the environmental ones in the study area, predicting a climatically suitable area of 274 km² (+21.2 %) (Fig. 3b–d). In particular, this model partly extends the current predicted range of *S. lanzai* towards lower elevations (mean = 2136 ± 262 m). However, more than two-thirds (70.4 %; 193 km²) of the climatically suitable area overlap with the potential range identified by the environmental suitability model, which in turn is mostly a subset of the eligible area from a bioclimatic point of view (i.e. 85.4 % of overlap).

The suitable area outlined by the bioclimatic suitability model overlaps with protected areas on 114 km² (41.6 %), showing also in this case a major contribution of Natura 2000 sites (88 km²; 32.1 %), while 80 km² (29.2 %) are covered by natural parks and reserves. The predicted bioclimatic EOO is 959 km², encompassing an AOO of 656 km².

Model validation in the field

Overall, during the targeted field surveys 108 km were walked within the suitable area predicted by the ensemble model. However, the occurrence of the Lanza's alpine salamander was confirmed in only two of the nine inspected sectors, namely the Balma Valley (Sangone Valley) and the Rodoretto Valley (Germanasca Valley) (Fig. 2). In particular, only two individuals were observed in the Balma Valley, while a relatively abundant population was detected in the Rodoretto Valley (N = 12). Despite the predicted high environmental suitability, *S. lanzai* appears to be absent in the Chisone Valley and on the southern slope of the Monviso massif (Varaita Valley).

Future scenarios

The future projections of the bioclimatic suitability model forecast a dramatic reduction and fragmentation of the climatically suitable area for *S. lanzai*, especially in the western and northern portions of its range, with considerable effects already predicted in the short term, even considering an optimistic emissions scenario (Fig. 4–5). Indeed, a -95.6 % reduction of the potential range of this species is expected in the study area for the period 2041–2060 in the SSP126 projection, limiting the bioclimatic suitability for the Lanza's alpine salamander to 12.1 km². In the same period, applying the worst emissions scenario (SSP585), the predicted range contraction is -98.9 %, resulting in only 3 km² of suitable area. In the long term (2081–2100), a further range reduction is foreseen, leading to a climatically suitable area of 5.8 km² in the SSP126 scenario (-52.1 % from 2041–2060; -97.9 % from 2000–2020), while the bioclimatic suitability for *S. lanzai* is expected to run out from the study area in the SSP585 projection. Looking at the expected future trend of the predictors involved in the modelling procedure (Bio06, Bio09, Bio12 and Bio15), the bioclimatic suitability for the Lanza's alpine salamander in the study area will be largely compromised by increasing temperatures, together with a reduction in annual precipitations (predicted by the SSP585 scenario), and progressively higher values of precipitation seasonality (Fig. S2.3).

A declining trend is also highlighted by the IUCN distribution parameters calculated on the bioclimatic suitability model outputs (Fig. 6a–b). A -64.1 % EOO reduction is predicted in the short term in the SSP126 scenario (344 km²), reaching the -95.1 % in the SSP585 condition (47 km²). Then, the EOO is expected to further shrink (SSP126: -74.9 %; 241 km²) in 2081–2100, even going to zero if the worst emissions scenario becomes a reality. Accordingly, the AOO shows a sudden decrease already in the 2041–2060 period while considering an optimistic emissions scenario (SSP126; -84.8 %; 100 km²), even exacerbated in the long term (-95.7 %; 28 km²). A -91.5 % AOO reduction (56 km²) is expected in the short term according to the SSP585 projection, coming to a theoretical extinction of *S. lanzai* in 2081–2100.

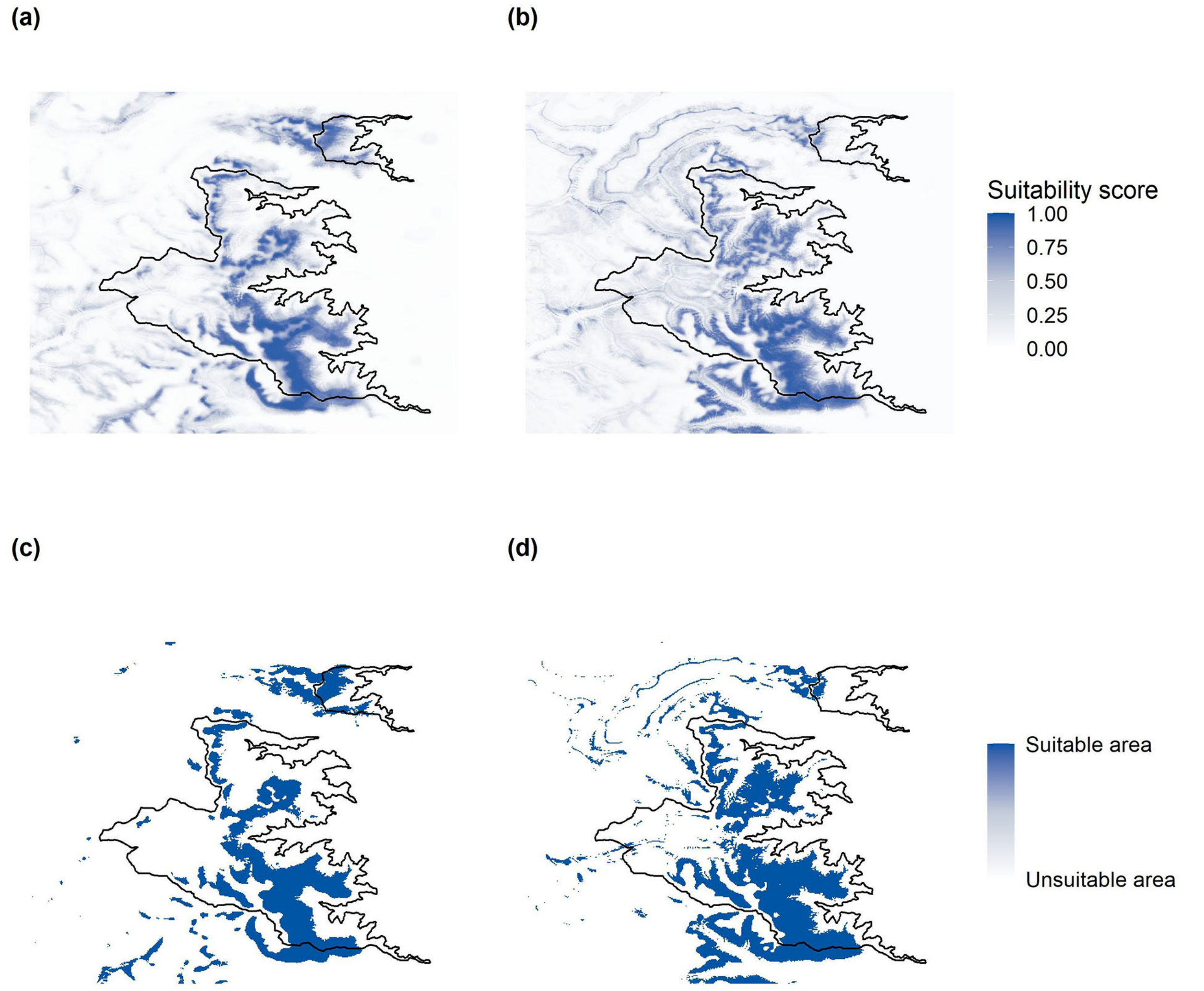

Figure 3. Current potential range of *S. lanzai* within the calibration area, as predicted by the environmental (a-c) and bioclimatic (b-d) suitability models. The suitable area is further highlighted in blue in the binary maps (c-d), according to the occurrence probability threshold that maximizes the TSS values. The black line identifies the perimeter of the study area considered in spatial analyses.

Following the predicted range contraction for the Lanza's alpine salamander and considering the current extent of protected areas (natural parks, natural reserves, and Natura 2000 sites), the climatically suitable area under formal environmental protection is expected to decrease in 2041–2060 (-10.2 %; 3.8 km²) according to the SSP126 scenario, but then slightly increasing (+3.1 %; 2 km²) towards the end of the century (Fig. 6c). Conversely, more than a half (56.7 %) of the remaining suitable area in 2041–2060 is forecasted to fall within protected areas in the SSP585 condition, although corresponding to an area of only 1.7 km². In this context, the Natura 2000 network is expected to fail in its contribution for *S. lanzai*'s protection, covering only the 12.4 % of the eligible area in 2041–2060 and the 3.4 % in 2081–2100 (SSP126), while no overlap between the species potential range and Special Areas of Conservation (SACs) is forecasted according to the SSP585 projections (Fig. 6c).

Together with a general reduction of the climatically suitable area for the Lanza's alpine salamander, future projections forecast an upward shift in the altitudinal range of the species (Fig. 6d). In particular, the mean predicted elevation is foreseen to shift of +324 m considering the SSP126 scenario in 2041–2060, increasing to +460 m in the SSP585 condition. Then, no significant changes in mean elevation are expected in 2081–2100 according to the SSP126 projection (-5 m from 2041–2060). Furthermore, predictions suggest that this upward shift will be more pronounced at the lower elevational limit for *S. lanzai*, where a +542 m shift is expected for 2041–2060 in the SSP126 scenario, compared with a change of only +4 m at the upper limit of the climatically suitable area for the species. This trend is even more apparent in the SSP585 projection, with a variation of +880 m and +4 m foreseen in the short term at the lower and the upper range limits respectively. In the long term, the lower elevational limit for the species is still expected to move upwards in the SSP126 condition (+141 m from 2041–2060), while the model forecasts no changes in the maximum elevation of the suitable area.

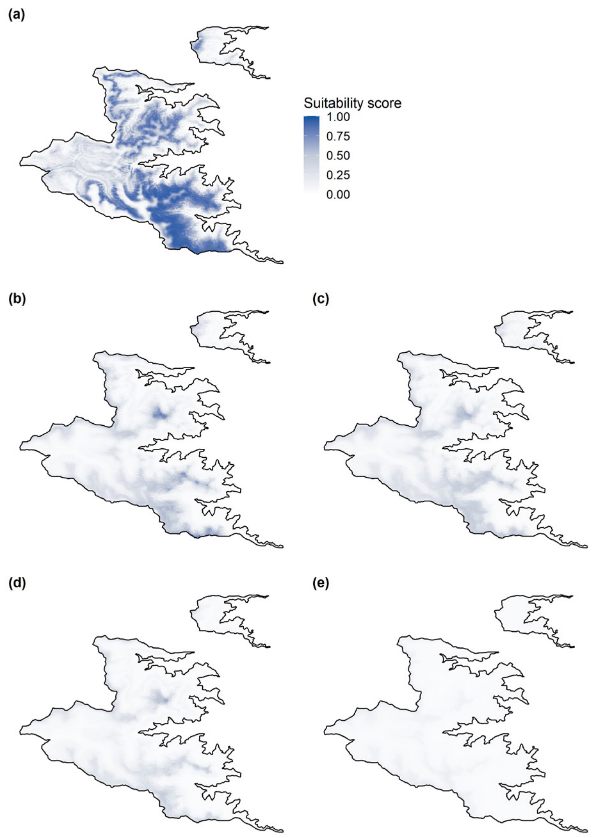

Figure 4. Future projections of the bioclimatic suitability for *S. lanzai* within the study area, according to two Shared Socio-economic Pathways (b, c: SSP126; d, e: SSP585) and two time periods (b, d: 2041–2060; c, e: 2081–2100). In a, the current bioclimatic suitability map is reported for comparison.

DISCUSSION

The SDM approach applied in this study provides a high-resolution statistical evaluation of the environmental and bioclimatic preferences of *S. lanzai* considering its whole distribution range. Although the effect of many fine-scale habitat features (e.g. micro-climatic conditions, availability of subterranean habitats, vegetation composition, etc.) and the species' thermoregulatory behaviour are not accounted for in the modelling procedure, the response of the Lanza's alpine salamander to the selected predictors is in accordance with the known ecological requirements of the species (Andreone, 2006), allowing for reliable projections in space and time. In particular, the overall propensity of this urodele for alpine grasslands and shrublands in relatively cold and humid (rainy) areas is confirmed, with cloud cover in summer positively affecting the occurrence probability of *S. lanzai*, highlighting a response to solar radiation similar to other mountain salamanders (e.g. Campbell Grant et al., 2018; Jacobsen et al., 2020).

Accordingly, the current environmental and bioclimatic suitability predicted by the ensemble models largely overlaps with the actual distribution range of the Lanza's alpine salamander, as confirmed by the new

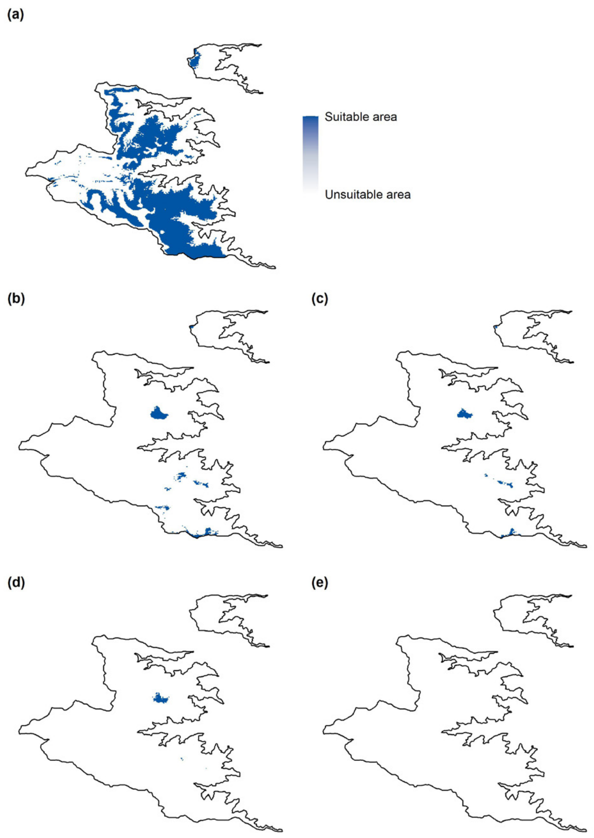

Figure 5. Future projections of the bioclimatic suitability for *S. lanzai* within the study area, according to two Shared Socio-economic Pathways (b, c: SSP126; d, e: SSP585) and two time periods (b, d: 2041–2060; c, e: 2081–2100). In this case, the predicted suitable area (in blue) is calculated according to the occurrence probability threshold that maximizes the TSS values. In a, the binary map concerning the current bioclimatic suitability is reported for comparison.

occurrence data collected in previously little-explored suitable areas during the targeted field surveys. However, models appear to overestimate the suitability for this amphibian in some portions of the calibration area, where observations are still lacking despite the specific field research carried out to validate model projections, leading to the exclusion of some environmentally and climatically suitable mountain sectors from the study area (likely falling outside the G_0 area, i.e. the actually occupied species range; Peterson et al., 2011; see Appendix S3).

In this context, although in previous studies on vertebrates the accuracy of climate-based SDMs was little improved after the addition of non-climatic predictors (Bucklin et al., 2015), the environmental suitability model shows a higher fitting with occurrence data than the bioclimatic suitability one (see AUC values), likely due to the major effect of June's solar radiation and July's NDVI in explaining *S. lanzai*'s distribution, exceeding the predictive capacity of most bioclimatic predictors. This results in a more conservative estimate in terms of extent of suitable area compared with bioclimatic model

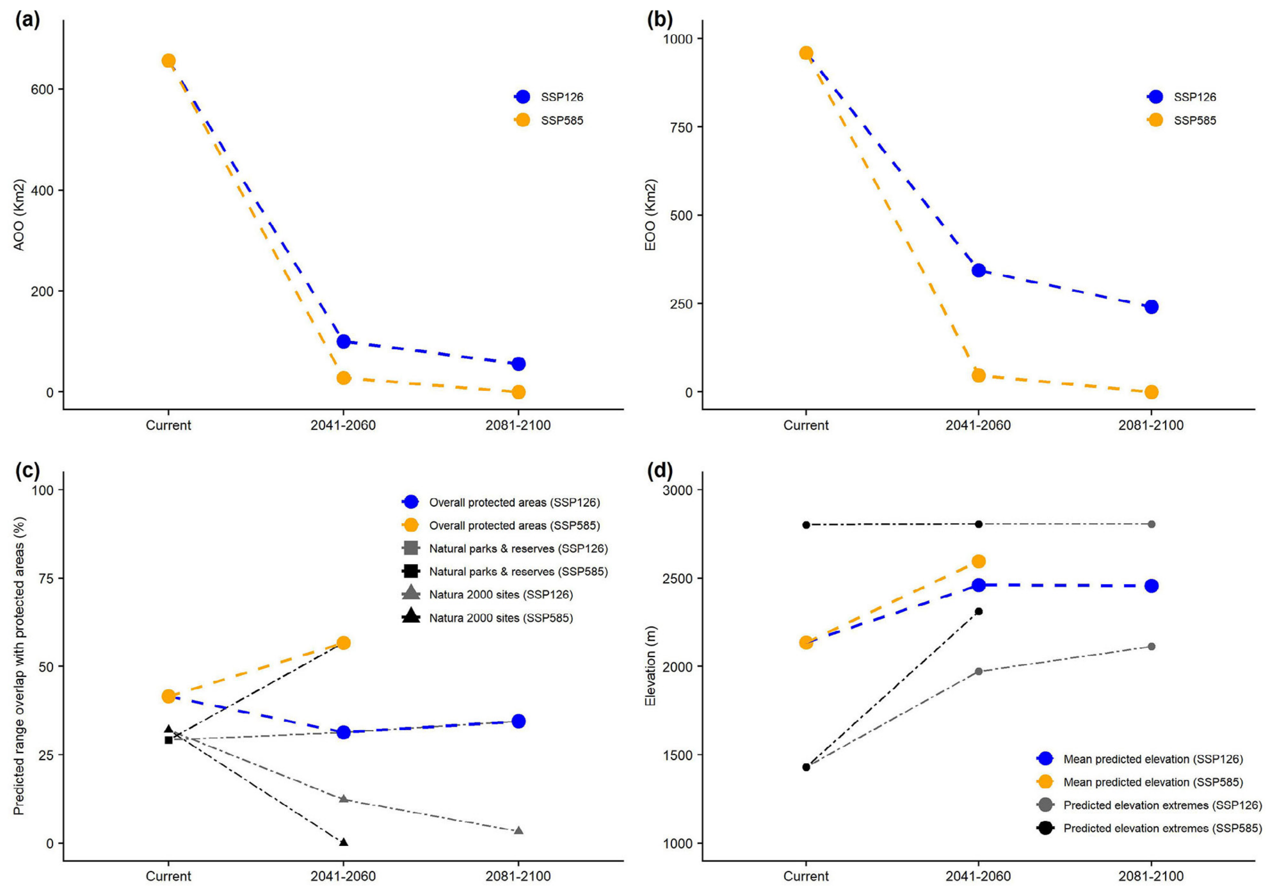

Figure 6. Plots reporting the current and predicted (2041–2060; 2081–2100) values concerning (a) the area of occurrence (AOO), (b) the extent of occurrence (EOO), (c) the range overlap with protected areas (natural park, natural reserves and Natura 2000 sites), and (d) the mean, minimum and maximum elevation of the distribution range for *S. lanzai* within the study area, according to the bioclimatic model outputs. Future projections are based on two Shared Socio-economic Pathways (SSP126 and SSP585).

outputs (226 km² vs. 274 km²), to be used as reference to outline the current Lanza's alpine salamander's potential distribution, especially when planning short-term management and conservation projects or land use changes within the study area.

On the other hand, despite its lower accuracy, the bioclimatic suitability model represents an essential baseline to perform future predictions on *S. lanzai*'s suitable area, owing to the lack of future projections for all the parameters considered in the environmental suitability model. However, the relationship observed in the current scenario between environmental and bioclimatic suitability has to be taken into account when analysing future projections, especially considering that the environmentally suitable area may continue to represent a subset of the climatically suitable one through time (Kearney & Porter, 2009). Therefore, the dramatic range reduction predicted by the ensemble models in the next decades might be even worse, since the actual eligible area for Lanza's alpine salamanders in the future may cover only a portion of the remaining climatically suitable mountain sectors.

According to ensemble model forecasts, the suitable area for *S. lanzai* from a bioclimatic point of view will already decline significantly in the next 20–40 years, even considering an optimistic CO_2 emissions scenario, leading to a theoretical extinction of this species within the study area in 2100 in case the SSP585 condition will be actualised. As documented for other montane salamanders (e.g. Jacobsen et al., 2020; Lyons & Kozak, 2020), rising temperatures are one of the major factors in limiting the future bioclimatic suitability for the Lanza's alpine salamander, confirming global warming as an additional threat to the conservation of this critically endangered amphibian. Furthermore, changes in precipitation regimes within the study area are also expected to impact this species, especially if a reduced amount of rain will fall in few months throughout the year, thus increasing precipitation seasonality.

Together with an overall range contraction, ensemble models predict a considerable shift towards higher elevations of future suitable bioclimatic conditions for *S. lanzai*, as already predicted in central Alps for the alpine salamander *Salamandra atra* (Feldmeier et al., 2020), with pronounced changes expected in particular at the lower elevational limit for this species (as for instance observed by Campbell Grant et al., 2018). Given the rapidity and the size of the process, the complex mountain topography and the limited dispersal capacity of Lanza's alpine salamanders, many low-altitude populations may not be able to successfully track the predicted changes in bioclimatic suitability, possibly exacerbating the extinction risk for this amphibian in some sectors of the study area (Forero-Medina et al., 2011). Moreover, environmental suitability may not necessarily follow bioclimatic suitability shifts, resulting in an increasing proportion of actually unsuitable habitats towards mountain tops (e.g. rocky slopes, cliffs, etc.), in a context already constrained by the limited surface available (Elsen & Tingley, 2015), adding further limitations to the future upslope dispersal of salamanders.

The dramatic scenario highlighted by model predictions underlines the urgent need of up-to-date conservation and management strategies to ensure a successful mitigation of climate change effects on *S. lanzai*'s populations. First of all, the role of climatic variations in threatening the Lanza's alpine salamander has to be officialised by including this factor in the IUCN extinction risk assessment, a recognised tool to support decision-makers in setting conservation priorities, although no regulatory value is given to IUCN Red Lists. Indeed, the current assessment is based only on the presumed effect of the spreading lethal fungal pathogen *Batrachochytrium salamandrivorans* (*Bsal*), precautionarily listing *S. lanzai* as Critically Endangered (CR) in accordance with the criterion E (IUCN SSC Amphibian Specialist Group, 2022). Thanks to the estimation of the current and future extent of occurrence (EOO) and area of occupancy (AOO), this study provides useful data to incorporate also the criterion B (geographic range; IUCN Standards and Petitions Committee, 2022) in the Lanza's alpine salamander's assessment, although the expected changes in bioclimatic suitability cannot worsen the current threat category. However, a future range contraction and fragmentation have to be taken into account when considering the *Bsal*'s threat on *S. lanzai*, also considering the increased risk of contact with other upslope-dispersing amphibian species (Tiberti et al., 2021), including the congeneric European fire salamander *Salamandra salamandra* (Sillero, 2021).

A second important issue is that Lanza's alpine salamander populations should be adequately covered by protected areas, given its threat category and the strict protection regime required by the EU Directive 92/43/EEC, ensuring the application of effective and legally binding conservation measures. Today, less than 50 % of the environmentally suitable area for this urodele is included within natural parks and reserves or in Natura 2000 sites (with considerable gaps, especially in Italy), and this proportion seems to be maintained or even reduced in the future, according to the projected bioclimatic suitability. In this context, the EU Biodiversity Strategy for 2030 provides the opportunity for the designation of new protected areas in the western Alps, since Italy (as all EU member states) is asked to increase its proportion of legally protected land up to 30 % (i.e. +10 %; MITE, 2021) in the next few years. The current and future suitability maps provided in this study can guide decision-makers in identifying the most important sectors where *S. lanzai* needs protection, considering possible future range shifts due to climate change and candidate refuge areas (e.g. on the Cialancia massif and in the Po Valley), possibly including this species among the priorities considered to justify the enlargement or new designation of natural parks or Natura 2000 sites.

However, the establishment of new protected areas is not sufficient itself to increase the actual conservation status of the Lanza's alpine salamander. Active management, surveillance, and research are required to transform "paper parks" into effective biodiversity conservation authorities. For instance, all the known additional threats for *S. lanzai*'s conservation should be removed immediately, in order to prevent future detrimental combined effects with climate change. This is the case of roadkill mortality, severely affecting some Italian populations at low elevations along mountain roads, despite their overlap within Natura 2000 sites. As resulted by ensemble model predictions, low-altitude Lanza's alpine salamander populations are expected to be the most impacted by changes in bioclimatic suitability, thus requiring an urgent regulation addressed to minimise the passage of vehicles along roads intercepting suitable areas for salamanders, especially by night and in rainy or foggy days between May and September. Furthermore, since *S. lanzai* occurs in habitats that can be altered by overgrazing and livestock trampling, local grazing exclusion areas can be identified, as already implemented in France.

In conclusion, this study attempts to forecast a future reaction of the Lanza's alpine salamander to climate change, focusing in particular on space (i.e. possible range shifts following changes in bioclimatic suitability). However, this should be considered only as a first step towards a complete understanding of the process, since this species may track future climatic variations also by shifting its climatic niche along two other non-exclusive directions (Bellard et al., 2012): time (i.e. phenology) and self (i.e. physiology and behaviour). Thus, future bioclimatic suitability shifts might be buffered by phenological, physiological or behavioural adaptations, that should be considered as the next research target on this endemic urodele. In addition, continued monitoring is essential to follow through time the response of *S. lanzai*'s populations to changing climate and environment, confirming or rejecting the model predictions provided here, evaluating the effects of possible additional threatening factors (e.g. overgrazing, shrub encroachment, etc.) and hopefully testing the feasibility of possible climate change mitigation measures.

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The tadpole of *Telmatobius fronteriensis* (Anura, Telmatobiidae), from Puquios, western of the Andes Range, northern Chile

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INTRODUCTION

Frogs of the genus *Telmatobius,* Wiegmann, are typical inhabitants of high Andean aquatic environments, such as small streams, thermal springs, lakes and lagoons (Lavilla & De la Riva, 2005). This diverse taxon (63 species; Frost, 2023) extends from the south of Ecuador to the North of Argentina, and its altitude ranges from 1,000 to 5,000 m.a.s.l. (Barrionuevo, 2017; Cei, 1986). The taxonomy of this genus has been considered remarkably complex due to low interspecific and intraspecific variations (De la Riva, 2005; Barrionuevo, 2017). Seven endemic species have been described in the Chilean Andes Range's western slopes: *T. chusmisensis*, *T. dankoi*, *T. fronteriensis*, *T. halli*, *T. pefauri*, *T. philippii* and *T. vilamensis*. Among these species, only the larva of *T. fronteriensis* is unknown, which prevents a better understanding of the importance of larval morphology for the systematics of the genus; so their tadpole is described for the first time in this work.

The morphological characters of anuran tadpoles have been successfully used for taxonomic purposes (Larson & de Sá, 1998; Aguilar & Pacheco, 2005; Aguilar & Valencia, 2009; Aguilar et al., 2007). Thus, Lavilla (1985; 1988) and Altig & McDiarmid (1999b) redefined the genus *Telmatobius* considering larvae's external characters. Consequently, based on the oral morphology of tadpoles, Lavilla (1985) proposed two phenetic groups in *Telmatobius* species: "the meridional" and the "septentrional" groups. On the other hand, Sáez et al. (2014), based on molecular analyses, studied the phylogenetic relationships of central Andes *Telmatobius* species in Chile and Bolivia, showing they form three phylogenetic groups: the *Telmatobius marmoratus* group, widespread in the Chilean and Bolivian Altiplano; the *Telmatobius hintoni* group, including the species *T. philippii*, *T. fronteriensis* and *T. huayra*, occurring in the south-western Altiplano of Chile and Bolivia; and the *Telmatobius pefauri* group, a new clade which includes *T. chusmisensis*, *T.dankoi* and *T. vilamensis*, restricted to the western slopes of the Andes in Chile.

In this study, we describe for the first time the tadpole of *T. fronteriensis*, a small-sized frog (mean snout-vent length 39.28 ± 2.56 mm; Benavides et al., 2002), endemic to the type locality of Puquios (northern Chile). We also comparatively analysed the external morphology of the larvae of the Chilean species of *Telmatobius*, and we explore the degree of similarities/inequalities that exist among the phylogenetic groups proposed by Sáez et al. (2014) and corresponding species tadpoles. Furthermore, the presence of *Batrachochytrium dendrobatidis* and *Ranavirus* is explored.

MATERIALS & METHODS

Specimens

Five tadpoles and 21 adults of *T. fronteriensis* were captured (permit N° 301/2015 from Agricultural and Livestock Service) in the type locality (Puquios, Fig. 1A). For comparative purposes we analysed the larvae of another six Chilean *Telmatobius* species (data in Table 1). At the time of capture (19–20 October 2015, austral spring), adults (Fig. 1B) and larvae were detected on the edge or in the middle of the stream (Fig. 1C), in cavities or among aquatic vegetation. Five tadpoles of *Telmatobius* species and two adults were euthanised in a solution of 4 g/L of tricaine methanesulfonate and preserved in 10 % formalin for posterior analysis. Tadpoles were assigned to *T. fronteriensis* because no further amphibian species were found in this area and its surroundings. Collected and examined specimens (tadpoles and adults) are housed in the Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Valdivia, IZUA. Measurement data of tadpoles are presented in Table 2.

Methods

Larval stages were determined according to Gosner (1960). Measurements and terminology follow Altig & McDiarmid (1999a) for total length (TL), body length (BL), and tail length (TAL); Lavilla & Scrocchi (1986) for body width (BW), body width at the narial level (BWN), body width at eye level (BWE), body height (BH), eye-snout distance (ESD), eye-nostril distance (END), nostril-snout distance (NSD), eye diameter (ED), narial diameter (ND), snout-spiracular distance (SSD) and oral disc width (ODW); Grosjean (2005) for dorsal fin height (DFH) and

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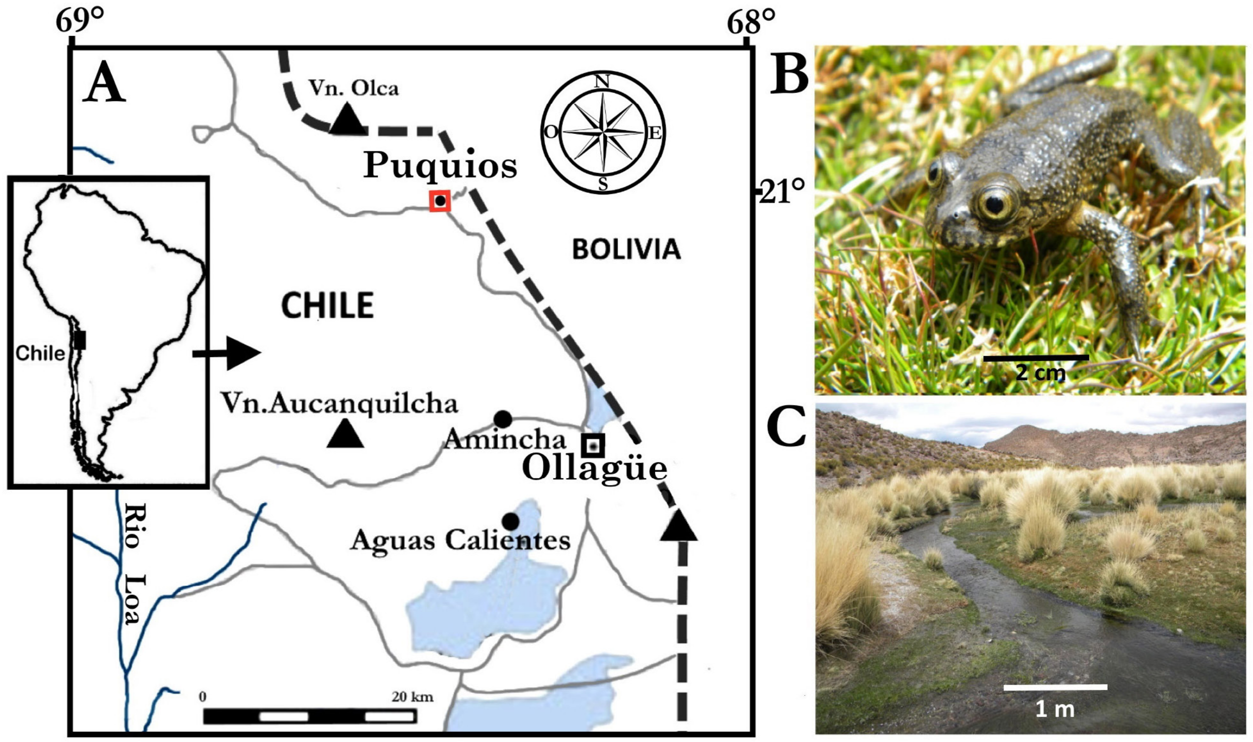

Figure 1. **(A)** Puquios, type locality of *T. fronteriensis*, **(B)** Adult male of *T. fronteriensis*, and **(C)** Stream where adult and tadpoles were captured.

Table 1. Tadpoles of the *Telmatobius* species and capture localities

Species	Voucher	n	High (m.s.n.m)	Localities/Province/Region	Co-ordinates
T. fronteriensis	IZUA 3657	5	4,104	Puquios (Type Locality)/El Loa/Antofagasta	21° 00'08,70'' S; 68° 22'42,40'' W
T. chusmisensis	IZUA 3662	5	3,365	Chusmisa/Iquique/Tarapacá	19° 40'44.92'' S; 69° 10'47.26'' W
T. dankoi	IZUA 3657	2	2,169	Las Cascadas/El Loa/Antofagasta	22° 30'14.75'' S; 68° 58'21.67'' W
T. halli	IZUA 3558	2	3,717	NearOllague/El Loa/Antofagasta	21° 17'49,39'' S, 68° 20'08,68'' W
T. pefauri	IZUA 3657	3	3,535	Chapiquiña/Parinacota/Arica y Parinacota	18° 20'46.60'' S; 69° 33'18.67'' W
T. philipii	IZUA 3657	1	3,845	Quebrada de Amincha/El Loa/Antofagasta	21° 11'55.10'' S; 68° 20'09,30'' W
*T. vilamensis**	IZUA 3082	5	3,110	Río Vilama/El Loa/Antofagasta	22° 52'05.80'' S; 68° 10'54,90'' W

* Data from Formas et al. (2003)

ventral fin height (VFH). We use the configuration of the spiracle and vent, according to Altig & McDiarmid (1999a). The neuromast topography was named according to Lannoo (1987). The shape of the tail tips was determined following Savage (2002). All measurements were taken to the nearest 0.1 mm. Collected tadpoles and additional 21 adults of *T. fronteriensis* were non-invasive sampled (skin and oral swabs respectively) and tested through specific real-time PCR assays for the presence of two pathogens: *Batrachochytrium dendrobatidis* and *Ranavirus*, following previously described methods (Soto-Azat et al., 2016).

Taxonomic criteria and number of specimens

To avoid nomenclatural confusion, we use the taxonomic proposition of Fibla et al. (2017), which suggests a synonymy involving priority of the name *Telmatobius pefauri* over *T. zapahuirensis*. Consequently, we use *T. pefauri* for the species and clade previously named *Telmatobius zapahuirensis*. The presence of *T. fronteriensis* in this restricted area (Puquios), and the inaccessibility of this place during some periods of the year prevent the capture of additional individuals. Furthermore, the critical state of conservation of the Chilean species of *Telmatobius* (Lobos & Rojas, 2020) justifies the description of the tadpoles *T. fronteriensis* based on scant material (five tadpoles).

RESULTS

The larvae of *T. fronteriensis* correspond to a morphological generalised type (Orton, 1953) and belong to the benthic ecomorphological guild type of Altig & McDiarmid (1999b). The body length corresponds to 40, 2 % of the total length (BL/TL = 0.40). Body shape oval in dorsal view, with maximum body width at abdominal region.

Table 2. Measurements (mm) of five tadpoles of *T. fronteriensis* (stages according to Gosner, 1960.) Characters are defined in the maintext.

Specimens	Stages				
Character (mm)	**32**	**34**	**35**	**36**	**41**
TL	68.5	71.1	71.5	65.2	66.0
BL	26.0	25.5	28.1	24.2	23.8
TAL	42.5	46.2	43.4	41.0	42.2
BMW	12.5	15.0	18.2	19.8	14.0
BWN	9.0	9.0	10.1	9.4	9.0
BWE	17.0	12.5	14.8	12.0	11.0
BMH	17.5	14.8	14.9	12.0	11.1
DFH	4.0	4.0	4.0	2.8	2.6
VFH	3.0	3.0	2.8	1.5	2.0
RSD	17.0	15.5	19.0	20.0	13.9
FN	4.6	4.7	3.0	4.1	4.0
END	2.9	2.5	3.1	2.0	2.6
IO	4.6	4.9	6.2	4.5	6.4
E	1.8	1.7	2.1	2.1	2.0
EN	2.1	2.0	3.0	2.9	2.8
OD	6.1	7.0	7.8	6.0	6.0
DG	4.8	4.9	5.0	3.9	3.2

The body is slightly rounded in lateral view (BMH/BMW = 0.77); the ventral contour of the body is flat at the level of the head and body (Fig. 2A). The dorsal contour is convex from the oral disc's anterior edge to the eyes' posterior border. In the dorsal view, the snout is slightly rounded (Fig. 2B). The nares are oval without protuberances. Dorsolaterally oriented and situated dorsally (BWN/ BWE = 0.74), closer to the snout than the eye (FN/END =66.0). Eyes relatively small (E/BWE = 0.10), situated dorsolaterally (IO/BWE = 0.47) (Fig. 2B). The spiracle is single, lateral, sinistral, directed posterodorsally, almost as long as broad, and placed approximately halfway between the snout and posterior margin of the body (Fig. 2A). Its inner wall presents as a slight ridge and is visible in dorsal and lateral views; its opening is oval, located below the body midline, and its diameter is smaller than the tube diameter, opening only visible in lateral view. The inner wall is attached to the body but perfectly distinguishable. Vent tube dextral, with an opening not visible laterally, concealed by a fold of vent tube wall; right wall attached anteriorly to ventral fin (Fig. 2C). The oral disc is anteroventral located and transversally elliptical (Fig. 2D). Medium-sized (OD/BMW = 0.43), not emarginated, and with a medium dorsal gap (DG/OD = 0.62) in marginal papillae; with mental papillae (Fig. 2D). Marginal papillae are simple, small, and conical, arranged in a single row in the anterior (upper) labium; placed in a single row in the posterior (lower) labium. There are 15–16 submarginal papillae in the anterior and posterior lip. The papillae are small, round, or conical with rounded or pointed tips. Submarginal papillae in mental area absent (Fig. 2D). Jaw sheaths are robust, more expansive than high, there are 15–17 /mm of finely serrated triangular

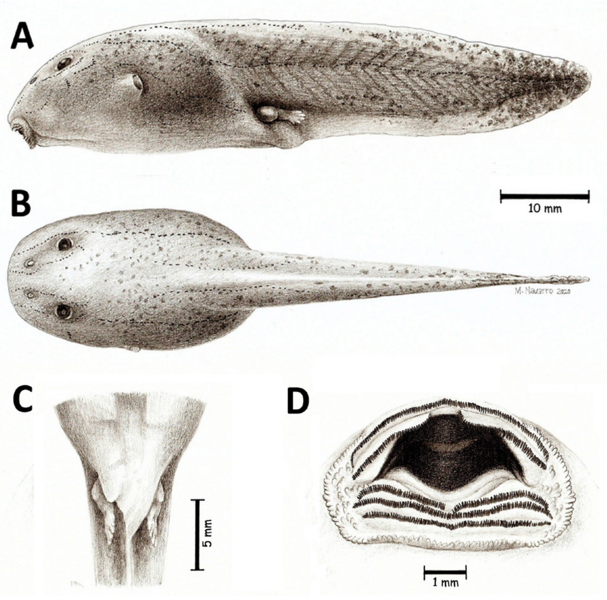

Figure 2. The tadpole of *T. fronteriensis* (IZUA N° 3657, stage 36) – **(A)** Lateral, **(B)** dorsal, and **(C)** ventral views. **(D)** Oraldisc.

serrations on the upper jaw, wide and brown pigmented. The upper jaw sheath is arc-shaped, and the lower jaw sheath is V-shaped. The free margin of the upper jaw sheath is widely arch-shaped, with a short lateral process. Lower jaw sheath V-shaped. The labial tooth row formula 2(2)/3(1); A2 = A1, P1 = P2 > P3; P4; gaps in A-2 and P-1 about 1.0 and 0.5 mm respectively. A2 > A1, P1 = P2 > P34; gaps in A-2 and P-1 about 1.4 and 0.8 mm. Tooth density on A1 23–55 teeth /mm.

The tail is large (TaL/TL = 0.43), with well-developed musculature, almost reaching the tail tip. The tail axis is straight and the tail tip is gently rounded. Dorsal and ventral fins of similar height; dorsal fin not extending onto the body; ventral fin starting at the end of vent tube; both fins almost as high as body height (MTH/BMH = 1.1). Lines of the neuromasts are present on the tail (dorsal and medial) (Fig. 2A), dorsum (dorsal) (Fig. 2B), around the eyes (anterior oral) (Fig. 2B) and belly (ventral).

Tadpole colouration

When the larvae were observed among the aquatic vegetation, they were olive green in colour (Fig. 3). In life, out of the water and exposed to the sun (Fig. 4A,B,C) the body and tail are leaden blues, covered with fine golden spots. In fixative (10% formalin) the body and tail are greyish and the golden colouration disappears.

Natural History Notes

Puquios belongs to the Puna biogeographic province, which is characterised by its aridity, rocky substrate, and xerophytic vegetation: Yareta (*Azorella compacta*) and low grasses and bushes (*Festuca ortophylla*, *Stipa nardoides*). In Ollagüe, a town near Puquios (15 km), the temperature ranges between 1.6 °C (July) and 10.2 °C (January). During two years (1989 and 1998) of observation, no rainfall was recorded, and in the period 1972–2014, only 69.2 mm of rainfall was recorded at the Ollagüe weather

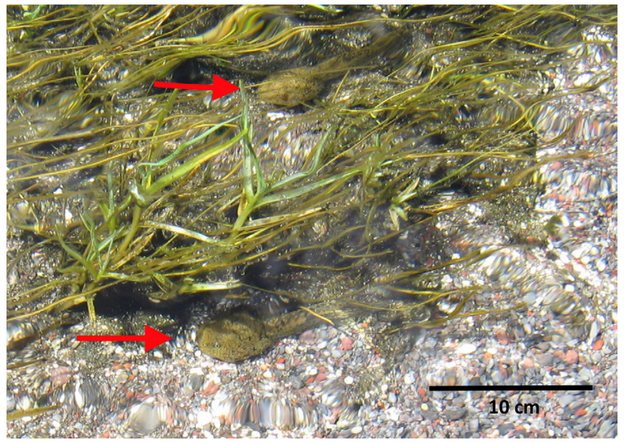

Figure 3. Tadpoles (red arrows) of *T. fronteriensis* under aquatic plants of the genus *Potamogeton*.

Figure 4. Colour of live larvae. **(A)** Lateral and **(B)** dorsal views of tadpoles of *T. fronteriensis* (stage 31). **(C)** Tadpole of *T. fronteriensis* (stage 39).

station (Sarricolea et al., 2017). On 19 October 2015, in Puquios during fieldwork, between 1230 h and 1300 h the temperature was 20 °C and the humidity was 23.4 %.

Adults and tadpoles of *T. fronteriensis* were captured among the aquatic vegetation (*Potamogeton* sp) of a low-flow stream (3 km long, 1 m wide, and 40 cm deep) (Fig. 3); the water temperature was 22 °C. The bottom consisted of mud (<1 mm), (0.5–2.0 mm) and was large (11–100 mm).

Disease screening

The five collected tadpoles and additional 21 adults, all gave negative results for both *B. dendrobatidis* and *Ranavirus*.

Variation

Among the tadpoles examined we found one (Fig. 4C) that differed from the rest. From the posterior third of the caudal fin, its edge drops steeply. In the other tadpoles, this edge descends gently. During the fieldwork, we found larvae in different stages of development (Gosner, 1960) which may be evidence of a larval development period of more than one year.

DISCUSSION

Díaz & Valencia (1985) studied the external morphology of four *Telmatobius* species (*T. halli* [specimens from Vilama river now *T. vilamensis*], *T. pefauri*, *T. marmoratus* and *T. peruvianus*) present in Chile, and noted the remarkable homogeneity of their external morphological characteristics. So, the bauplan of these larvae corresponds to a lentic type, e.g. their larvae have in tendency a globular body, median to high fins, a non-robust tail musculature, and a pointed tail end (Altig & McDiarmid, 1999a; Laudor et al., 2021) (See Figs. 2 & 5). In northern Chile, these prevailing morphological traits are frequently found in lentic and lotic environments. Probably because lotic environments do not present great differences in flow compared to lentic environments (generally streams 1 m wide and 20 cm deep, see Fig. 1). This situation differs from those of lotic environments with fast running waters (mountain streams) where highly modified reophilous tadpoles are found, such as *T. espadai* and *T. sanborni* (suctorial oral disc, and tail muscle more robust) (Aguilar et al., 2007). However, in this work, we found variations in some of their external characters. For example, the tadpoles *T. halli* differ from the analysed tadpoles in the following characteristics: the spiracular aperture is rounded, the inner wall is wholly attached to the body wall and the dorsal fin originates in the anterior third of the tail. On the other hand, the BL/TL index ranges between 0.36 (*T. chusmisensis*) and 0.51 (*T. halli*). This comparative analysis showed that at the interspecific level, some characters are "variable" (e.g. spiracle, the tip of the tail) and others "more constant" (e.g. tooth row formula, oral disc emarginate/not emarginate). Thus, to improve the interspecific comparisons of Chilean *Telmatobius* tadpoles, it would be helpful to standardise the characters to be considered (variable/constant, developmental states) so that comparisons help to establish their taxonomic identity and the morphological patterns involved. Some characteristics of those tadpoles are presented in Table 3.

Among the generalised (sensu Orton, 1953) *Telmatobius* tadpoles, Lavilla (1985) recognised two well-defined phenetic groups: the first, with only one row of sub-marginal mental papillae ("meridional" group), whereas the second ("septentrional") lacks this row. All species examined in this work belong to the "septentrional" group. Barrionuevo & Baldo (2009) and Barrionuevo (2017) questioned the phylogenetic validity of Lavilla's hypothesis. However, the significance of these structures should be evaluated appropriately.

Sáez et al. (2014) studied the phylogenetic relationships of 19 *Telmatobius* species from the central Andes of Chile and Bolivia, including individuals from 12 undescribed populations of Chile. This analysis based on mitochondrial DNA 16S and cytochrome b shows that the Chilean endemic species belong to two groups (clade) species: (a) the *T. hintoni* (*T. fronteriensis* and *T. philippii*) and

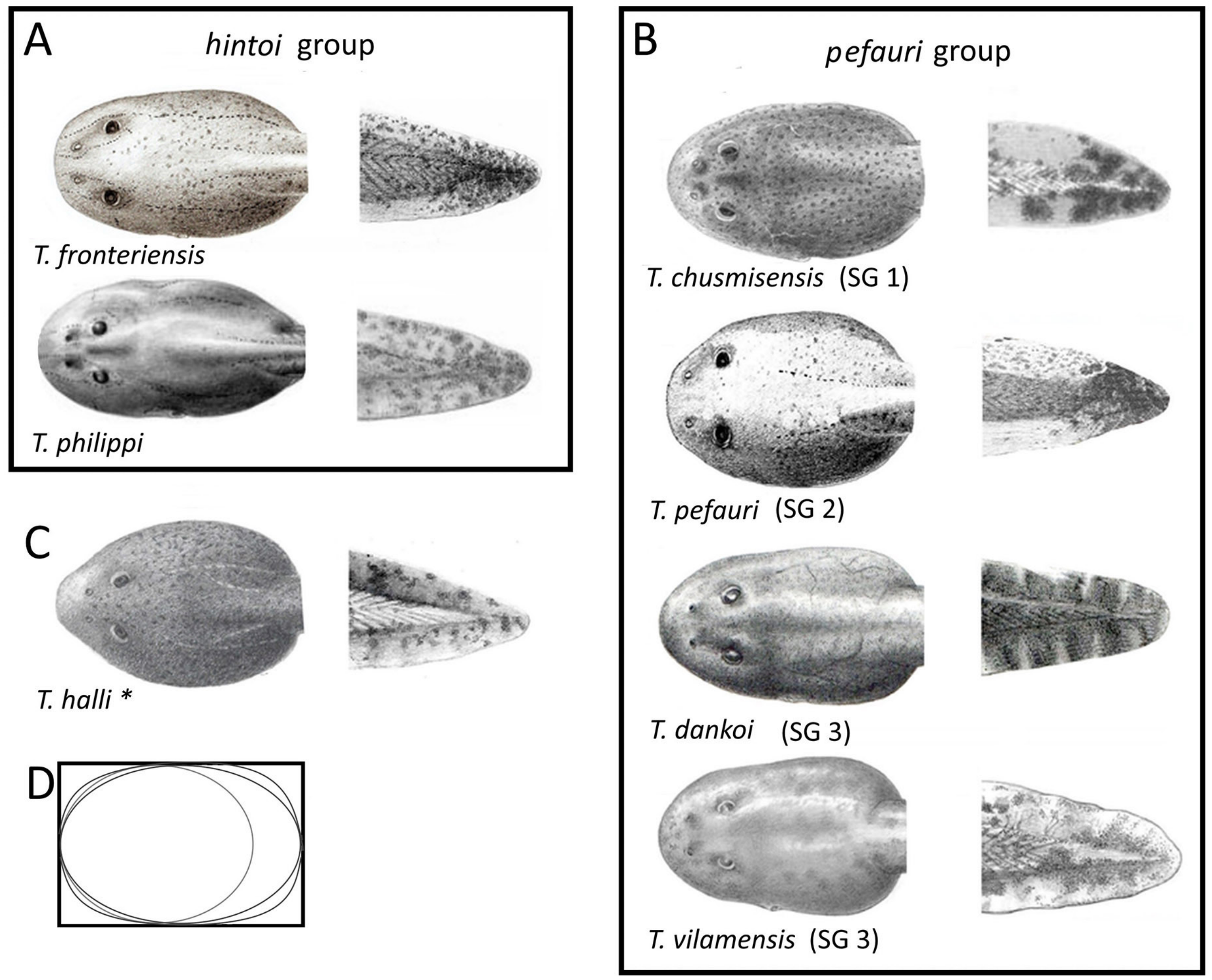

Figure 5. Dorsal view of body and tail tips of seven Chilean endemic *Telmatobius* tadpoles – **(A)** hintoi group, and **(B)** pefauri group. **(C)** *T. halli* not in Sáez et al. **(D)** Body shape criteria (frame from inside out): rounded, ovoid, rectangular. Drawings (not to scale) taken from original publications, except *T. fronteriensis* and *T. pefauri*. SG Subgroup.

Table 3. External morphological comparisons among the tadpoles of seven Chilean *Telmatobius* species. Stages according to Gosner (1960). BL = body length, LT = total length.

Species	Stage	BL/ TL	Spiracle: direction and shape of its aperture	Dorsal fin origin	Bodyform	Tip tail form and pigmentation	Source
T. fronteriensis	36	0.39	posterior, ovoid	contact the body	ovoid	gently rounded, pigmented	This paper
T. philippii	33	0.42	posterior, ovoid	contact the body	rectangular	gently rounded, gently pigmented	Cuevas & Formas, 2002; This paper
T. pefauri	36	0.39	posterior, ovoid	contact the body	ovoid	gently rounded, pigmented	This paper
T. chusmisensis	36	0.40	posterior, ovoid	contact the body	rectangular	gently rounded, pigmented	Formas et al., 2006; This paper
T. dankoi	37	0.36	posterior, ovoid	contact the body	ovoid	rounded, pigmented	Formas et al., 1999; This paper
*T. vilamensis**	35	0.44	posterior, ovoid	contact the body	ovoid	rounded, gently pigmented	Formas et al., 2003
T. halli	33, 34	0.51	lateral, rounded	anterior third tail	approximately rounded	bluntly pointed, gently pigmented	Cuevas et al., 2020; This paper

* Data from Formas et al. (2003)

(b) the *T. pefauri*, the latter having three subgroups: the first, *T. chusmisensis*, the second, *T. pefauri*; and the third includes, *T. dankoi* and *T. vilamensis*. This group (b) is restricted to the western slopes of the Andes. In this work tadpoles of the six exclusive Chilean *Telmatobius* species (*T. fronteriensis*, *T. philippi*, *T. chusmisensis*, *T. pefauri*, *T. dankoi* and *T. vilamensis*) were distributed in each group proposed by Sáez et al. (2014) (Fig. 5AB), and their external morphological characteristics compared (Table 3). As a result of this exercise, we observed differences between the members of different groups. For example, the members of the "hintoi" group differ in body shape: ovoid in *T. fronteriensis* and rectangular (elongated) in *T. philippii*. Among the tadpoles of the "pefauri" group, in *T. chusmisensis* (subgroup 1) and *T. pefauri* (subgroup 2) differ just in body form, ovoid (*T. chusmisensis*) and rounded (*T. pefauri*). Finally, both members of subgroup 3 share the body and tail form (ovoid and rounded respectively), however, the tail is gently pigmented in *T. vilamensis*, and strongly pigmented in *T. dankoi*, being the more consistent group respecting Sáez et al. proposition. *Telmatobius halli* (not in Sáez et al., 2014) (Fig. 5C) is the most dissimilar tadpole (body rounded with snout acuminate, tail shape bluntly pointed and the opening of the spiracle tube directed perpendicular to the body wall). These observations showed the differences that exist among the external characters and suggest that heterogeneity is a frequent phenomenon in these tadpoles. Although our observations are preliminary, they suggest that the revision of the criteria of morphological homogeneity should be reconsidered in species of the genus *Telmatobius* tadpoles (and adults).

Telmatobius spp. has been one of the most impacted groups of amphibians by *B.dendrobatidis* (Scheele et al., 2019) and no published information exists on the impacts of *Ranavirus* to this amphibian group. It is likely that the apparent absence of both pathogens (all 26 samples negative) is due to the isolation of the stream used by this species. It is therefore useful to assess the potential threat posed by an unwanted introduction of both emerging pathogens to *T. fronteriensis*. Finally, to increase knowledge of the biology of *Telmatobius* species on the western slope of the Andes in northern Chile, it is convenient to determine the taxonomic identity of the populations analysed by Sáez et al. (2014), describe their tadpoles for a better understanding of the taxonomy of this genus, and thus be able to address future conservation actions.

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