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Spatial and temporal variation in detection probability and abundance of freshwater turtles in a peri-urban area of Midwestern Brazil

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Abstract. This study assessed spatial (across water bodies) and temporal (over months and years) variation in the detection probability and abundance of freshwater turtles, along with the effects of climatic variables such as precipitation and temperature. Fieldwork was conducted in four water bodies located at the headwaters of a stream in the peri-urban area of Campo Grande, Mato Grosso do Sul, in Midwestern Brazil. Over 38 months of sampling (2014–2017), we captured 94 individuals: 73 *Phrynops geoffroanus*, 19 *Mesoclemmys vanderhaegei*, and two *Trachemys dorbigni*. The detection probability of *M. vanderhaegei* was influenced by both precipitation and temperature, whereas detection of *P. geoffroanus* was affected only by temperature. The abundance of both species varied across water body types and sampling months, with *P. geoffroanus* being more abundant in water bodies where *M. vanderhaegei* was less common. *P. geoffroanus* reached its highest abundance during the rainy season (November to March), with fluctuations in the subsequent months. In contrast, *M. vanderhaegei* maintained low abundance throughout most of the year, with a peak in December. To our knowledge, this is the first study to examine population patterns of these two syntopic species. *Phrynops geoffroanus*, the more abundant species, is often recorded in anthropogenically altered environments and appears to possess greater competitive ability than *M. vanderhaegei*, which is less abundant and likely more sensitive to environmental degradation. Despite the urban context and the presence of an invasive species (*T. dorbigni*), the occurrence of *M. vanderhaegei* suggests that the study area may still retain important elements of habitat quality.

Keywords. Chelidae, chelonians, Testudines, urban ecology, *Phrynops geoffroanus, Mesoclemmys vanderhaegei*, *Trachemys dorbigni.*

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INTRODUCTION

Knowledge about chelonian ecology is still incipient in South America. Population studies are now more frequent, but studies on the structure of assemblages or on the temporal partition of resources of the different species of freshwater turtles are rare, even in the Amazon basin, where the diversity of these reptiles high (Mittermeier et al., 2015). This highlights the challenge of conducting studies on freshwater turtles in natural environments.

Due to their ease of access, water bodies in urban areas can be considered excellent sites for obtaining information on different aspects of the ecology of freshwater turtles (Rocha et al., 2025; Dorado-Rodrigues et al., 2024). On the other hand, urbanization imposes significant pressure on wildlife, altering natural environments through deforestation, which leads to vegetation fragmentation, species loss, and biodiversity decline (Guzy et al., 2013; Liu et al., 2016; Rocha et al., 2025). Nevertheless, many turtles tolerate, adapt to, and survive in urbanized environments, even those of poor quality, as long as they have access to resources essential for their survival (e.g., food, shelter, and reproductive sites) (Gibbons et al., 2000; Luiselli, 2003; Stokeld et al., 2014; Spencer et al., 2018; Rocha et al., 2025).

Freshwater turtles inhabit different types of habitats, terrestrial and aquatic, which vary according to their basic ecological needs as a function of their semi-aquatic habitat (Bodie and Semlitsch, 2000). Terrestrial and aquatic habitats (whether lentic or lotic) undergo changes due to variations promoted by the hydrological cycle which, in turn, affect the availability of resources, and consequently the quality of the habitat (Alho and Pádua, 1982; Fachín-Terán et al., 2006). As a result of these environmental variations, freshwater turtles adjust their preexisting strategies – such as feeding, thermoregulation, and reproduction – to habitat alterations to ensure their survival (Moll and Moll, 2004). Thus, habitat use by freshwater turtles may vary according to periods of drought and rainfall, since these aspects directly influence the spatial distribution and structure of assemblages by affecting the distribution and supply of resources in the environment (Plummer et al., 1997; Luiselli, 2008).

In this study, we evaluated the effects of population parameters, such as detectability and abundance, for two species of freshwater turtles in relation to temporal (sampling period, rainfall, and temperature) and spatial (water bodies) characteristics. We tested the following hypotheses: (1) the detection probability of freshwater turtles varies with monthly precipitation and temperature; (2) The abundance of freshwater turtles varies spatially among water bodies and temporally with sampling months and temperature, as well as with the interaction between water bodies and sampling months; and (3) the abundance of a species varies with the area of the water body and/or the abundance of syntopic species.

MATERIALS AND METHODS

Study area

The study area is located in the urban perimeter of the municipality of Campo Grande (Mato Grosso do Sul, Midwestern Brazil), situated in the *Cerrado* biogeographic domain and the Upper Paraguay river basin (20°23’00”S and 54°36’00”W; Datum WGS84; Fig. 1). The study was conducted in a vegetation fragment of 191 hectares of which 20 hectares are designated for the protection area called legal reserve in Brazil (Cheung et al., 2016; Corral et al., 2021). The fragment is located in an area of urban expansion and is part of a region that includes both forest and open formations. The forest formations range from *cerradão* – a dense forest formation characterized by tall trees (8–15 m), a closed canopy, a sparse grassy understory, and occurrence in fertile, humid soils – to riparian vegetation, including gallery and riparian forests (Sano et al., 2008; Cheung et al., 2016). The open formations include *cerrado stricto sensu* and *campo sujo*. *Campo sujo* refers to herbaceous-shrubby habitats dominated by grasses, with few trees and shrubs (<2% of the land cover; Furley, 1999).

The climate in the region is of Aw type (tropical climate with a dry winter season), according to the Köppen’s classification (Peel et al., 2007). The rainy (October to March) and dry (April to September) seasons are well defined (see Marcuzzo and Mello, 2011). The average annual temperature is 24°C and the annual rainfall is between 1800 and 2000 mm.

Data collection

We sampled four water bodies located at the headwaters of the Segredo stream in a landscape with fragmented *Cerrado* vegetation (Fig. 1). We collected information on the freshwater turtle assemblage between March 2014 and July 2017, using funnel traps 1.2 m in length (see description in Brito et al., 2009; 2018; Balestra et al., 2016). The traps were baited with ground beef and remained in the water diurnally, being reviewed once a day over five consecutive days per month. The number of traps ranged from two to four traps per water body (Table 1). The minimum distance between traps within the same water body was 100 meters, while the maxi-

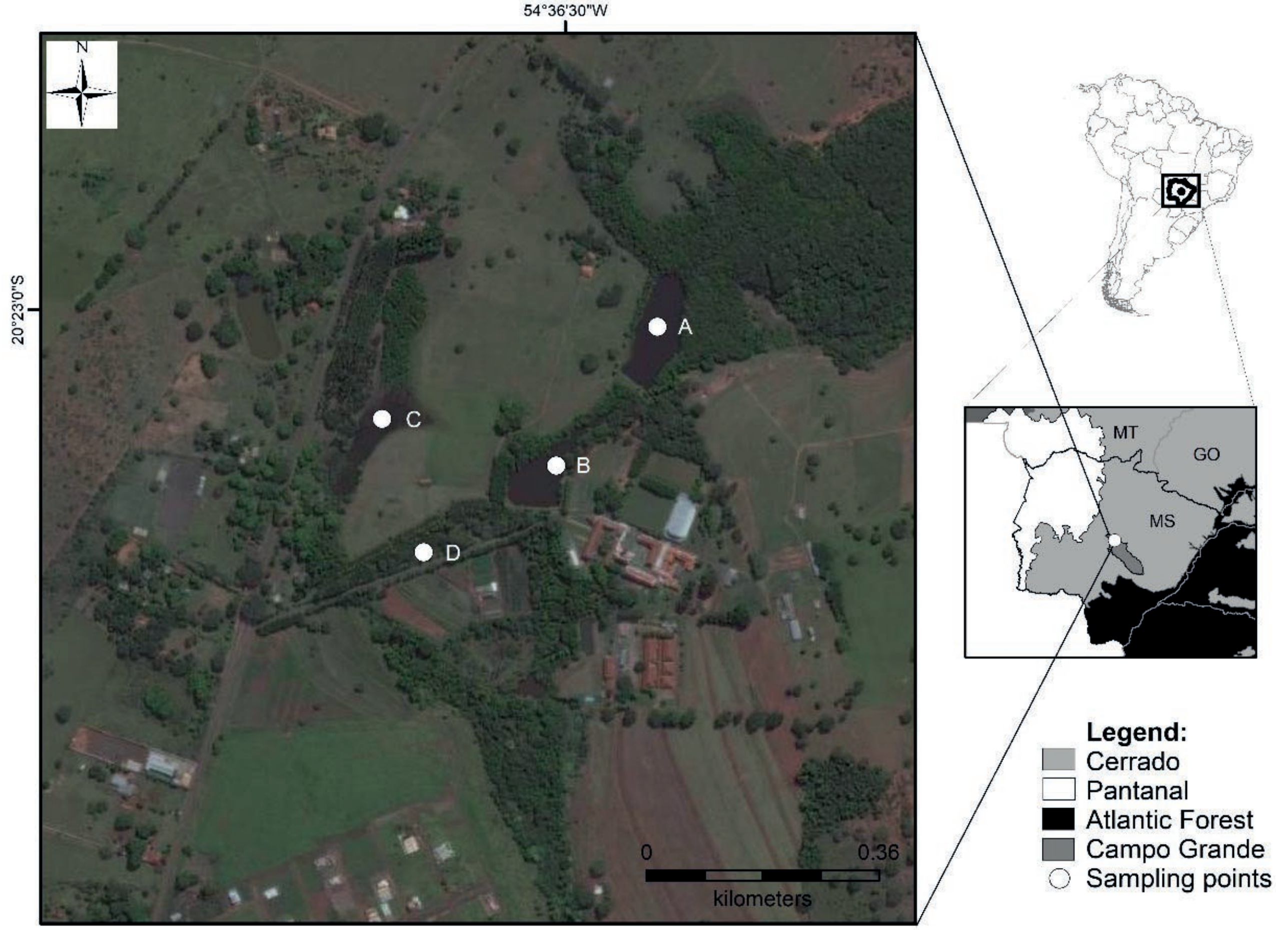

Fig. 1. Water bodies sampled, located at the Segredo stream, in forest fragments of an urban area of Midwestern Brazil.

Table 1. Water bodies located at the Segredo stream sampled for freshwater turtle species in an urban area of the *Cerrado* region, located in Midwestern Brazil.

Water bodies	Type	Habitat	Area (m^2)	Surrounding matrix	Vegetation cover	Substrate	Funnel traps
A	Dam	Lentic	7543,6	Riparian forest	Native arboreous plants	Sandy	4
B	Dam	Lentic	9171,6	Riparian forest with exotic vegetation	Exotic arboreous plants	Sandy	4
C	Dam	Lentic	8089,1	Grassland flooded	Herbaceous and aquatic plants	Clay	2
D	Lagoon	Lentic	6542,1	Riparian forest	Native arboreous plants	Sandy	2

mum distance varied up to 150 meters in water body A, the largest of the four. The traps were installed along the margins of each water body.

Considering the possibility of imperfect detection, we performed five repeated samplings per month (five consecutive days), necessary to estimate the probability of detection. Since each species could be detected (1) or not detected (0), we constructed a detection history denoting the sequence of detections and non-detections for each species (e.g., 10100 - five sampling occasions within a session, where the species was detected on two and not detected on three). The assumption was that the site occupancy status for each species did not change during the period, or that the changes occurred completely at random (i.e., the species present at the sites were constant during sampling) (MacKenzie et al., 2006).

We identified, marked, sexed, and performed biometrics on all of the captured turtles. We marked each captured turtle individually using a system of rectangular cuts in marginal scutes of the carapace, adapted from Cagle (1939). We determined the sex of the captured individuals by examining secondary sexual characteristics (males having a more elongated tail than females) and measured carapace length (CL; to the nearest 0.05 mm) with a 150 mm or 300 mm Vernier caliper. The sexed individuals were classified as either female or male, but their sexual maturity status was not determined, as this information is unknown for *M. vanderhaegei* and *P. geoffroanus*. We could not determine the sex of some individuals smaller than 100 mm CL for both species investigated. Therefore, we classified them as juveniles. We obtained the body mass with Pesola® spring balances of the following capacities: 2.5 kg (50g precision) and 5.000 g (100g precision).

Finally, we obtained information on monthly precipitation and temperature from the INMET (National Institute of Meteorology) database obtained from the meteorological station in Campo Grande, Mato Grosso do Sul (20°27’00”S; 54°43’12”W), located 25 km from the study area.

Characterization of the water bodies

Among the four water bodies sampled at the headwaters of the Segredo stream, three are artificial dams (A, B and C) and one is a natural pond (D) that remained with water during the entire study period. The area of the water bodies, estimated using Google® Earth Pro software, ranged from 6,542 m^2 to 9,171 m^2, are located in the *Cerrado*. The surrounding matrix is formed by floodable riparian forest and *campo sujo*, with tree and shrub vegetation, also containing exotic plant species (e.g., bamboo, eucalyptus, and mango trees). The margin and the interior of the water bodies present vegetation cover by herbaceous and aquatic plants and the substrate is sandy or clayey (Table 1). Despite being located in an area of urban expansion, none of the water bodies receive sewage effluents.

Data analysis

Sex ratio. We used a chi-square test to determine whether the sex ratio differed from 1:1 in each of the turtle populations, employing only those individuals sexed and captured for the first time.

Detectability, temporal, and spatial variations. To assess the probability of detection of each species of freshwater turtles, we used the “single-season, single-species” occupancy model, which explicitly accounts for imperfect detection [$\psi(.)\rho(.)$]. Since our goal is focused on comparing only how much the detection pattern changes according to the temporal characteristics of rainfall and daily temperature occurrence, the detection probability was estimated without the effect of environmental variables on occupancy (ψ Näive). Thus, we tested the effect of rainfall occurrence and daily temperature on the detection probability (from the history of detection [1] and non-detection [0]), considering the other parameters constant [$\psi(.)\rho(\text{variable})$] (MacKenzie et al., 2006). We considered the most parsimonious detection probability model, with the smoothing method with 100 Bootstrap interactions (to compute the standard error of the estimated derived parameters) (MacKenzie et al., 2006). We obtained the parameter estimates by means of maximum likelihood estimation (MLEs).

We used AIC (Akaike Information Criterion; Burnham and Anderson, 2002) to rank and compare the candidate models for each species. We selected the best models (i.e., the model with the parameter that best describes the data as a function of the variable) by considering those with ΔAIC values ≤ 2. We examined the relevance of the variables in the best selected models for uncertainty from analysis of the 95% confidence interval of the parameter estimates (i.e., the overlap of the confidence interval to zero).

We assessed the normality of species abundance data in relation to water bodies and climatic variables using the Shapiro-Wilk test. To evaluate variations in freshwater turtle abundance (by species and total) in relation to temporal (month sampled, mean daily temperature) and spatial (water bodies) characteristics, we fitted Generalized Linear Mixed Models (GLMMs). The months sampled, mean daily temperature and water bodies, as well as the interaction between them, were considered to be fixed factors. To assess whether species abundance varied across sampling months and between water bodies, random factors, we fitted Generalized Linear Mixed Models (GLMMs) separately for each species and treated fixed and random factors in the same way. Due to the overdispersion in abundance data, we used the quasi-Poisson distribution, which has a better fit to data with inflated variance (Hoef and Boveng, 2007).

To assess whether the abundance of a freshwater turtle is affected by the abundance of another species and whether it varies with the area of the water bodies, we used Generalized Linear Models (GLMs). We evaluated the variation for each sampling year (2014, 2015, 2016, 2017) and considering the total catches taken over the four years. Generalized Linear Models present a major advantage over classical multiple regression approaches in that they integrate data from different statistical distributions with the appropriate modeling of statistical error. We verified by means of the Shapiro-Wilk test the absence of overdispersion in the abundance data, and thus we used the Poisson distribution to estimate abundance.

All statistical analyses were performed in the R 3.6.0 programming environment (R Development Core Team, 2019), using the Occu function in the unmarked package (Fiske and Chandler, 2011) in the AIC analysis, and the MASS package (Venables and Ripley, 2002) for the GLMMs analyses (Venables and Ripley, 2002). We adopted a significance level of $P < 0.05$.

RESULTS

We recorded three species of freshwater turtles in the four water bodies sampled: two native (*Phrynops geoffroanus* and *Mesoclemmys vanderhaegei*) and one introduced species (*Trachemys dorbigni*). In all, we captured 94 individuals: 73 were *P. geoffroanus* (78% of the total; 31 females, 30 males, and five juveniles), 19 *M. vanderhaegei* (20% of the total; 10 females, nine males, and one juvenile) and two *T. dorbigni* (2% of the total). Both *T. dorbigni* individuals were adults (156 and 190 mm CL), but their sexes were not determined. Sex ratio did not differ from 1:1 for either *P. geoffroanus* (0.96M:1F; x2 = 0.016, df = 1, P = 0.898) or *M. vanderhaegei* (1M:1F; x2 = 0, df = 1, P = 1.000). Juveniles represented a small proportion of captures for both *P. geoffroanus* and *M. vanderhaegei* (Fig. 2). Of the 94 individuals captured and marked, we recorded 49 (52%) recapture events – 55% involving *P. geoffroanus* and 47% involving *M. vanderhaegei*. We did not record recaptures of *T. dorbigni* (Table 2).

Table 2. Total number of captures and recaptures of chelonians recorded in the Segredo stream, located in an urban area of the *Cerrado* region, located in Midwestern Brazil.

Sampled water bodies	*Phrynops geoffroanus*		*Mesoclemmys vanderhaegei*		*Trachemys dorbigni*	
	Capture	Recapture	Capture	Recapture	Capture	Recapture
A	23	10	3	2	0	0
B	23	21	0	2	0	0
C	2	0	14	3	0	0
D	25	9	2	2	2	0
Total	73	40	19	9	2	0

The detection probability of *Mesoclemmys vanderhaegei* did not include precipitation or temperature in the best model. However, the second-best model, which included precipitation (ΔAIC = 0.05), and the third-best model, which incorporated temperature (ΔAIC = 1.99), were equally informative for the species. On the other hand, *Phrynops geoffroanus* showed a higher detection probability only with increasing temperature, as both the naïve model and the model including precipitation had ΔAIC > 3, indicating lower support compared to the temperature model (ΔAIC = 0; Table 3; Fig. 3).

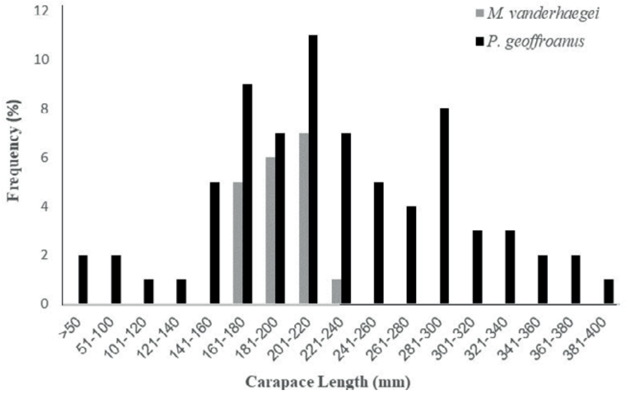

Fig. 2. Frequency distribution of size classes of *Phrynops geoffroanus* and *Mesoclemmys vanderhaegei* captured at the Segredo stream, in forest fragments of an urban area of Midwestern Brazil.

The abundance of *M. vanderhaegei* and *P. geoffroanus* varied among water bodies, across sampling months, and also considering the interaction between these two factors (Table 4; Fig. 4, 5). However, the average daily temperature influenced abundance only for *M. vanderhaegei* ($X^2 = 22.676$, df = 2, $P < 0.001$; Table 4). When both species were modeled together, the average daily temperature also did not have a significant effect on species abundance ($X^2 = 3.3153$, df = 1, $P = 0.068$; Table 4). The variation in abundance over the sampling months was different for *P. geoffroanus* and *M. vanderhaegei*, with high capture rates in the wettest and warmest months of the year for *P. geoffroanus* (November, December, January, February, and March) (Fig. 4). For both species, no new individuals were captured in May (Fig. 4).

Table 3. Probability models of detection of *Phrynops geoffroanus* and *Mesoclemmys vanderhaegei* in relation to daily temperature and occurrence of precipitation, considering constant occupancy (Ψ).

Taxon	K	AIC	ΔAIC	AICw	Beta
Mesoclemmys vanderhaegei					
p()Ψ()	2	198.54	0	0.46	-3.62
p(prec) Ψ()	3	199.04	0.5	0.36	0.56
p(temp) Ψ()	3	200.53	1.99	0.17	-0.0088
Phrynops geoffroanus					
p(temp) Ψ()	3	490.21	0	0.756	0.103
p() Ψ()	2	493.44	3.22	0.151	-2.3
p(prec) Ψ()	3	494.41	4.2	0.093	0.264

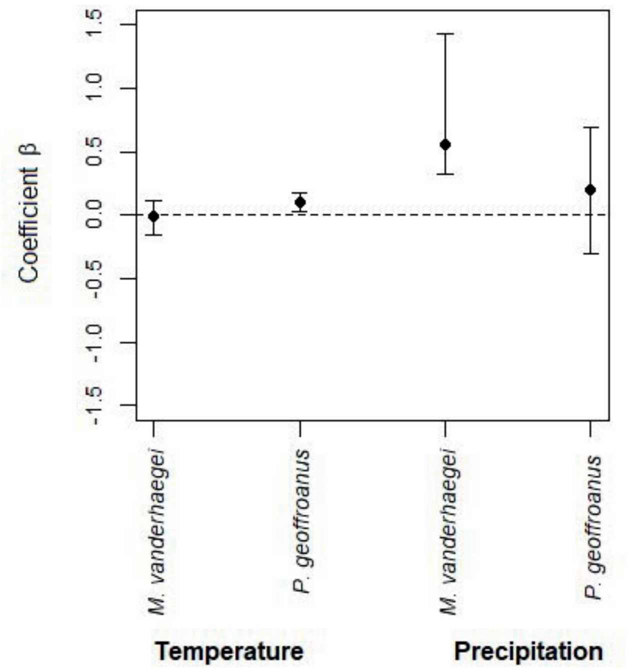

Fig. 3. Detection estimates for *Mesoclemmys vanderhaegei* and *Phrynops geoffroanus* in relation to daily temperature and precipitation positive beta values indicate higher detections and negative lower detections of the species when the confidence intervals do not overlap the dotted line, we consider that the environmental variables interfere with species detection.

Table 4. Fixed-effect parameters in a Generalized Linear Mixed Model (GLMM) accounting for the abundance of *Phrynops geoffroanus*, *Mesoclemmys vanderhaegei* and total turtle abundance in urban vegetation fragment, in relation to spatial effect (water bodies sampled), temporal effect (month sampled and temperature) and their interaction (water bodies sampled*month sampled).

Variable	df	X^2	P
Abundance of *Phrynops geoffroanus*			
Water bodies	3	3458.4617	<0.001
Month	11	1123.1046	<0.001
Average temperature	1	7.2644	0.007
Interaction water body*month	33	4384.3083	<0.001
Abundance of *Mesoclemmys vanderhaegei*			
Water bodies	3	12840.363	<0.001
Month	11	11455.841	<0.001
Average temperature	2	22.676	<0.001
Interaction water body*month	33	44687.955	<0.001
Both species			
Water bodies	3	527.0880	<0.001
Month	11	1150.3408	<0.001
Average temperature	1	3.3153	0.068
Interaction water body*month	33	5100.7831	<0.001

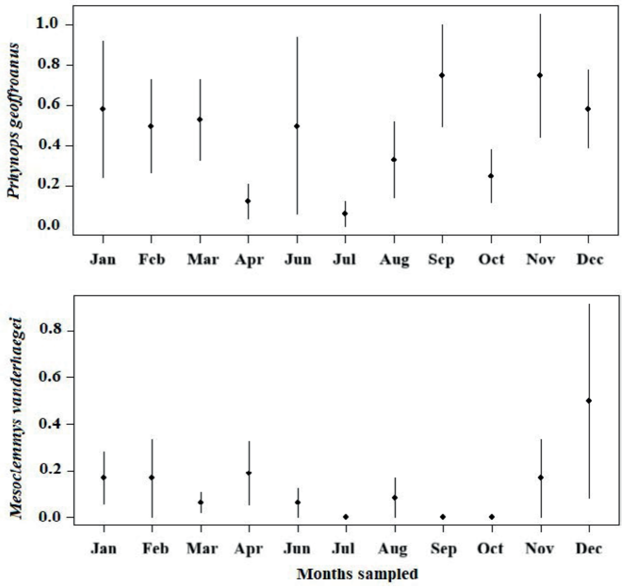

Fig. 4. Abundance of *Phrynops geoffroanus* and *Mesoclemmys vanderhaegei* in the months sampled at the Segredo stream, Midwestern Brazil.

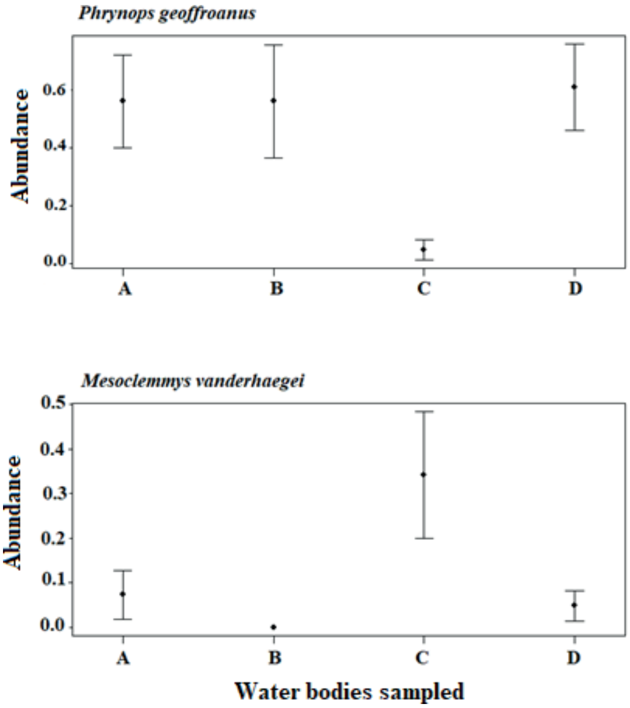

Fig. 5. Abundance of *Phrynops geoffroanus* and *Mesoclemmys vanderhaegei* in three artificial dams (A, B, C) and one natural pond (D) sampled at the Segredo stream, Midwestern Brazil. Description of each water body (A, B, C, D) is shown in Table 1.

Table 5. Fixed-effect parameters in a Generalized Linear Model (GLM) to assess whether the abundance of each turtle species (*Phrynops geoffroanus* and *Mesoclemmys vanderhaegei*) affects the abundance of the other and whether this variation is related to water body area.

Year	Variable	df	X^2	P
Abundance of *Phrynops geoffroanus*				
2014	Water body area	3	1.17	<0.001
	Mesoclemmys vanderhaegei abundance	3	0.02	0.81
2015	Water body area	3	0.31	0.36
	Mesoclemmys vanderhaegei abundance	3	-0.06	0.46
2017	Water body area	3	-0.66	0.16
	Mesoclemmys vanderhaegei abundance	3	0.03	0.92
Total	Water body area	3	-0.19	0.10
	Mesoclemmys vanderhaegei abundance	3	-0.15	<0.001
Abundance of *Mesoclemmys vanderhaegei*				
2014	Water body area	3	2.74	0.01
	Phrynops geoffroanus abundance	3	-0.34	0.01
2015	Water body area	3	0.39	0.70
	Phrynops geoffroanus abundance	3	-0.15	0.22
2017	Water body area	3	0.18	0.79
	Phrynops geoffroanus abundance	3	-0.06	0.77
Total	Water body area	3	-0.84	0.18
	Phrynops geoffroanus abundance	3	-0.12	<0.001

When analyzing, year by year (2014, 2015, and 2017), whether the water bodies (A, B, C, and D) and the abundance of *M. vanderhaegei* affected the abundance of *P. geoffroanus*, we observed a significant influence of the area only in 2014 ($X^2 = 1.17$, df = 3, $P < 0.001$). However, when all years were analyzed together, the sampled area did not affect the abundance of *P. geoffroanus*, which was significantly associated only with the abundance of *M. vanderhaegei* ($X^2 = -0.15$, df = 3, $P < 0.001$; Table 5). Conversely, when examining whether the abundance of *P. geoffroanus* or the area affected the abundance of *M. vanderhaegei*, we found that both the area ($X^2 = 2.74$, df = 3, $P = 0.01$) and the abundance of *P. geoffroanus* ($X^2 = -0.34$, df = 3, $P = 0.01$) influenced *M. vanderhaegei* in 2014, but not in the other years when analyzed individually. When all years were considered together, the area did not significantly affect the abundance of *M. vanderhaegei* ($X^2 = -0.84$, df = 3, $P = 0.18$), which was influenced exclusively by the abundance of *P. geoffroanus* ($X^2 = -0.12$, df = 3, $P < 0.001$; Table 5). Abundance was not estimated for 2016, as no individuals of *M. vanderhaegei* were recorded that year. Although the area was not significantly related to the abundance of either species, graphical analysis showed a trend: *P. geoffroanus* tended to be more abundant in water bodies with fewer individuals of *M. vanderhaegei*, and similarly, *M. vanderhaegei* was more abundant where *P. geoffroanus* occurred at lower abundance (Figure 5).

DISCUSSION

In the present study, we found a higher abundance of *Phrynops geoffroanus* compared to *Mesoclemmys vanderhaegei*. The abundance of *P. geoffroanus* was higher from November to March, coinciding with the rainy season, while *M. vanderhaegei* showed a peak in abundance in December and remained at low levels during the other months. The probability of detection was influenced by climatic factors such as precipitation and temperature, but the effect differed between the two most abundant species. Overall, considering all sampling years, the abundance of the two species varied in relation to each other; however, water bodies and sampling months also influenced the individual abundance of each species.

Phrynops geoffroanus was the dominant species in the study. This pattern was expected, since *P. geoffroanus* seems to be more adapted to urban environments (e.g. Souza and Abe, 2000; Souza et al., 2008). *Phrynops geoffroanus* does not appear to be selective regarding habitat quality, using both lentic and lotic water bodies of various sizes (Souza et al., 2008; Magalhães et al., 2015; Silva et al., 2016; Rocha et al., 2025). *Mesoclemmys vanderhaegei*, on the other hand, is often found in oligotrophic, small, lentic and lotic water bodies, in conserved and higher altitude environments (Brito et al., 2009, 2018; Maffei et al., 2016) and, more rarely, in anthropic environments (Marques et al., 2013, 2014).

Precipitation and temperature affected the detection probability of *M. vanderhaegei*, while for *P. geoffroanus*, only temperature influenced its detection probability. However, when abundance was considered, average temperature affected only *M. vanderhaegei*. Freshwater turtles usually respond to climatic variables linked to temperature and humidity (Moll and Moll, 2004; Souza, 2004). Both variables are considered equally important for turtles, as demonstrated in other studies, such as those on flooding cycles for *Podocnemis expansa* (Eisemberg et al., 2016), or temperature for activity of the turtles in general (Plummer, 2003; Pittman and Dorcas, 2009; Tamplin and Cyr, 2011). Chelonians are ectothermic animals that depend directly on the temperature of the environment to regulate their internal temperature. Some species are more active when the environment is warmer (Tamplin and Cyr, 2011) or, less active during warmer periods of the day to avoid overheating (Hailey and Coulson, 1996). Our study showed that, although temperature and rainfall are equally important for turtle species, different responses to these variables may occur depending on the species analyzed. Additionally, it is important to note that our study area is located in a tropical Aw climate zone, characterized by consistently high temperatures and minimal seasonal variation, with dry winters. Therefore, other environmental factors – such as aquatic habitat type and resource availability – may play a more significant role in determining species abundance.

Phrynops geoffroanus was more abundant in water bodies with lower abundances of *M. vanderhaegei*, particularly in water bodies A, B, and D. In contrast, *M. vanderhaegei* reached its highest abundance in water body C, where only a few individuals of *P. geoffroanus* were captured. Behavioral differences, resource use, or morphological traits between the two species may lead to variation in habitat use. Larger species, such as *P. geoffroanus*, generally require more resources and tend to occupy larger home ranges (Perry and Garland, 2002; Jaeger and Cobb, 2012; Müller et al., 2019). Although the total area of these water bodies is relatively similar, they may vary in depth, resource availability, and degree of eutrophication. For instance, *M. vanderhaegei* is frequently recorded in small, oligotrophic water bodies, whereas *P. geoffroanus* appears to be less selective in habitat use, occupying a variety of aquatic environments (Brito et al., 2009, 2018; Souza and Abe, 2000, 2001). However, *P. geoffroanus* is more commonly found in eutrophic rather than oligotrophic systems (Souza and Abe, 2000; Souza et al., 2008; Ferronato et al., 2009; Rocha et al., 2025). In addition, both species are omnivorous, with a tendency toward carnivory, feeding on aquatic insects, fish, and amphibians (Martins et al., 2010; Brito et al., 2016). In general, species tend to partition resources to coexist, and omnivores generally feed opportunistically, according to food availability. However, interspecific competition can determine exclusion through aggressive interactions or through the exploitation of resources with higher protein content by more competitive species (Luiselli, 2008).

Finally, the presence of *Trachemys dorbigni* does not seem to be a problem for native species yet, due to its low abundance. However, it is an exotic species, found outside its natural range, and with great potential for dispersal and adaptation, especially in human-altered environments (Rachemys, 2009; Molina et al., 2016; Ciccheto et al., 2018; Fonseca et al., 2021). *Trachemys dorbigni* occurs naturally in Brazil (Rio Grande do Sul), Uruguay, and Argentina, inhabiting large floodplains (Alcalde et al., 2012). If its population increases outside its natural range, it can quickly occupy the niche of native species, leading to a decrease in native species abundance or even local extinction if population control is not taken (Zalba and Ziller, 2007). Exotic species usually have greater niche width (Pérez-Santigosa et al., 2011), competing directly with native species and affecting the structure of assemblages with changes in the foraging process and the dissemination of pathogens (Lowe et al., 2000; Zalba and Ziller, 2007). In the case of *Phrynops geoffroanus*, a recent study conducted on a turtle assemblage in an urban area in southern Brazil indicates that the species may alter its activities in response to the coexistence with invasive turtles, such as *Trachemys dorbigni* and *T. scripta elegans* (Rocha et al., 2025). This adaptation may occur mainly due to its generalist habits and tolerance to anthropogenic pressure (Souza and Abe, 2001; Rocha et al., 2025). However, in the case of *M. vanderhaegei*, less abundant than *P. geoffroanus*, our study indicates that it may be less effective as a competitor and, consequently, less able to compete with *T. dorbigni*.

We show here the syntopic occurrence of *Phrynops geoffroanus* and *Mesoclemmys vanderhaegei* with different patterns of abundance. This varies for both species over time, and the abundance of *P. geoffroanus* is reflected in the abundance of *M. vanderhaegei*. Our findings suggest that *P. geoffroanus* is the dominant species in the study area and, according to other studies in urban areas (Souza and Abe, 2001; Souza et al., 2008; Ferronato et al., 2009; Rocha et al., 2025), should persist in this small protected fragment, as well as in other urban environments near the study area (Souza et al., 2008; Martins et al., 2010; Müller et al, 2019). Despite the challenges of living in fragmented urban environments, the presence of *M. vanderhaegei* suggests that the area still offers suitable conditions for species with higher habitat quality requirements. Both species seem to have an overlapping trophic niche, and resource partitioning is a hypothesis to be investigated.

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Atlas of the herpetofauna of Batna Province and the Belezma Biosphere Reserve, north-eastern Algeria

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Abstract. Algeria, the largest country in Africa, exhibits significant gaps in knowledge regarding species' geographical distributions, particularly for herpetofauna. This deficiency is attributed in part to the country's vast geographical expanse, limited local engagement in herpetological research, and persistent underfunding for biodiversity studies. This study presents the first comprehensive atlas of the herpetofauna of Batna Province, northeastern Algeria, including the Belezma Biosphere Reserve, marking a critical step toward developing a national herpetofaunal atlas. Based on 12 years of field surveys and a critical review of historical records spanning nearly 150 years, we document 47 species (four amphibians and 43 reptiles), including seven new provincial records and five species not reported in Batna for over 130 years. Our findings indicate that Batna harbours over two-fifths of Algeria's known herpetofaunal diversity, highlighting its significance as a biodiversity hotspot. This study also provides Arabic common names for the recorded species to enhance public engagement while offering insights into species richness distribution patterns, conservation, and biogeography. We believe this atlas addresses critical knowledge gaps and will contribute to more accurate biodiversity assessments, while informing effective conservation planning in Algeria and the Mediterranean region.

Keywords. Amphibia, Reptilia, diversity, conservation, biogeography, North Africa.

INTRODUCTION

The Wallacean shortfall, which refers to the paucity of information on species' geographical distribution, poses limitations to both our understanding of biodiversity and the implementation of effective conservation strategies (Hortal et al., 2015). This knowledge gap is particularly evident in least developed countries and those with vast territories and remote locations (Titley et al., 2017), with arid regions being especially illustrative of this shortfall. The Saharo-Arabian Desert, in particular, suffers from strong sampling biases driven by limited accessibility (Velo-Antón et al., 2022). Moreover, within these arid environments, desert ecotones are topographically

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complex and biologically diverse transitional zones that remain critically under-surveyed despite their high ecological relevance (Liz et al., 2025). In this context, Algeria, Africa's largest country, over 80% of which lies within the Sahara Desert, epitomizes this knowledge gap when it comes to vertebrates (Brito et al., 2014), notably amphibians and reptiles (Tolley et al., 2016; Beddek et al., 2018). The scarcity of data on Algerian herpetofauna is partly an artefact of the country's vast geographical expanse and the remoteness of many of its regions (Weiss et al., 2018), both of which make comprehensive data collection challenging. Moreover, there appears to be a lack of interest among local researchers in herpetology, which is further compounded by Algeria's position as one of the ten most severely underfunded nations for biodiversity conservation (Waldron et al., 2013). Notwithstanding these challenges, the last decade has witnessed a notable local resurgence of interest in Algeria's herpetofauna, as evidenced by several recent published papers. These works have contributed to expanding our knowledge of range distributions of various herpetofaunal taxa (e.g., Saoudi et al., 2017; Bouam et al., 2019; Bouam and Merzougui, 2021; Bouam et al., 2022, 2024; Chedad et al., 2024), and to the discovery of previously undocumented species within the country (Rouag et al., 2016; Mouane et al., 2021; Boulaouad et al., 2023).

The province of Batna in northeastern Algeria has been the subject of few intermittent herpetological surveys. Noteworthy early explorations include Fernand Lataste's expedition to Algeria in 1880, which included Batna (Lataste, 1881). Lataste's notes and collections were later scrutinized by George Albert Boulenger, who reported additional taxa from the province (Boulenger, 1891). In the ensuing decade, Franz Werner conducted two herpetological surveys across eastern Algeria, encompassing Batna as well (Werner, 1892, 1894). Following Werner's work, herpetofaunal research in Batna remained scarce for much of the 20th century, with only limited records provided by Gauthier (1932) and museum collections. More recent contributions include the investigations undertaken by Ulrich Joger and Wolfgang Bischoff in various parts of the Maghreb region (Joger and Bischoff, 1989). Their findings within Batna were extensively detailed in subsequent work by Bischoff and In Den Bosch (1991). The most comprehensive study on the reptiles of the province was carried out by Laurent Chirio, who conducted intensive surveys in the Aurès region, which covers most of southeastern Batna (Chirio and Blanc, 1997b). The recent phylogeographic study by Beddek et al. (2018) provided additional records for the province. While these contributions have collectively established a foundational understanding of the province's herpetofaunal diversity, their focus has been largely restricted to mountainous massifs, with steppic plains receiving considerably less attention. Furthermore, much of the data from these studies are constrained by quality issues, including spatial biases such as insufficient geographical details, and taxonomic uncertainties resulting from subsequent nomenclatural and taxonomic changes (e.g., Wade, 2001; Carranza et al., 2006; Wagner et al., 2011; Rato et al., 2012; Denzer, 2021; Martínez-Freiría et al., 2021).

This study aims to provide the first comprehensive atlas of the herpetofauna of Batna Province, including the Belezma Biosphere Reserve. Based on data gathered over a decade of field surveys and a critical review of existing records spanning nearly 150 years, it offers an updated taxonomic list, reliable distribution maps, and insights into species richness patterns, conservation, and biogeography. Additionally, Arabic common names are included to enhance public engagement. This work serves as a foundational reference for future research and conservation efforts in Algeria and the broader Mediterranean region.

MATERIAL AND METHODS

Study area

The study was conducted in Batna Province (hereafter Batna), northeastern Algeria, which covers 12,039 km^2 and includes the Belezma Biosphere Reserve (hereafter Belezma BR), recognized as a Key Biodiversity Area (UNESCO, 2015) (Fig. 1). Batna lies at the junction of two major geographical domains: the Tell Atlas to the north and the Saharan Atlas to the south, resulting in significant topographic variation, with elevations ranging from 74 m in the extreme south to 2,322 m at Djebel Mahmel (evaluated from a 30 m Copernicus elevation model; European Space Agency, 2024).

Physiographically, Batna can be broadly divided into three distinct regions: the northern high Tellian plains, characterized by flatlands interspersed with shallow brackish and saline lakes; the western high steppic plains, exhibiting pre-Saharan features and encompassing the eastern extent of Chott El Hodna, a large saline dry lake; and the central and southern mountainous massifs, notably the Aurès and Belezma ranges (Bernard and Ficheur, 1902).

Following Emberger's climatic classification (Daget, 1977), Batna's climate is predominantly Mediterranean with pronounced Saharan influences, leading to significant climatic variability across the province. The inferior and superior arid stages dominate the southern and western lowlands, the inferior and superior semi-arid stages characterize the northern regions and areas surrounding

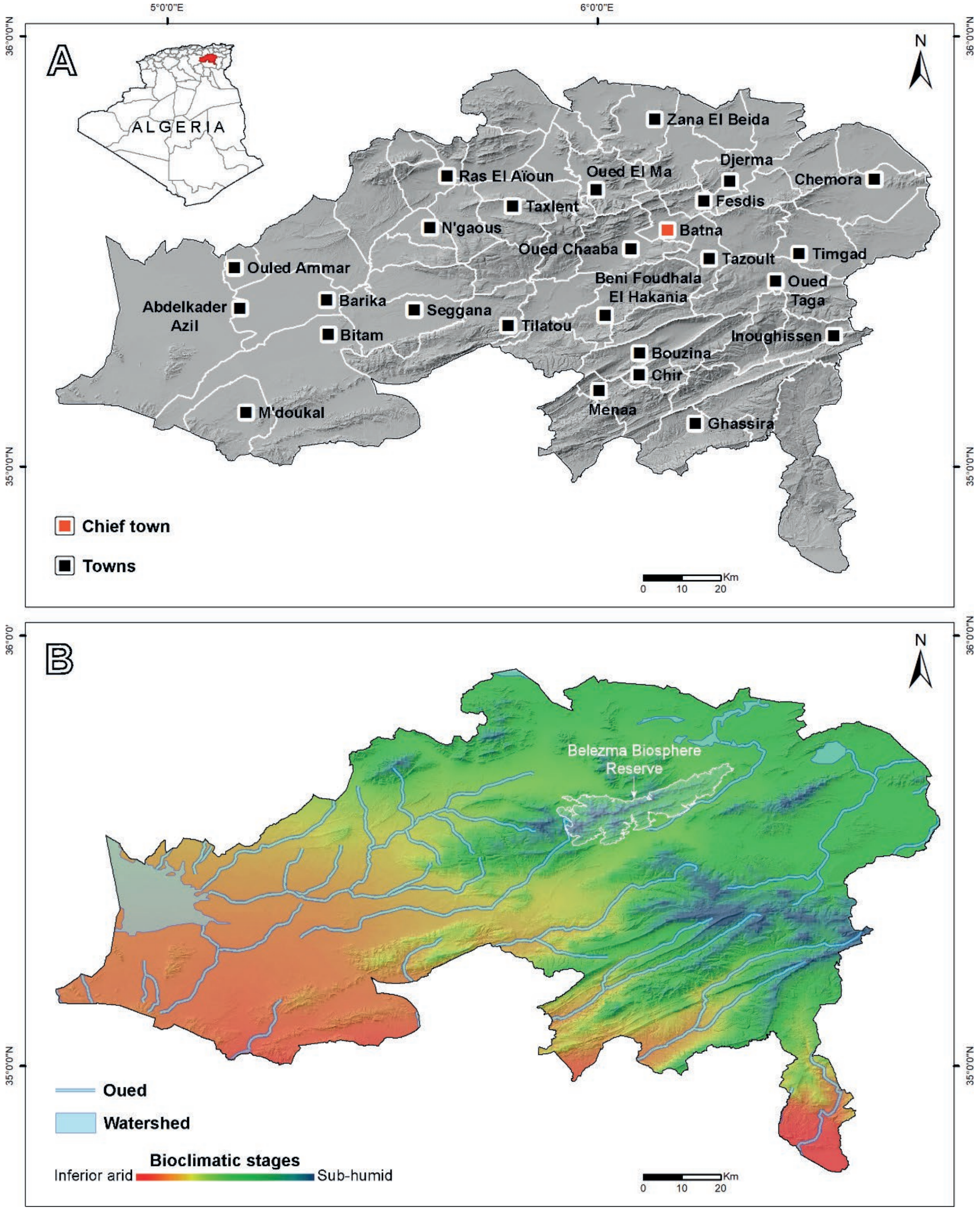

Fig. 1. Map of Batna Province, northeastern Algeria, showing (A) the administrative division and the municipalities referenced in the text, and (B) the Belezma Biosphere Reserve, bioclimatic stages, major hydrographic networks, and watershed boundaries. Bioclimatic stages were derived from Emberger's pluviothermic quotient (Q), obtained from the ENVIREM dataset (Title and Bemmels, 2018), and reclassified according to the bioclimatic thresholds defined by Daget (1977).

the mountainous massifs, while the sub-humid bioclimate prevails at higher elevations (Fig. 1B).

The hydrographic network of Batna is shaped by a relatively dense system of oueds – an Arabic term referring to river channels that experience brief, intense runoff during rainfall and prolonged dry periods. These oueds originate primarily in the mountains and drain into three main watersheds: Chott El Hodna, the shallow lakes of the high Tellian plains, and Chott Melrhir, located beyond the province's southern boundary. Most oueds exhibit weak, highly seasonal flow, with the entire network dependent on precipitation and significantly affected by high evaporative rates (Mebarki, 2007).

Batna encompasses four ecoregions: Mediterranean conifer and mixed forests, Mediterranean woodlands and forests, Mediterranean dry woodlands and steppe, and North Saharan steppe and woodlands (Olson et al., 2001). These ecoregions host diverse habitats, from alpine grasslands and Atlas cedar forests at higher elevations to desert landscapes in the western lowlands (Fig. 2).

Sampling and data collection

Data for this study were collected through opportunistic observations and systematic fieldwork by the authors from 2012 to 2024. Surveys were conducted diurnally and nocturnally employing various sampling methods, primarily visual surveys, rock flipping, and hand capture (McDiarmid et al., 2012). The dataset also included identifiable dead specimens and snake shed skins. Most individuals were photographed, and all had their precise geographic locations recorded via Global Positioning System (GPS) with corresponding dates. When multiple individuals of the same species were found nearby, a single spatial data point was recorded. This methodology resulted in 565 observations. Additionally, 15 observations were provided by local residents and colleagues through identifiable species photographs.

To complement our database, we integrated data from GBIF (2025), available literature, and undigitized museum or institutional collections (Appendices 1-2). Throughout the text, the following institutional abbreviations are used for museum collections, following Sabaj (2020): Museum of Comparative Zoology, Harvard University, Cambridge, USA (MCZ), Musée des Confluences, Lyon, France (MHNL), Muséum National d'Histoire Naturelle, Paris, France (MNHN), Natural History Museum, London, UK (NHMUK), Naturhistorisches Museum Basel, Basel, Switzerland (NMB), Natuurhistorisch Museum Rotterdam, Rotterdam, Netherlands (NMR), Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany (ZFMK). Following Chowdhury et al. (2024), we also incorporated data sourced from Facebook. Species identification for photographic records, primarily from iNaturalist via GBIF (2025) and Facebook, was double-checked. Photographs that were of poor quality, lacked key diagnostic features, or corresponded to individuals that could not be reliably identified were systematically excluded. Only records with confident taxonomic identification, precise locality descriptions, and/or GPS coordinates with at least two decimal digits were retained. Records with coordinate uncertainties exceeding 10 km or those generically referencing "Batna" were also excluded, and duplicate records were removed. This process yielded a dataset of 231 occurrence records, with 49% originating from museum and institutional collections, 29% from online repositories (GBIF and Facebook), and 22% from published literature.

Taxonomy and nomenclature followed Frost (2024) for amphibians and Uetz et al. (2024) for reptiles. Authorship references for all taxa cited are provided in Appendix 3. English common names were sourced from Frost (2024), IUCN (2024), and Uetz et al. (2024). Arabic common names were primarily derived from El Maalouf (1985) and Ghaleb (1986). While several species had Arabic names listed in these references with scientific names consistent with current taxonomy, others appeared under synonyms, were represented only at the genus level, or were entirely absent. In the latter two cases, we retained the available Arabic name for the genus and translated the species epithet from the scientific or English name. For taxa not listed at all, complete Arabic names were constructed through direct translation from their scientific or English equivalents. IUCN conservation categories for the species reported in this study were obtained from the IUCN Red List of Threatened Species website (IUCN, 2024).

Chorotypes for each species were assigned following Chirio and Blanc (1997a), who applied this classification to the reptile fauna of the Aurès region, providing a relevant biogeographic framework for the study area. Eight distinct affinity groups were identified: Mediterranean taxa sensu stricto (MSS), distributed throughout the entire Mediterranean region; Mediterranean taxa sensu lato (MSL), extending their range eastward to the Caspian Sea region; West Mediterranean taxa (MWE), restricted to the western Mediterranean; Mediterranean taxa endemic to the Maghreb region (MAG); Saharan taxa sensu stricto (SSS), exclusively distributed in the African Sahara; Saharan taxa sensu lato (SSL), whose range extends into the deserts of Asia Minor; West Saharan taxa (SWE), confined to the western part of the Sahara; and Tropical taxa (TRO), predominantly found in the steppes and savannas south of the Sahara. For spe-

Fig. 2. Examples of habitat types from Batna Province, northeastern Algeria: (A) alpine grassland in Djebel Mahmel, (B) Gadaïne wetland, (C) Atlas cedar (*Cedrus atlantica*) forest in Djebel Chélia, (D) Aleppo pine (*Pinus halepensis*) forest in Djebel Afoughal, (E) open maquis in Bouzina, (F) Ghoufi canyon, (G) gravel plain with sparse vegetation in Bitam, and (H) sand dunes in Bitam. Photographs by Mohamed Ali Ramoul (A), Amar Kherchouche (B), Idriss Bouam (C, D, E, H), and Ahmed Abdennebi (F, G).

cies exhibiting multiple chorotypes, we retained only the dominant biogeographic affinity that corresponds to the largest portion of the species' natural range, while marginal affinities were not considered.

Distribution maps

Distribution maps for individual species, the number and dominant source of observations, species richness, and chorotypes were generated using 6-arc-minute grids (~11 × 9 km), yielding 162 cells. This resolution offers a balance between spatial detail and sampling adequacy, minimizing false absences without compromising data quality, as recommended for atlas-based mapping (Robertson et al., 2010). Moreover, a similar grid size has been applied in herpetofaunal atlases for regions of comparable extent (Sillero et al., 2005; Cogălniceanu et al., 2014). All spatial analyses were conducted using ArcGIS v.10.8.

RESULTS

A total of 47 herpetofaunal species were documented from Batna. These are systematically presented in Table 1, along with their common names, IUCN conservation categories, and chorotypic classifications. The amphibian assemblage includes four anuran species – two frogs and two toads – from three families: Alytidae Fitzinger, 1843, Bufonidae Gray, 1825, and Ranidae Batsch, 1796, all of which occur in Belezma BR. The reptilian fauna comprises 43 species, 20 of which occur in Belezma BR. Snakes (Serpentes Linnaeus, 1758) account for 32.55% of the reptile species, spanning seven families: Colubridae Oppel, 1811 (seven species), Viperidae Oppel, 1811 (three species), and Erycidae Bonaparte, 1831, Lamprophiidae Fitzinger, 1843, Psammophiidae Dowling, 1967, and Elapidae Boie, 1827 each represented by a single species. Lacertid lizards (Lacertidae Batsch, 1788) follow at 27.90% (12 species), geckos (Phyllodactylidae Gamble, Bauer, Greenbaum & Jackman, 2008 and Gekkonidae Oppel, 1811) at 11.62% (five species), skinks (Scincidae Gray, 1825) at 9.30% (four species), and agamids (Agamidae Spix, 1825) at 6.97% (three species). The families Testudinidae Batsch, 1788, Geoemydidae Theobald, 1868, Trogonophidae Bonaparte, 1838, Chamaeleonidae Gray, 1825, and Varanidae Gray, 1827 are each represented by a single species.

In terms of conservation status, the herpetofauna of Batna encompasses six IUCN categories. Most species (74.46%, 35 species) are classified as Least Concern, while 10.63% (five species) are Near Threatened. *Testudo graeca* Linnaeus, 1758 and *Vipera monticola* Saint Girons, 1953 are listed as Vulnerable, *Acanthodactylus blanci* Doumergue, 1901 as Endangered, and *Spalerosophis dolichospilus* (Werner, 1923) as Data Deficient. Three species, namely *Tarentola* sp., *Stenodactylus mauritanicus* Guichenot, 1850, and *Tropiocolotes chirioi* Ribeiro-Júnior, Koch, Flecks, Calvo & Meiri, 2022, remain Not Evaluated.

Our results show that 105 out of 162 grid cells (64.81%) were sampled (Fig. 3). Most of the province was covered, with unsampled grids mainly along the borders and additional gaps in Chott El Hodna in western Batna. Despite these gaps, the overall sampling effort is considered substantial.

The number of observations per grid cell ranged from 1 to 44 (Fig. S1A), with original field records representing the dominant data source in most cells, whereas museum and institutional records predominated in parts of the Aurès region; cells where online and literature-based records were dominant were comparatively scarce and spatially scattered (Fig. S1B). Species richness per cell varied from 1 to 17 species (Fig. 3A). The spatial patterns of both observation counts and species richness were broadly congruent, with the highest concentrations within and around the Belezma range, extending south to the northern Aurès Mountains. Notable richness also occurs near Tazoult and within the quadrilateral delineated by Seggana, Djebel Metlili in Tilatou, Bitam, and Barika. The remaining areas of the province exhibit moderate to low species richness, with a marked contrast between the Aurès and the arid western regions, the latter displaying lower herpetofauna richness.

Biogeographically, Batna's herpetofauna is predominantly Mediterranean, comprising about two-thirds of the recorded species, while Saharan taxa account for the remaining third. One species of tropical origin, *Naja haje* (Linnaeus, 1758), was also documented. Among Mediterranean taxa, Maghrebian species are the most prevalent, representing approximately 40%. Within Saharan taxa, all chorotypic categories are evenly distributed (Table 1). A clear spatial separation is observed (Fig. 3B), with Mediterranean species concentrated in the eastern half, while Saharan and Tropical species are primarily found in the southern and western regions. Notably, these chorotypes interdigitate along the western periphery of the Aurès and Belezma mountain ranges.

Species accounts

Discoglossus pictus Otth, 1837 (Figs 4A; S2A). Algerian populations belong to a single lineage, except those in the extreme northwest (Beddek et al., 2018). In Batna, the species was first recorded by Lataste in Boulenger (1891), followed by Werner (1892, 1894) and Olivier (1894). The

Table 1. Systematic list of the documented herpetofauna in Batna Province, northeastern Algeria, including common names in English and Arabic (with Latin transcription in parentheses), IUCN Red List categories, and chorotypes. Underlined species represent Algerian endemics; (*) Species present in the Belezma Biosphere Reserve; (**) Species documented for the first time in the province; (‡) Tentatively assigned species pending taxonomic resolution (see species accounts); (NA) Not available. IUCN Red List categories: (LC) Least Concern; (NT) Near Threatened; (VU) Vulnerable; (EN) Endangered; (DD) Data Deficient; (NE) Not evaluated. Chorotypes: (MSS) Mediterranean sensu stricto; (MSL) Mediterranean sensu lato; (MWE) West Mediterranean; (MAG) Maghrebian; (SSS) Saharan sensu stricto; (SSL) Saharan sensu lato; (SWE) West Saharan; (TRO) Tropical.

Taxa	English name	Arabic name	IUCN Status	Chorotype
Class: Amphibia				
Order: Anura				
Family: Alytidae				
Discoglossus pictus Otth, 1837*	Painted Frog	ضَفَّاج مُلَوَّن (Ḍaffāj Mulawwan)	LC	MWE
Family: Bufonidae				
Sclerophrys mauritanica (Schlegel, 1841)*	Moorish Toad	عُلْجُوم موريطاني (ʻUlǧūm Mūrīṭānī)	LC	MAG
Bufotes boulengeri (Lataste, 1879)*	African Green Toad	عُلْجُوم بولونجي (ʻUlǧūm Būlūnjī)	LC	MSS
Family: Ranidae				
Pelophylax saharicus (Boulenger, 1913)*	North African Green Frog	ضِفْدَع أخضر شمال إفريقي (Ḍifdaʻ ʼAkhḍar Shamāl ʼIfriqī)	LC	MSS
Class: Reptilia				
Order: Testudines				
Family: Testudinidae				
Testudo graeca Linnaeus, 1758*	Spur-thighed Tortoise	سُلَحْفَاة اغريقية (Sulḥafāh Ighrīqiyyah)	VU	MSL
Family: Geoemydidae				
Mauremys leprosa (Schweigger, 1812)*	Mediterranean Pond Turtle	حَمَسَة مُتَوَسِطية (Ḥamasah Mutawassiṭiyyah)	NT	MWE
Order: Squamata				
Suborder: Amphisbaenia				
Family: Trogonophidae				
Trogonophis wiegmanni Kaup, 1830*	Checkerboard Worm Lizard	قُهَيْقَرَان ويغمان (Quhayqarān Wayghmān)	LC	MAG
Suborder: Lacertilia				
Family: Agamidae				
Agama bibronii A. Duméril in Duméril & Duméril, 1851*	Bibron's Agama	حِرْذَون بيبرون (Ḥirḏawn Bībrūn)	LC	MAG
Trapelus mutabilis (Merrem, 1820)**,‡	Desert Agama	عَضْرَفُوط مُتَلَّوّن (ʻAḍrafūṭ Mutalawwin)	LC	SSS
Uromastyx acanthinura Bell, 1825**	North African Spiny-tailed Lizard	ضب شمال إفريقي (Ḍabb Shamāl ʼIfriqī)	NT	SWE
Family: Chamaeleonidae				
Chamaeleo chamaeleon (Linnaeus, 1758)*	Common Chameleon	حِرْبَاء شائعة (Ḥirbāʼ Shāʼiʻah)	LC	MSS
Family: Phyllodactylidae				
Ptyodactylus oudrii Lataste, 1880	Oudri's Fan-footed Gecko	أبو بُرَيْص أودري مروحي الأرجل (Abū Burayṣ ʼAudrī Marwḥī al-Arjul)	LC	SWE
Tarentola deserti Boulenger, 1891	Desert Wall Gecko	أبو بُرَيْص صحراوي (Abū Burayṣ Ṣaḥrāwī)	LC	SWE
Tarentola sp.*	NA	NA	NE	MAG

(Continued)

Table 1. (Continued).

Taxa	English name	Arabic name	IUCN Status	Chorotype
Family: Gekkonidae				
Stenodactylus mauritanicus Guichenot, 1850	Moorish Sand Gecko	أبو بُرَيْص رملي موريطاني (Abū Burayṣ Ramlī Mūrīṭānī)	NE	MSS
Tropiocolotes chirioi Ribeiro-Júnior, Koch, Flecks, Calvo & Meiri, 2022**, ‡	Chirio's Gecko	أبو بُرَيْص شيريو (Abū Burayṣ Shīryū)	NE	SWE
Family: Varanidae				
Varanus griseus (Daudin, 1803)**	Desert Monitor	ورل صحراوي (Warl Ṣaḥrāwī)	LC	SSL
Family: Lacertidae				
Acanthodactylus boskianus (Daudin, 1802)	Bosc's Fringe-toed Lizard	ثُعْبَة بوسك (Thuʿbah Būsk)	LC	SSL
Acanthodactylus blanci Doumergue, 1901	Blanc's Fringe-toed Lizard	ثُعْبَة بْلَان (Thuʿbah Blān)	EN	MAG
Acanthodactylus erythrurus (Schinz, 1833)*	Spiny-footed Lizard	ثُعْبَة حمراء الذيل (Thuʿbah Ḥamrāʾ al-Dhayl)	LC	MWE
Acanthodactylus bedriagai Lataste, 1881	Bedriaga's Fringe-fingered Lizard	ثُعْبَة بَدْرِياغا (Thuʿbah Badriyāghā)	NT	MAG
Mesalina guttulata (Lichtenstein, 1823)	Small-spotted Desert Racer	وَحِيرَة رَقْطَاء (Waḥīrah Raqṭāʾ)	LC	SSS
Mesalina olivieri (Audouin, 1829)*	Olivier's Desert Racer	وَحِيرَة أوليفيي (Waḥīrah ʾŪlīfiyyī)	LC	MSS
Ophisops elegans Ménétries, 1832	Snake-eyed Lizard	سِحْلِيِّة عين الثعبان الأنيقة (Siḥliyyat ʿAyn al-Thuʿbān al-ʾAnīqah)	LC	MSL
Ophisops occidentalis (Boulenger, 1887)*	Western Snake-eyed Lizard	سِحْلِيِّة عين الثعبان الغربية (Siḥliyyat ʿAyn al-Thuʿbān al-Gharbiyyah)	LC	MAG
Podarcis vaucheri (Boulenger, 1905)*	Vaucher's Wall Lizard	سِحْلِيِّة فوشي الجدارية (Siḥliyyat Fūshī al-Jidāriyyah)	LC	MWE
Psammodromus algirus (Linnaeus, 1758)*	Algerian Sand Racer	سُنْدُوَاة جزائرية (Sunduwāh Jazāʾiriyyah)	LC	MWE
Psammodromus blanci (Lataste, 1880)*	Blanc's Sand Racer	سُنْدُوَاة بلان (Sunduwāh Blān)	NT	MAG
Timon pater (Lataste, 1880)*	North African Ocellated Lizard	سِحْلِيِّة مَعْيُونَة شمال افريقية (Siḥliyyat Maʿyūnah Shamāl Ifrīqiyyah)	LC	MAG
Family: Scincidae				
Chalcides mertensi Klausewitz, 1954	Algerian Three-toed Skink	دَسَّاسَة جزائرية ثلاثية الأصابع (Dassāsah Jazāʾiriyyah Thulāthiyyat al-Aṣābiʿ)	LC	MAG
Chalcides ocellatus (Forskål, 1775)*	Ocellated Skink	دَسَّاسَة مَعْيُونَة (Dassāsah Maʿyūnah)	LC	MSS
Heremites vittatus (Olivier, 1804)	Bridled Skink	حُكْأَة مخططة (Ḥukʾah Mukhaṭṭaṭah)	LC	MSS
Scincus scincus (Linnaeus, 1758)**	Common Skink	سَقَنْقَور شائع (Saqanqūr Shāʾiʿ)	LC	SSL
Suborder: Serpentes				
Family: Erycidae				
Eryx jaculus (Linnaeus, 1758)	Javelin Sand Boa	دَسَّاس رُمحي (Dassās Rumḥī)	LC	MSL
Family: Lamprophiidae				
Malpolon insignitus (Geoffroy Saint-Hilaire, 1809)*	Eastern Montpellier Snake	خضاري مونبلييه شرقي (Khuḍārī Mūnblīyah Sharqī)	LC	MSL
Family: Psammophiidae				
Psammophis schokari (Forskål, 1775)	Schokari Sand Racer	شُقارِيّ (Shuqārīy)	LC	SSL

(Continued)

Table 1. (Continued).

Taxa	English name	Arabic name	IUCN Status	Chorotype
Family: Colubridae				
Coronella girondica (Daudin, 1803)*	Southern Smooth Snake	حُفَّاثْ أَمْغَر (Ḥuffāth Amghar)	LC	MWE
Hemorrhois algirus (Jan, 1863)	Algerian Whip Snake	حَنَش جزائري (Ḥanash Jazā’irī)	LC	SSS
Hemorrhois hippocrepis (Linnaeus, 1758)*	Horseshoe Whip Snake	حَنَش حدوة الحصان (Ḥanash Ḥidwat al-Ḥiṣān)	LC	MWE
Lytorhynchus diadema (Duméril, Bibron & Duméril, 1854)	Crowned Leaf-nosed Snake	حَوْفَث مُتَوَّج (Ḥawfath Mutawwağ)	LC	SSL
Macroprotodon mauritanicus Guichenot, 1850*	Moorish False Smooth Snake	بسباس موريطاني (Bisbās Mūrītānī)	LC	MAG
Natrix maura (Linnaeus, 1758)*	Viperine Snake	حِنْفِش (Ḥinfish)	LC	MWE
Spalerosophis dolichospilus (Werner, 1923)**	Werner’s Diadem Snake	أرقم وارنر (Arqam Wārnar)	DD	SWE
Family: Viperidae				
Cerastes cerastes (Linnaeus, 1758)	Desert Horned Viper	أفعى قرناء (Afʿā Qarnā’)	LC	SSL
Daboia mauritanica (Gray, 1849)*	Moorish Viper	أفعى موريطانية (Afʿā Mūrītānīyah)	NT	MAG
Vipera monticola Saint Girons, 1953	Mountain Viper	أفعى جبلية (Afʿā Jabalīyah)	VU	MAG
Family: Elapidae				
Naja haje (Linnaeus, 1758)**	Egyptian Cobra	صِلّ مصري (Ṣill Miṣrī)	LC	TRO

painted frog is the least frequently encountered amphibian in Batna, with a seemingly restricted distribution limited to mountain streams in sub-humid areas.

Sclerophrys mauritanica (Schlegel, 1841) (Figs 4B; S2B). Harris and Perera (2009) included a specimen from Batna in their genetic study, revealing minimal intraspecific variation across the species’ range in the Maghreb. Werner (1892) provided the earliest record, referring to it as *Bufo mauritanicus* Schlegel, 1841. The Moorish toad is common throughout the province and was recorded in a variety of habitats, particularly agricultural landscapes and stagnant sections of streams, and was occasionally found road-killed along mountain roads.

Bufotes boulengeri (Lataste, 1879) (Figs 4C; S2C). Dufresnes et al. (2019) confirmed that North African populations belong to the nominotypical subspecies, reporting three localities in Batna. The African green toad is relatively common in the province, occurring across arid to sub-humid areas.

Pelophylax saharicus (Boulenger in Hartert, 1913) (Figs 4D; S2D). Molecular studies (Beddek et al., 2018; Dufresnes et al., 2024) identified two lineages in Algeria: a western and an eastern lineage, the latter including Batna populations. Werner (1892) first recorded the species in Batna as *Rana esculenta* var. *ridibunda* Boettger, 1880. The North African green frog is the most common amphibian in the province and is encountered in virtually all types of aquatic habitats.

Testudo graeca Linnaeus, 1758 (Figs 5A; S2E). Molecular analyses identified two subspecies in Algeria, including *Testudo graeca whitei* Bennett in White, 1836 in Batna (Escoriza et al., 2022). Werner (1892, 1894) first documented the species in Lambesa (presently and hereafter referred to as Tazoult) as *Testudo ibera* Pallas, 1814, later reassigned to *Testudo graeca graeca* Linnaeus, 1758 by Loveridge and Williams (1957). The spur-thighed tortoise is increasingly rare in Batna, with remaining populations severely threatened by illegal wildlife trade driven by local demand for pet tortoises.

Mauremys leprosa (Schweigger, 1812) (Figs 5B; S2F). Algerian populations are recognized as the subspecies *Mauremys leprosa saharica* Schleich, 1996 (Bertolero and Busack, 2017). In Batna, it was first recorded by Lataste in Boulenger (1891) and later by Gauthier (1932) as *Clemmys leprosa* Schoepff in Schweigger, 1812. Subsequently, Loveridge and Williams (1957) assigned Batna’s populations to the subspecies *Clemmys caspica leprosa* Schoepff in Schweigger, 1812. The Mediterranean pond turtle persists in small, fragmented populations within a few northeastern wetlands.

Trogonophis wiegmanni Kaup, 1830 (Figs 6A; S2G). Phylogenetic analyses suggest that northeastern Alge-

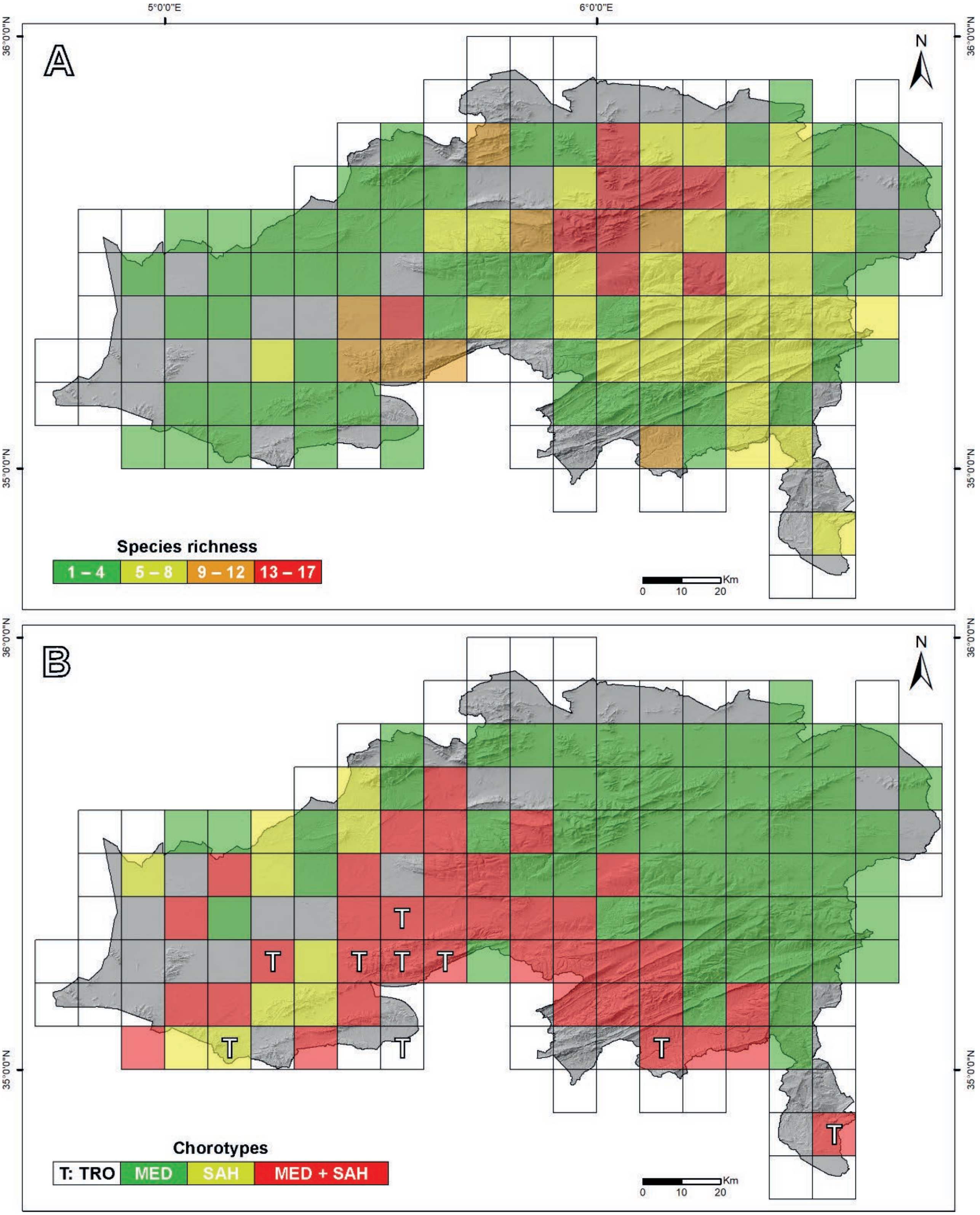

Fig. 3. Distribution patterns of the herpetofauna in Batna Province, northeastern Algeria: (A) species richness, and (B) chorotypes categorized as Mediterranean (MED), Saharan (SAH), and Tropical (TRO). Blank grids represent either unsampled cells or those lacking recorded observations.

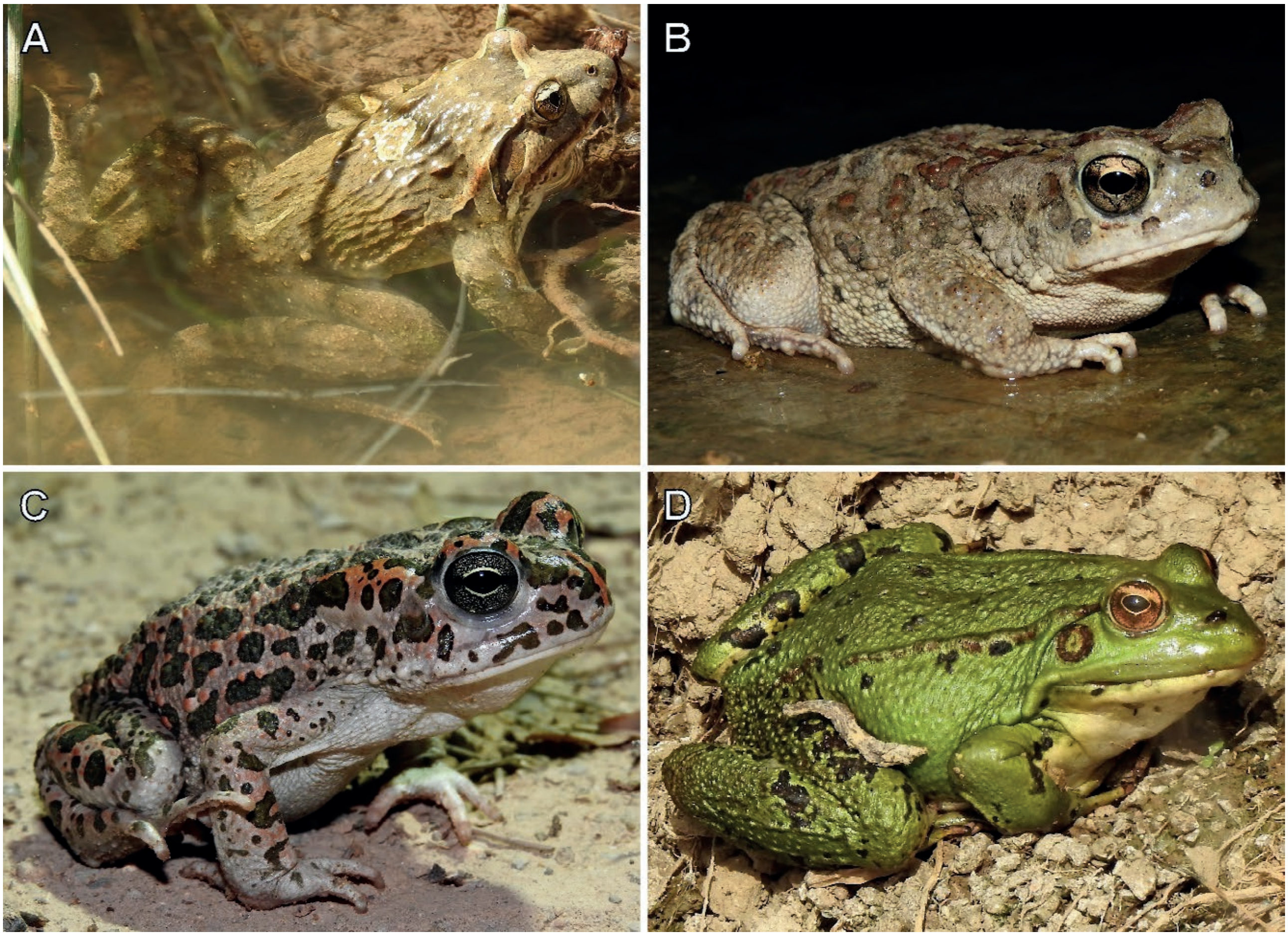

Fig. 4. Amphibians of Batna Province, northeastern Algeria, (A) *Discoglossus pictus* from Oued Taga, (B) *Sclerophrys mauritanica* from Ghassira, (C) *Bufotes boulengeri* from Seggana, (D) *Pelophylax saharicus* from Ghassira. Photographs by Idriss Bouam (A, B, C) and Amar Kherchouche (D).

rian populations belong to the nominotypical subspecies (Salvi et al., 2018). The species' earliest record in Batna dates to Strauch (1882), who reported two specimens acquired from Mr. Deyrolle in 1879. Strauch (1882) also mentioned *Amphisbaena cinerea* Vandelli, 1797 [=*Blanus cinereus* (Vandelli, 1797)] in Batna, a species confined to the Iberian Peninsula (Uetz et al., 2024). Boulenger (1891) noted that Fernand Lataste had not found *B. cinereus* in Algeria, suggesting a misidentification with *T. wiegmanni*. This was corroborated by Ineich et al. (2003) through re-examination of two specimens from Batna housed in the MHNL, originally catalogued as *B. cinereus*, which they identified as *T. wiegmanni*. The checkerboard worm lizard is sporadically encountered in Batna, where both the 'mauve' and 'yellow' forms, sensu Salvi et al. (2018), are documented.

Agama bibronii A. Duméril in Duméril & Duméril, 1851 (Figs 6B; S2H). Denzer (2021) posits that *A. bibronii* is the correct nomenclature, rather than *Agama impalearis* Boettger, 1874. The first formal record in Batna was documented by Saoudi et al. (2017). This species is common in rocky habitats but absent from the arid steppic plains in the province's west. Batna populations may represent the species' easternmost limit, as no records exist further east.

Trapelus mutabilis (Merrem, 1820) (Figs 6C; S3A). We report the first record of this species from Batna, specifically from multiple localities in the arid, rocky habitats of the province's western region. Wagner et al. (2011) did not include genetic data from northeastern Algeria in their revision of the North African *T. mutabilis* complex; later, Wagner and Wilms (2013) provisionally assigned populations from this region to *Trapelus boehmei* Wagner, Melville, Wilms & Schmitz, 2011, though they acknowledged uncertainty. The closest populations, from Biskra – a province bordering Batna to the south – were

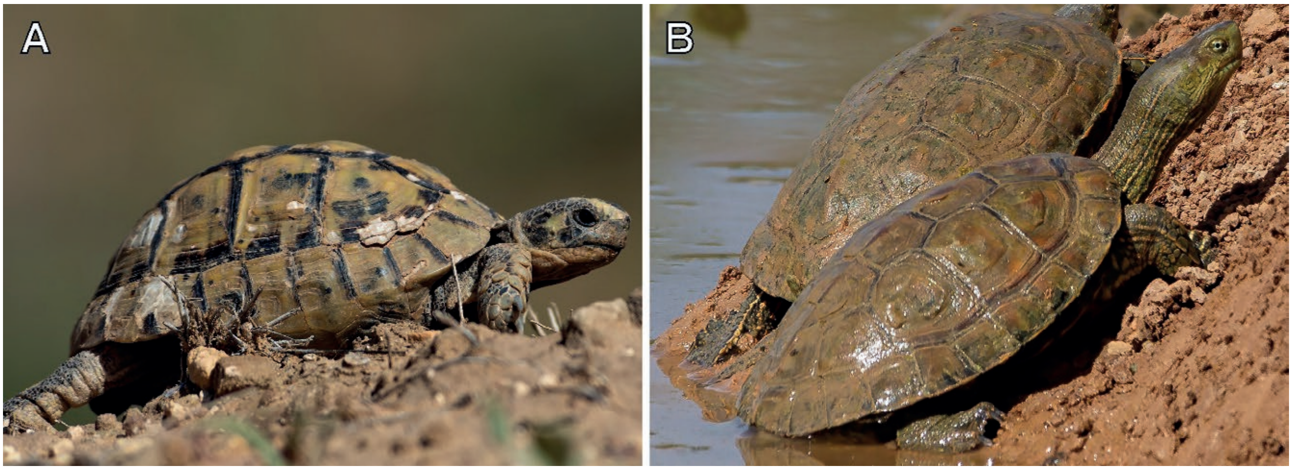

Fig. 5. Testudinidae and Geoemydidae of Batna Province, northeastern Algeria, (A) *Testudo graeca* from Timgad, (B) *Mauremys leprosa* from Timgad. Photographs by Toufik Lemoufek.

identified by Werner (1893) as *Agama aspera* Daudin, 1802, a taxon Wagner et al. (2011) considered likely valid and distinct from *T. boehmei*. Chirio and Blanc (1997b) identified Biskra's populations as *T. mutabilis*, although Wagner et al. (2021) questioned its occurrence in Algeria, while acknowledging its possible presence near the Libya-Tunisia-Algeria border. Given this ongoing taxonomic uncertainty, we provisionally assign Batna's populations to *T. mutabilis*, pending further research.

Uromastyx acanthinura Bell, 1825 (Figs 6D; S3B). Tamar et al. (2018) assigned northeastern Algerian populations to *U. acanthinura*. Although documented in the neighbouring provinces of M'sila (Benelkadi et al., 2021) and Biskra (Chirio and Blanc, 1997b; as *Uromastyx acanthinurus* Bell, 1825), it had not been recorded in Batna. Our study provides the first formal record of this species in Batna, where it is notably prevalent in the rocky habitats of the western region.

Chamaeleo chamaeleon (Linnaeus, 1758) (Figs 6E; S3C). Although historical records of this species in Batna are scarce – limited to four specimens at the NMB collected by K. Seiler in 1946 without precise locality data, and a single specimen at the MNHN (RA-1997.6480) collected by Laurent Chirio – recent iNaturalist records (GBIF, 2025) and our observations indicate that it is more widespread than previously documented, occurring in arboreal habitats across all bioclimatic zones.

Ptyodactylus oudrii Lataste, 1880 (Figs 7A; S3D). Molecular studies identified North Algerian populations of the genus as belonging to *P. oudrii*, while also suggesting it may represent a species complex (Perera and Harris, 2010; Metallinou et al., 2015). The first record in Batna was documented by Lataste in Boulenger (1891), based on specimens collected in 1880 between Biskra and Batna, initially identified as *Ptyodactylus lobatus* (Geoffroy Saint-Hilaire, 1809) [=*Ptyodactylus hasselquistii* (Donndorff, 1798)]. Strauch (1887) referred to a specimen obtained from Mr. Deyrolle in 1879 from Batna as *Ptyodactylus gecko* Hasselq. [=*Ptyodactylus hasselquistii*], while another, collected by Ms. Westphal in 1893, is preserved at the ZFMK (HERP 002294) under *P. oudrii*. Loveridge (1947) treated Batna's populations as the subspecies *Ptyodactylus hasselquistii oudrii* Anderson, 1898. Subsequent records from Batna (Chirio and Blanc, 1997b; Perera and Harris, 2010; Beddek et al., 2018) consistently identified it as *P. oudrii*. In Batna, Oudri's fan-footed gecko is common in rocky habitats within semi-arid to arid bioclimatic zones, especially in the south.

Genus *Tarentola* Gray, 1825 (Figs 7B-C; S3E-F). Rato et al. (2012) included two specimens from Batna in their genetic analyses – one from Belezma BR and another from M'doukal in southwestern Batna. Both specimens were assigned to Clade XV sensu Rato et al. (2012) within the *Tarentola fascicularis/deserti* complex, a distinct lineage spanning Djelfa to Batna, with morphological evidence supporting its recognition as a separate species. Historically, two species have been recognized in Batna; *Tarentola mauritanica* (Linnaeus, 1758) (Werner, 1894; Bischoff and In Den Bosch, 1991; Chirio and Blanc, 1997b) and *Tarentola deserti* Boulenger, 1891 (MNHN-RA-1997.5057; MNHN-RA-1999.9460). Chirio and Blanc (1997b) assigned *T. mauritanica* from Batna to the nominotypical subspecies but noted that individuals from the eastern Aurès exhibited intermediate traits between *T. mauritanica* and *Tarentola fascicularis* (Daudin, 1802). Pending further research, and based on preliminary

Fig. 6. Trogonophidae, Agamidae, and Chamaeleonidae of Batna Province, northeastern Algeria, (A) *Trogonophis wiegmanni* from Djerma, (B) *Agama bibronii* from Tilatou, (C) *Trapelus mutabilis* from Bitam, (D) *Uromastyx acanthinura* from Seggana, (E) *Chamaeleo chamaeleon* from Beni Foudhala El Hakania. Photographs by Elalmi Benmokhtar (A), Tahar Mebarki (B), Toufik Lemoufek (C, E), and Amar Kherchouche (D).

genetic analyses (pers. obs.), we provisionally recognize two species in Batna: *Tarentola* sp., encompassing all prior records of *T. mauritanica*, and *T. deserti*.

Stenodactylus mauritanicus Guichenot, 1850 (Figs 7D; S3G). Previously considered a subspecies of *Stenodactylus sthenodactylus* (Lichtenstein, 1823), this species was later

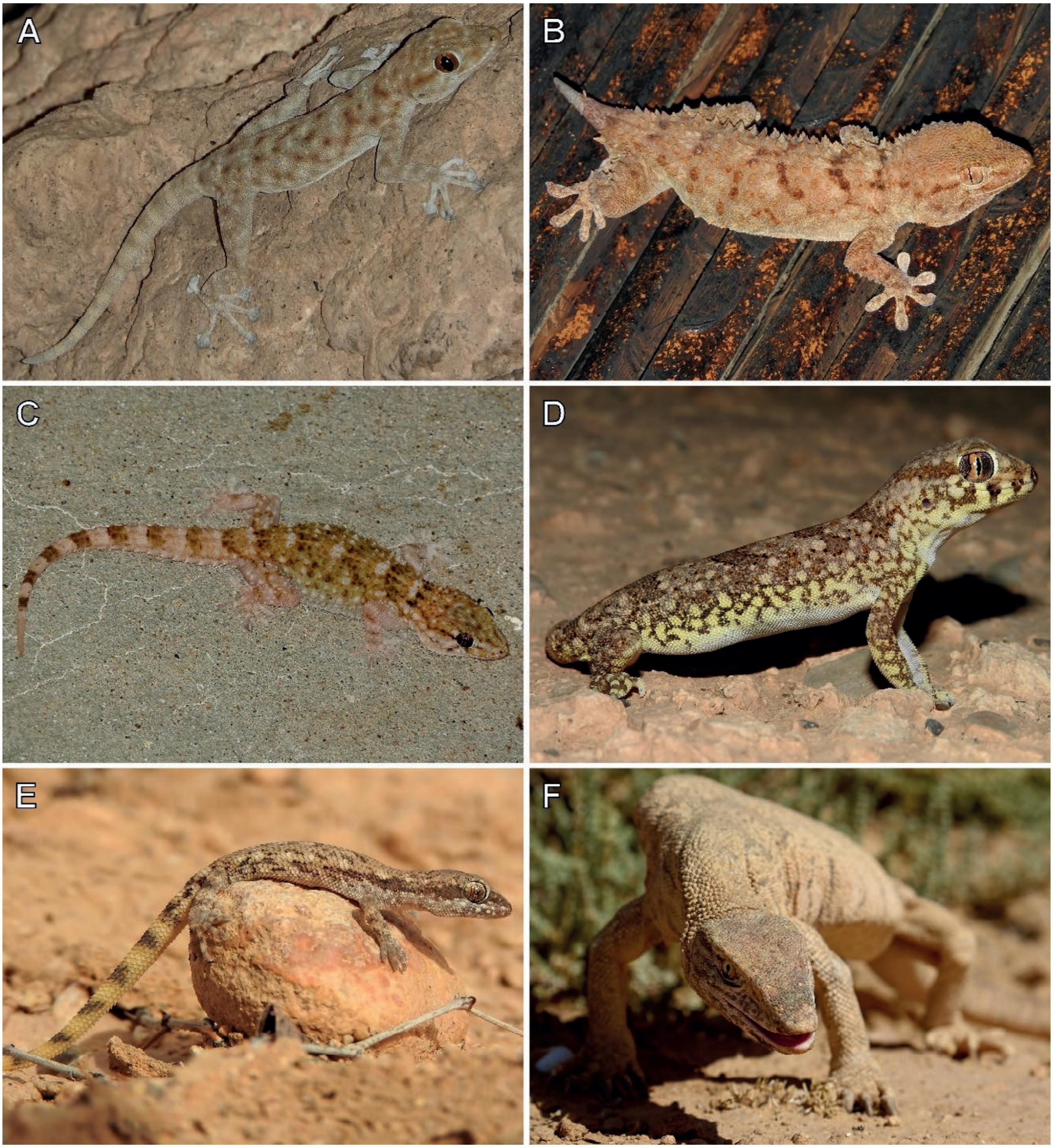

Fig. 7. Phyllodactylidae, Gekkonidae, and Varanidae of Batna Province, northeastern Algeria, (A) *Ptyodactylus oudrii* from Ghassira, (B) *Tarentola deserti* from Bitam, (C) *Tarentola* sp. from Bitam, (D) *Stenodactylus mauritanicus* from Seggana, (E) *Tropiocolotes chirioi* from M'doukal, (F) *Varanus griseus* from Seggana. Photographs by Idriss Bouam (A, B, C, D, E) and Elalmi Benmokhtar (F).

confirmed as distinct through morphological and genetic analyses (Baha El Din, 2006; Metallinou et al., 2012). Historical records from Batna include Strauch's (1887) report of two species, *Stenodactylus wilkinsonii* (Gray, 1842) and *Stenodactylus guttatus* Cuvier, 1829, based on specimens obtained from Mr. Deyrolle in 1879. Boulenger (1891) later synonymized both under *S. guttatus* [=*S. sthenodactylus*]. Olivier (1894) also reported *S. guttatus* from Batna based on a specimen collected by Mr. Pic. However, none of these historical records provide precise locality data. Our study confirms the presence of the Moorish sand gecko in several areas of the arid western steppes of Batna, where it is frequently observed at night.

Tropiocolotes chirioi Ribeiro-Júnior, Koch, Flecks, Calvo & Meiri, 2022 (Figs 7E; S3H). Described by Ribeiro-Júnior et al. (2022) based solely on morphological data, this micro-endemic species was initially known only from Biskra Province. Chirio and Blanc (1997b) had previously identified these populations as the nominotypical subspecies of *Tropiocolotes tripolitanus* Peters, 1880. Following Ribeiro-Júnior et al. (2022), we tentatively assign the Batna populations to *T. chirioi* due to their close geographic proximity to the type locality in Biskra, pending further phylogenetic analyses to confirm their taxonomic status. Our study provides the first record of Chirio's Gecko in Batna, documenting multiple individuals under stones in rocky habitats across three localities in the southwestern region near the Biskra border.

Varanus griseus (Daudin, 1803) (Figs 7F; S4A). The desert monitor was recorded during our surveys in the arid steppic plains of the province's western region. These observations represent the first formal records of the species in Batna. Notably, one specimen was found as a roadkill.

Acanthodactylus boskianus (Daudin, 1802) (Figs 8A; S4B). *Acanthodactylus boskianus* constitutes a species complex comprising considerable undescribed diversity, including nine potential candidate species (Liz et al., 2021). Tamar et al. (2016) assigned Algerian populations to the subspecies *Acanthodactylus boskianus asper* (Audouin, 1829). Lataste in Boulenger (1891) documented a single occurrence from N'gaous, referring to it as the 'variety' *asper*. In Batna, Bosc's fringe-toed lizard predominantly inhabits sandy areas in the western region, where several populations appear well established.

Acanthodactylus erythrurus (Schinz, 1833) species complex (Figs 8B-C; S4C-D). The taxonomy of this species complex remains unresolved (Fonseca et al., 2009; Tamar et al., 2016; Miralles et al., 2020; Harris et al., 2024). Miralles et al. (2020) identified five main lineages across the Maghreb, suggesting the existence of at least five species, two of which occur in Algeria. Genetic analyses have included only a single specimen from Batna, which was assigned to the Central Algeria clade (Beddek et al., 2018; Harris et al., 2024). Based on available data, we provisionally recognize two taxa from this complex in Batna: *Acanthodactylus blanci* and *Acanthodactylus erythrurus.*

Acanthodactylus blanci, which may represent a valid species (Miralles et al., 2020), is rare in Batna. Two specimens (MNHN-RA-1997.6308 and RA-1997.6317) were collected by Laurent Chirio in 1993 from Col de Teniet El Hamadi in Chir. In 2024, we recorded a juvenile at this locality, identified as *A. blanci* by Philippe Geniez.

Populations of *A. erythrurus* from Batna are assigned to the subspecies *Acanthodactylus erythrurus belli* Gray, 1845 (Salvador, 1982; Chirio and Blanc, 1997b; Beddek et al., 2018), distinguished by extensive contact between the subocular scale and the upper lip (*bellii* conformation sensu Miralles et al. 2020). The first record of this species from Batna dates to 1893, based on a specimen housed at the ZFMK (HERP 022724), collected by Alexander Koenig in Tazoult. The species inhabits the eastern mountainous regions of Batna, particularly in semi-arid to sub-humid areas, and is the only member of the genus recorded within Belezma BR.

Acanthodactylus bedriagai Lataste, 1881 (Figs 8D; S4E). Endemic to Algeria (Nouira et al., 2022), this species belongs to the *Acanthodactylus pardalis* (Lichtenstein, 1823) complex, a species-group with intricate phylogenetic relationships (Fonseca et al., 2008; Tamar et al., 2016). Lataste (1881) first described *A. bedriagai* from multiple localities, including Batna, but later reclassified it as a 'variety' of *Acanthodactylus savignyi* (Audouin, 1829) (Lataste, 1885). Boulenger (1891, 1921) treated it as a 'variety' of *A. pardalis*, while Salvador (1982) reinstated it to species rank, including specimens from Batna. Chirio and Blanc (1997b) considered Batna populations as a subspecies of *Acanthodactylus maculatus* (Gray, 1838). Bedriaga's fringe-fingered lizard is relatively common across the high Tellian plains and foothills of northern Batna but does not extend into the adjacent mountainous massifs.

Mesalina guttulata (Lichtenstein, 1823) (Figs 8E; S4F). Genetic studies (Kapli et al., 2008, 2015) indicate that *M. guttulata* represents a species complex. Sindaco et al. (2018) restricted its distribution to Africa, uncovering significant genetic variability and unresolved taxonomic issues. In Batna, only two specimens are preserved in the MNHN: one collected by Henri Martin without a precise locality (RA-0.8561), identified as *Eremias guttulata watsonana* (Stoliczka, 1872) [=*Mesalina watsonana* (Stoliczka, 1872)], and another from southern Batna collected by Laurent Chirio in 1984 (RA-1997.5864). We document several additional localities for the small-spotted desert racer, mainly in the southern half of the province.

Mesalina olivieri (Audouin, 1829) (Figs 8F; S4G). The *Mesalina olivieri* species complex exhibits considerable, yet undescribed, intraspecific diversity (Kapli et al., 2015; Pizzigalli et al., 2021). In Batna, it is known from few MNHN specimens collected by Laurent Chirio in the southeastern province, identified as the nomino-

Fig. 8. Lacertidae of Batna Province, northeastern Algeria, (A) *Acanthodactylus boskianus* from Bitam, (B) *Acanthodactylus blanci* from Chir, (C) *Acanthodactylus erythrurus* from Taxlent, (D) *Acanthodactylus bedriagai* from Djerma, (E) *Mesalina guttulata* from Seggana, (F) *Mesalina olivieri* from Belezma Biosphere Reserve. Photographs by Idriss Bouam (A, B, C), Elalmi Benmokhtar (D), Toufik Lemoufek (E), and Ahmed Abdennebi (F).

typical subspecies. We report two additional localities, including one within the Belezma BR, where the species was observed in open habitats with patchy shrub cover.

Ophisops elegans Ménétries, 1832 (Figs 9A; S4H). It was first reported from Batna by Chirio and Blanc (1993), who identified it as the nominotypical subspecies, distinguishing it from *Ophisops occidentalis* (Boulenger, 1887) by its higher counts of supraocular scales and femoral pores. This species forms an isolated population in the Aurès Mountains, approximately 1,500 km from the nearest population in Libya. In Batna, it is confined to the southeastern mountain ranges, inhabiting drier, more steppic biotopes than *O. occidentalis*.

Ophisops occidentalis (Boulenger, 1887) (Figs 9B; S5A). It was first reported from Batna by Lataste in Boulenger (1891) as *Ophiops occidentalis*, based on specimens collected in 1880, followed by Werner (1892). Subsequent records include Bischoff and In Den Bosch (1991) and Chirio and Blanc (1993). The Western snake-eyed lizard is the most frequently observed reptile in Batna, occupying diverse biotopes but absent from the western arid steppic plains and higher elevations.

Podarcis vaucheri (Boulenger, 1905) (Figs 9C; S5B). Phylogeographic studies of the *Podarcis hispanicus* (Steindachner, 1870) complex suggest that Batna's populations represents a distinct lineage (Lima et al., 2009; Kaliontzopoulou et al., 2011; Caeiro-Dias et al., 2018), which remains undescribed (Nouira et al., 2022). Due to their genetic and morphological similarity to *P. vaucheri* (Kaliontzopoulou et al., 2012), we provisionally assign these populations to this species. The earliest records from Batna, collected by Alexander Koenig in 1893, are housed in the ZFMK. Subsequent studies referred to Batna specimens as a subspecies of *P. hispanicus* (Bischoff and In Den Bosch, 1991; Chirio and Blanc, 1997b), and later *P. vaucheri* (Beddek et al., 2018). Vaucher's wall lizard is common in Batna, particularly in the eastern half of the province, where it primarily inhabits elevated mountainous areas.

Psammodromus algirus (Linnaeus, 1758) (Figs 9D; S5C). Phylogenetic analyses confirm that all North African populations of *P. algirus* belong to the African clade (Verdú-Ricoy et al., 2010; Mendes et al., 2017, Faria and Harris, 2020). This species was initially documented in Batna by Werner (1892), with subsequent records provided by Bischoff and In Den Bosch (1991), Chirio and Blanc (1997b), Bouam et al. (2016), and Khelfaoui et al. (2023). The Algerian sand racer is mainly found in forested habitats across the mountain ranges of the eastern half of the province.

Psammodromus blanci (Lataste, 1880) (Figs 9E; S5D). In their phylogeographic study of the genus, Mendes et al. (2017) included two specimens of *P. blanci* from Belezma BR, revealing significant genetic differentiation between Batna and Tunisian populations. Originally described as *Zerzoumia blanci* Lataste, 1880 from Algiers and Batna, Boulenger (1891) later reclassified it as *P. blanci*. A specimen collected by Henri Martin in Batna in 1881 is housed in the MNHN (RA-0.8551). Early records from Batna, mainly from Tazoult, were documented by Lataste (1880a) and Werner (1892, 1894), with specimens deposited in NHMUK and ZFMK, including those collected by Alexander Koenig between 1892 and 1893. More recent records include specimens collected by Laurent Chirio (1984–1993) in the MNHN and those reported by Bischoff and In Den Bosch (1991), with several deposited in the ZFMK. Blanc's sand racer has a limited distribution in Batna, primarily inhabiting Atlas cedar forests at higher elevations in the eastern half of the province. While Joger et al. (2009) reported an upper elevation limit of 1,200 m for *P. blanci*, we observed the species at 1,892 m on Djebel Chélia.

Timon pater (Lataste, 1880) (Figs 9F; S5E). Ahmadzadeh et al. (2016) confirmed the Batna populations as *T. pater* through phylogenetic analysis. The species was first reported in Batna by Lataste (1880b) as *Lacerta ocellata pater* Lataste, 1880. Subsequent records identified it as *Lacerta pater* Lataste, 1880 (Werner, 1892, 1894; Bischoff and In Den Bosch, 1991), *Lacerta lepida pater* (Lataste, 1880) (Chirio and Blanc, 1997b), and *T. pater* (Ineich et al., 2005). The North African ocellated lizard has a continuous distribution across the sub-humid mountain ranges of the eastern province, where it favours forested, elevated habitats (Bouam et al., 2017).

Chalcides mertensi Klausewitz, 1954 (Figs 10A; S5F). Werner (1894) first recorded this species in Batna, from Tazoult, as *Chalcides tridactylus* Laurenti, 1768 [=*Chalcides chalcides* (Linnaeus, 1758)]. Chirio and Blanc (1997b) later identified it as *C. mertensi* from the same locality. Our findings confirm its presence in this area and document a new locality in southwestern Batna. According to Chirio and Blanc (1997a), the Algerian three-toed skink likely represents relictual populations in Batna, persisting in humid microhabitats within the province.

Chalcides ocellatus (Forskål, 1775) (Figs 10B; S5G). Werner (1894) first reported this species from Batna. Chirio and Blanc (1997b) assigned all specimens to the subspecies *Chalcides ocellatus tiligugu* (Hermann, 1783), distinguished by dark and light longitudinal laterodorsal bands. However, a specimen from Ouled Ammar (NMR 998800000275) was identified as *Chalcides ocellatus subtypicus* Werner, 1931, suggesting the presence of both subspecies. The ocellated skink is widespread across Batna, commonly found under stones or near rocky structures.

Heremites vittatus (Olivier, 1804) (Figs 10C; S5H). Baier et al. (2017) suggested that *H. vittatus* may represent a species complex, with populations from Tunisia, Libya, and likely Algeria forming a distinct mitochondrial

Fig. 9. Lacertidae of Batna Province, northeastern Algeria, (A) *Ophisops elegans* from Menaa, (B) *Ophisops occidentalis* from Chemora, (C) *Podarcis vaucheri* from Taxlent, (D) *Psammodromus algirus* from Fesdis, (E) *Psammodromus blanci* from Belezma Biosphere Reserve, (F) *Timon pater* from Oued El Ma. Photographs by Ahmed Abdennebi (A), Amar Kherchouche (B), Idriss Bouam (C, E), Elalmi Benmokhtar (D), and Tahar Mebarki (F).

lineage. While common in the Eastern Mediterranean, its distribution in North Africa is limited, with few records from Algeria. In Batna, a historical specimen (MNHN-RA-1974.322) collected by Henri Martin in 1884 was identified as *Mabuya vittata* (Olivier, 1804), although it lacks precise locality data. We confirm the species' presence in Batna through an observation of three individuals along an irrigation canal in the western arid steppes.

Scincus scincus (Linnaeus, 1758) (Figs 10D; S6A). The northernmost record of *S. scincus* in Algeria was reported by Olivier (1894) as *Scincus officinalis* Laurenti, 1768 from Oumache in southern Biskra Province. However, Chirio and Blanc (1997b) questioned its presence there, suggesting its northern limit lies in the Great Oriental Erg, about 100 km south of Biskra. We document the first confirmed record of the common skink in Batna, from an isolated sand dune area approximately 70 km northwest of Oumache. This population appears well-established and likely represents an isolated population due to the absence of suitable sand dune habitats in the surrounding areas.

Eryx jaculus (Linnaeus, 1758) (Figs 11A; S6B). Lataste in Boulenger (1891) first reported the Javelin sand boa in Batna from Barika and N'gaous. We observed this species only twice during our surveys. Its distribution in the province appears sporadic, likely due to its cryptic behaviour (Geniez, 2015).

Malpolon insignitus (Geoffroy Saint-Hilaire, 1809) (Figs 11B; S6C). The molecular study by Carranza et al. (2006) elevated *M. insignitus* to species status. Morphologically, it differs from *Malpolon monspessulanus* (Hermann, 1804) by the absence of a dark 'saddle' marking on the foreparts of males. Geniez (2015) and Trape (2023) assigned the northeastern Algerian populations to *M. insignitus*, although the recent IUCN assessment (Aghasyan et al., 2021) inadvertently reported the species as absent from Algeria. The first record of *M. insignitus* in Batna was provided by Werner (1894) as *Coelopeltis lacertina* (Wagler, 1830), with later reports by Bischoff and In Den Bosch (1991) as *M. monspessulanus* and Chirio and Blanc (1997b) as the subspecies *Malpolon monspessulanus insignitus* (Geoffroy Saint-Hilaire,

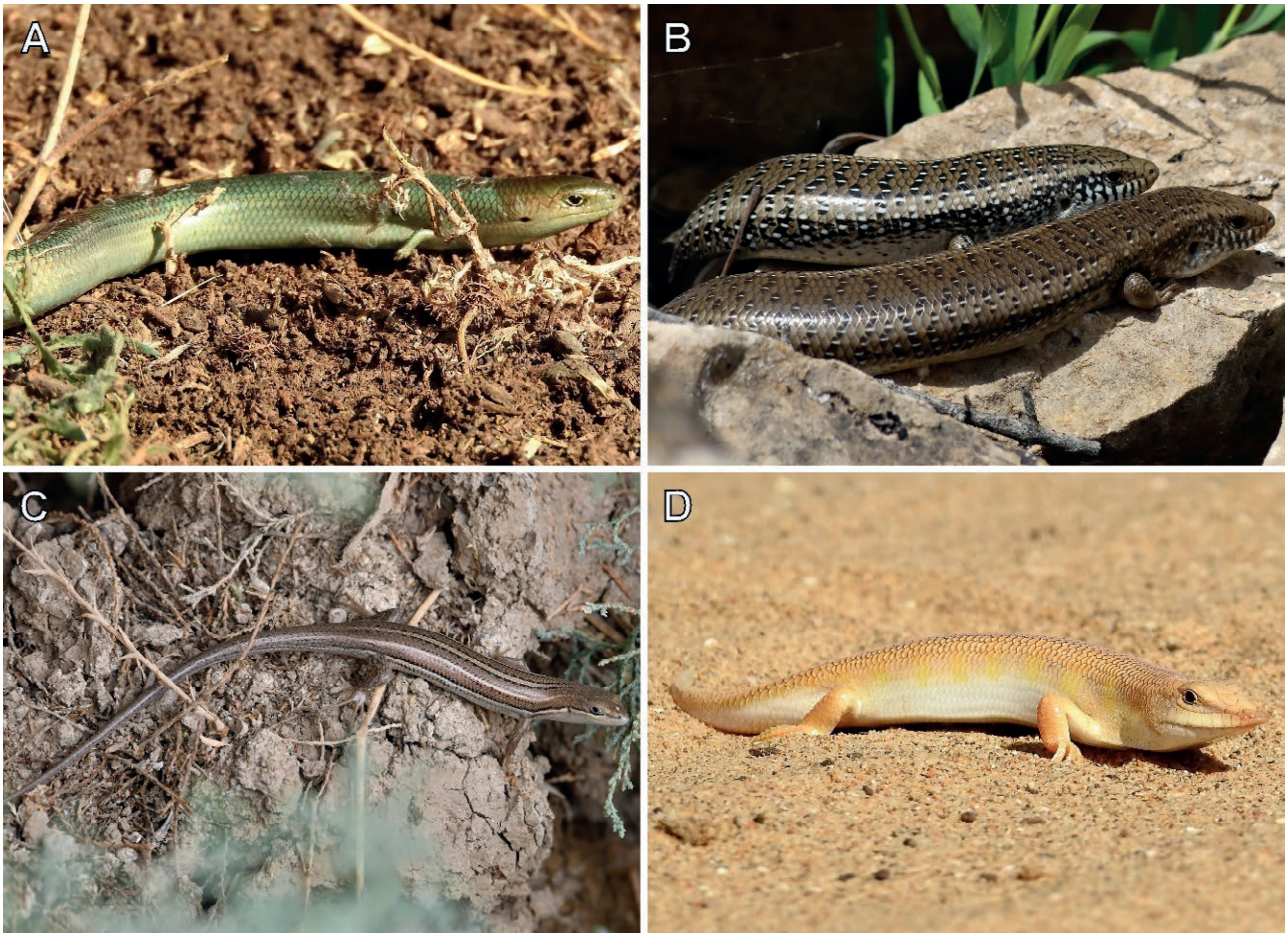

Fig. 10. Scincidae of Batna Province, northeastern Algeria, (A) *Chalcides mertensi* from Oued Taga, (B) *Chalcides ocellatus* from Zana El Beida, (C) *Heremites vittatus* from Abdelkader Azil, (D) *Scincus scincus* from Bitam. Photographs by Idriss Bouam (A), Tarek Messaoudi (B), Toufik Lemoufek (C), and Ahmed Abdennebi (D).

1809). In Batna, the Eastern Montpellier snake is relatively common, although road mortality poses a significant threat to its population.

Psammophis schokari (Forskål, 1775) (Figs 11C; S6D). A specimen collected from Ghoufi in Ghassira, southern Batna, was included in the genetic analyses by Rato et al. (2007) and Gonçalves et al. (2018), confirming that Algerian populations form a monophyletic lineage. This specimen represents the only recorded instance of the species in Batna. Our documentation is limited to three sightings in semi-arid to arid areas in the southern and northwestern parts of the province.

Coronella girondica (Daudin, 1803) (Figs 12A; S6E). The presence of this species in Batna is documented by two specimens housed at the ZFMK (HERP 023220–023221), collected by Alexander Koenig between 1892 and 1893, without specific locality data. The Southern smooth snake appears rare in Batna, as we encountered it only twice during our surveys at relatively high elevations of 1,433 and 1,833 m within the Belezma mountain ranges.

Hemorrhois algirus (Jan, 1863) (Figs 12B; S6F). The only previous record of this species in Batna was from Ghoufi in the province's south (Abreu, 2017). We document several new localities in semi-arid and arid environments across the southern half of Batna, including individuals with the characteristic 'horseshoe' mark and others with uniformly darker heads.

Hemorrhois hippocrepis (Linnaeus, 1758) (Figs 12C; S6G). This species was first recorded in Batna as *Zamenis hippocrepis* (Linnaeus, 1758) by Lataste in Boulenger (1891) and later by Werner (1892). Chirio and Blanc (1997b) documented it as *Coluber hippocrepis* Linnaeus, 1758 from two localities, with an additional site reported by Abreu (2017). The horseshoe whip snake is prevalent in the northeastern part of the province but absent from

Fig. 11. Erycidae, Lamprophiidae, and Psammophiidae of Batna Province, northeastern Algeria, (A) *Eryx jaculus* from Oued Chaaba, (B) *Malpolon insignitus* from Belezma Biosphere Reserve, (C) *Psammophis schokari* from Tilatou. Photographs by Tahar Mebarki (A, C) and Lazhar Moulahcene (B).

the arid western and southern regions, where *H. algirus* appears to replace it. It is frequently observed near urban areas and is often persecuted due to its large size and human fear, despite being non-venomous.

Lytorhynchus diadema (Duméril, Bibron & Duméril, 1854) (Figs 12D; S6H). This species, associated with desert sandy habitats (Geniez, 2015), was reported in Batna by Boulenger (1891) based on a specimen in the Saint Petersburg Museum, though specific details were not provided. The crowned leaf-nosed snake appears rare in Batna, as we documented it only once during a nocturnal survey in a sandy area of the province's western arid region.

Macroprotodon mauritanicus Guichenot, 1850 (Figs 12E; S7A). Molecular analyses by Carranza et al. (2004) identified the northeastern Algerian populations as *M. mauritanicus*. Initially reported in Batna by Lataste in Boulenger (1891) as *Macroprotodon cucullatus* (Geoffroy Saint-Hilaire, 1809), subsequent studies (Wade, 1988; Busack and McCoy, 1990) assigned Batna specimens to *Macroprotodon cucullatus mauritanicus* Guichenot, 1850. Wade (2001) later elevated this subspecies to species rank, considering six specimens from Batna, including Lataste's collections. Bischoff and In Den Bosch (1991) provided precise locality records, with one specimen preserved in ZFMK (HERP 049602). The Moorish false smooth snake is relatively common in Batna but appears absent in arid regions.

Natrix maura (Linnaeus, 1758) (Figs 12F; S7B). Genetic analyses by Barata et al. (2008), including a specimen from Batna, revealed significant genetic divergence between populations from eastern Algeria and Tunisia compared to those in western Algeria, Morocco, and Europe. Historical records of *N. maura* in Batna include a specimen collected by Alexander Koenig in 1893 and Werner's (1894) report from Tazoult as *Tropidonotus viperinus* (Sonnini & Latreille, 1802). The viperine snake is the only semi-aquatic snake species in Batna, frequently observed in aquatic habitats.

Spalerosophis dolichospilus (Werner, 1923) (Figs 12G; S7C). This species remains one of the least studied within its genus (Yadollahvandmiandoab et al., 2023) and had not been previously reported in Batna. It appears rare in the province, with only two nocturnal observations provided by local residents from the western arid regions. We recorded a single individual in the same locality indicated by one of these residents, in an arid, stony area sparsely vegetated with shrubs.

Cerastes cerastes (Linnaeus, 1758) (Figs 13A; S7D). Strauch (1862) mentioned this species in Batna, referring to it as *Vipera cerastes* (Linnaeus, 1758) and noting its purported abundance, though without personal observations or specific locality data. Our findings confirm the presence of the desert horned viper in the arid western and southern regions, where it primarily inhabits sandy substrates and gravel plains with sparse vegetation.

Daboia mauritanica (Gray, 1849) (Figs 13B; S7E). Martínez-Freiría et al. (2017) proposed treating all North African populations as *D. mauritanica*, invalidating *Daboia deserti* (Anderson, 1892). Lataste in Boulenger (1891) first reported this species near Batna as *Vipera lebetina* (Linnaeus, 1758) [=*Macrovipera lebetinus* (Linnaeus, 1758)] from M. Hénon's collection. Its presence in Batna was formally confirmed over a century later from a single documented locality north of the Belezma BR (Martínez-Freiría et al., 2017). Our findings indicate the Moorish viper is relatively common in rocky habitats across the province's eastern half.

Vipera monticola Saint Girons, 1953 (Figs 13C; S7F). Martínez-Freiría et al. (2021) confirmed that North African populations belong to *V. monticola*, while *Vipera latastei* Bosca, 1878 is restricted to the Iberian Peninsula, assigning Algerian populations to the subspecies *Vipera monticola saintgironsi* Martínez-Freiría, Fahd, Larbes & Brito in Martínez-Freiría et al., 2021. In Batna, the mountain viper has only been documented from Djebel Chélia in the eastern part of the province (Bouam et al., 2019), which remains the sole confirmed locality.

Naja haje (Linnaeus, 1758) (Figs 13D; S7G). This species has not been previously documented in Batna. We report its occurrence in the arid southern and western regions, particularly in palm groves and rocky landscapes. These records represent the first formal documentation of the Egyptian cobra in the province. Unfortunately, many observed specimens were deceased, often killed by local residents.

Unconfirmed or potentially erroneous records

Emys orbicularis (Linnaeus, 1758). Gauthier (1932) reported several individuals from "Fontaine-Chaude" (now Ain Skhouna) in northeastern Batna. However, despite extensive surveys, we found no evidence of this species, and the region's arid climate appears unsuitable for its persistence (Gherbi et al., 2023). Given its resemblance to *M. leprosa* (Schleich et al., 1996), the reported individuals were likely misidentified.

Chalcides boulengeri Anderson, 1892. Two historical specimens of *Sphenops boulengeri* (Anderson, 1892) [=Chalcides *boulengeri*] are tentatively attributed to Batna. The first, housed at the MNHN (RA-0.8578), was collected by Henri Martin in 1884 and labelled "Batna". The second, held at the MCZ (R-112204), was also collected by H. Martin but recorded as from "Biskra (prov-

Fig. 12. Colubridae of Batna Province, northeastern Algeria, (A) *Coronella girondica* from Belezma Biosphere Reserve, (B) *Hemorrhois algirus* from Bitam, (C) *Hemorrhois hippocrepis* from Belezma Biosphere Reserve, (D) *Lytorhynchus diadema* from M'doukal, (E) *Macroprotodon mauritanicus* from Ras El Aïoun, (F) *Natrix maura* from Ghassira, (G) *Spalerosophis dolichospilus* from Seggana. Photographs by Lazhar Moulahcene (A, E), Toufik Lemoufek (B), Idriss Bouam (C, G), Aziz Hadj-Aissa (D), and Ahmed Abdennebi (F).

Fig. 13. Viperidae and Elapidae of Batna Province, northeastern Algeria, (A) *Cerastes cerastes* from Bitam, (B) *Daboia mauritanica* from Fesdis, (C) *Vipera monticola* from Inoughissen, (D) *Naja haje* from Seggana. Photographs by Idriss Bouam (A), Elalmi Benmokhtar (B, C), and Tahar Mebarki (D).

ince of Batna)". The latter locality suggests that the historical records of *C. boulengeri* in Batna likely reflect the administrative organization of Algeria during the French colonial period, when Biskra was included as a district within the department of Batna. The occurrence of *C. boulengeri* in Biskra was confirmed by Chirio and Blanc (1997b), but we found no evidence of the species within Batna's current boundaries.

Tarentola neglecta Strauch, 1887. Strauch (1887) described *Tarentola neglecta* and *Tarentola angusticeps* Strauch, 1887 from Batna, based on two specimens purchased in Paris from Mr. Deyrolle, who claimed they originated from Batna. However, Strauch explicitly noted that he could not verify the accuracy of the locality. Boulenger (1887) considered both specimens to represent *T. neglecta*, while Olivier (1894) questioned their origin, arguing that *T. neglecta* is strictly desertic. Given the uncertain provenance, the species' current known distribution (Rato et al., 2012), and the results of our field surveys, we suggest that the occurrence of *T. neglecta* in Batna likely reflects an imprecise locality record.

Stenodactylus petrii Anderson, 1896. This species was reported from Batna by Angel (1923) and Loveridge (1947), both relying on Strauch (1887). Strauch, as in the case of *Tarentola neglecta*, obtained specimens from Mr. Deyrolle, identifying them as *Stenodactylus wilkinsonii* and *Stenodactylus guttatus*. However, Boulenger (1891) synonymized these 'forms' under *S. guttatus* [= *Stenodactylus sthenodactylus*]. Loveridge (1947) also suggested that Strauch's records likely originated from southern Algeria rather than Batna. Given uncertainties in specimen identification and locality, combined with our fieldwork findings, we propose that the occurrence of *S. petrii* in Batna likely reflects a misidentification with *S. mauritanicus* or an imprecise locality record.

DISCUSSION

This study marks a significant contribution to the understanding of Algerian herpetofauna, particularly given the lack of a national atlas and the reliance on distributional data from general works on North Africa (e.g., Schleich et al., 1996; Sindaco and Jeremčenko, 2008, Geniez, 2015; Trape, 2023), which, while providing valuable insights, are often based on outdated literature and museum collections, limiting their contemporary relevance.

The present work provides extensive spatial coverage, encompassing approximately two-thirds of the province's area. This estimate is conservative, as distinguishing between unsampled grid cells and those with no observations was challenging due to the opportunistic nature of many records. Most empty grids are located along the province's borders, a common limitation in atlases (e.g., Cogălniceanu et al., 2014; Burriel-Carranza et al., 2019). Since border grids cover less provincial area than interior grids, the actual spatial coverage is likely more comprehensive.

Original field data constituted the dominant source of observations in the majority of grid cells, reflecting the substantial sampling effort conducted across the province. The prevalence of museum and institutional records in parts of the Aurès region is unsurprising, reflecting the intensive herpetological work carried out in this region by Chirio and Blanc (1997a, b), whose surveys yielded numerous specimens now housed in the collections of the MNHN. Grid cells dominated by records from online or literature sources were both scarce and spatially scattered, emphasizing their more localized and opportunistic nature. These areas, where original field data remain limited, should be prioritized in future surveys to improve spatial completeness and data consistency.

The observed pattern of species richness across Batna is largely congruent with the distribution of observation records, which is expected given that species richness generally increases with sampling effort and spatial extent, highlighting the need for intensified surveys in the less-sampled western region. However, this apparent pattern should be interpreted with caution, as the number of records per grid cell is not always a reliable proxy for actual sampling effort in atlases (Robertson et al., 2010), and should therefore be assessed in conjunction with the individual species distribution maps, which in our study display contiguous patterns for numerous species, indicative of well-documented ranges across the province.

The amphibian assemblage of Batna, though seemingly species-poor, reflects the naturally depauperate amphibian diversity characteristic of North Africa (Escoriza and Ben Hassine, 2019). Nonetheless, these species collectively represent 25% of Algeria's documented amphibian fauna (Frost, 2024), highlighting the regional significance of Batna's amphibian diversity within the national context.

In contrast, the reptile assemblage of Batna comprises 43 species, including seven newly recorded for the province: *Trapelus mutabilis*, *Uromastyx acanthinura*, *Tropiocolotes chirioi*, *Varanus griseus*, *Scincus scincus*, *Spalerosophis dolichospilus*, and *Naja haje*, all representing genera previously unrecorded in the province, with *T. mutabilis* and *T. chirioi* tentatively assigned pending further taxonomic confirmation. With the exception of the Tropical *Naja haje*, these species are of Saharan chorotypes and were predominantly recorded in the arid western regions of Batna – an area that has remained virtually unexplored, with only sporadic historical records from the localities of Barika, M'doukal, N'gaous, and Ouled Ammar (Boulenger, 1891; Olivier, 1894; Salvador, 1982; Rato et al., 2012; GBIF, 2025). This finding represents a significant 19.44% increase in the known reptile diversity of Batna, further corroborating the pronounced Wallacean shortfall across the Algerian Sahara and its adjacent arid zones (Brito et al., 2014; Tolley et al., 2016). Additionally, our study confirmed the presence of *Acanthodactylus boskianus*, *Heremites vittatus*, *Coronella girondica*, *Lytorhynchus diadema*, and *Cerastes cerastes*, species that had not been reported in the region for over 130 years. Conversely, historical records of *Emys orbicularis*, *Chalcides boulengeri*, *Tarentola neglecta*, and *Stenodactylus petrii* were excluded due to potential misidentifications, vague locality data, or insufficient supporting evidence. However, the possible presence of *C. boulengeri* in the province merits further investigation, particularly in the sandy arid landscapes of the western region, where *Scincus scincus* – a species with comparable ecological requirements – is currently established.

The reptile fauna documented in Batna represents 42.57% of Algeria's known terrestrial reptile diversity (Rouag et al., 2024), an exceptional proportion considering that the province accounts for only 0.5% of the country's area. This richness surpasses that reported in the few recent regional herpetofaunal surveys conducted in Algeria (Rouag and Benyacoub, 2006; Benelkadi et al., 2021; Bezaz et al., 2021; Mouane et al., 2024), despite the limited number of such studies for comparison. Remarkably, Batna's reptile diversity also exceeds that of several Mediterranean countries (Cox et al., 2006), further highlighting its significance as a biodiversity hotspot for reptiles both within Algeria and across the Mediterranean region. Despite this exceptional diversity, substantial gaps remain in the genetic characterization of Batna's herpetofauna, a deficiency observed across much of Algeria (Beddek et al., 2018). Future molecular studies should focus on *Trapelus*, *Tarentola*, *Tropiocolotes*, *Acanthodactylus*, and *Ophisops* to resolve their unclear phylogenetic relationships.

Eight species (17.02%) of Batna's herpetofauna have an unfavourable conservation status, with *Acanthodactylus blanci*, *Testudo graeca*, and *Vipera monticola* being the most threatened. *Acanthodactylus blanci* and *V. monticola* are at risk due to their restricted extent of occurrence (Nouira and Joger, 2006; Martínez-Freiría et al. 2024), though no immediate threats were observed at their single recorded localities. In contrast, *T. graeca* faces significant pressure from illegal wildlife trade, a widespread issue across the Mediterranean region (Nijman and Bergin, 2017). Despite its legal protection in Algeria, its persistent presence in Batna's traditional markets (pers. obs.) underscores the urgent need for stricter law enforcement. In addition, the provincial records of species newly documented in Batna presented in this study provide crucial distributional data that can contribute significantly to future IUCN assessments, offering more accurate estimates of Extent of Occurrence (EOO) and Area of Occupancy (AOO), two critical parameters in Red List evaluations.

Human persecution, particularly targeting snakes, and road mortality were among the most pervasive threats observed during our fieldwork. These pressures affect numerous species, including those currently classified as Least Concern, highlighting the need for a national Red List assessment for Algerian herpetofauna to better inform conservation priorities. Moreover, increasing public awareness on snake conservation – including educational campaigns promoting the distinction between venomous and non-venomous species – and implementing road mortality mitigation measures are crucial to addressing these threats (Colley et al., 2017; Roshnath and Divakar, 2019).

The Belezma BR is the only protected area within Batna, supporting 20 of the 47 documented herpetofaunal species and representing the most species-rich area in the province (Fig. 3A). Notably, it harbours 50% of species with unfavourable conservation statuses, all belonging to the Mediterranean chorotype. In contrast, the conservation of Saharan species should prioritize areas such as Djebel Metlili in Tilatou and the sand dunes of Bitam and M'doukal, and further west toward the Abdelkader Azil region. These unique ecological zones support species of particular conservation concern, including the Data Deficient *Spalerosophis dolichospilus*, the micro-endemic *Tropiocolotes chirioi*, and the only known populations of *Heremites vittatus*, *Scincus scincus* and *Lytorhynchus diadema* in the province.

The east/west distribution of chorotypes across Batna (Fig. 3B) highlights the role of the Aurès and Belezma ranges as biogeographic barriers, restricting herpetofaunal dispersal and contributing to regional faunal differentiation. During hyper-arid phases of the Quaternary, Mediterranean habitats in North Africa were largely confined to montane refugia, including these massifs, profoundly shaping the region's biogeographic and taxonomic structure (Husemann et al., 2014). The humid montane environments of the Aurès and Belezma served as glacial refugia, sustaining relict populations of moisture-dependent species such as *Chalcides mertensi*, *Coronella girondica*, and *Vipera monticola* (Chirio and Blanc, 1997a; Bouam et al., 2019). Additionally, prolonged geographic isolation within these ranges likely fostered genetic divergence in reptile lineages, as evidenced by studies on the *Podarcis hispanicus* complex and *Psammodromus blanci* (Kaliontzopoulou et al., 2011; Mendes et al., 2017). In contrast, the arid western region of Batna may have acted as a climatic refugium for hyper-arid specialists such as *Scincus scincus*, consistent with evidence that peripheral Saharan zones have served as refugia fostering the persistence of isolated populations, as supported by paleodistribution models in wide-ranging taxa (Liz et al., 2024).

This study provides a critical piece of the puzzle toward the development of a national atlas of Algerian herpetofauna. However, achieving comprehensive coverage remains challenging due to limited national and international funding for biodiversity research (Waldron et al., 2013). The growing involvement of naturalist photographers in documenting Algeria's biodiversity offers a promising avenue to address these gaps. We also advocate for public institution-led initiatives, such as coordinated mapping campaigns, to facilitate systematic surveys across other provinces, thereby enhancing knowledge not only of Algeria's herpetofauna but also of its overall biodiversity.

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SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at: https://oaj.fupress.net/index.php/ah/article/view/17427/14573.

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Metazoan endoparasites of *Erythrolamprus poecilogyrus caesius* (Cope, 1862) (Serpentes: Dipsadidae) in South American Chaco: Structural attributes at different organization levels

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Abstract. In this study, the structure of metazoan endoparasites infracommunities and component community in *Erythrolamprus poecilogyrus caesius* (Cope, 1862) (Serpentes: Dipsadidae) is presented. From 21 specimens (15 females; 6 males) collected between February 2017 and March 2022 at Ingeniero Juárez, Formosa, Argentina, 90.5% of individuals surveyed (n= 19) were infected with at least one species of metazoan endoparasite. Nine hundred sixty specimens were collected (117 adults; 843 larvae), from 11 taxa. *Kalicephalus appendiculatus* (Nematoda), *Raillietiella furcocerca* (Pentastomida), *Ophiotaenia joanae, Catadiscus uruguayensis, Opisthogonimus lecithonotus* (Platyhelminthes) were found as adults. Larvae identified were *Oligacanthorhynchus* sp. (Acanthocephala), *Physaloptera* sp. (Nematoda), *Strigea* spp. and *Alaria* sp. (Digenea). Digenea was the richest group (S= 6). Adults were collected in intestines, trachea and esophagus, while larvae were found in mesenteries and body cavity. *Ophiotaenia joanae* was the most prevalent species (38.1%) and *K. appendiculatus* had the highest mean abundance (3.7). Considering larvae, *Strigea* spp. were dominant and *Oligacanthorhynchus* sp. was subdominant. Between pairs of metazoans, positive and negative covariations were obtained. In general, intensity of metazoan taxa had positive correlation to host weight and body condition but had negative relationships with snout-vent length. Mean infracommunity richness (including larvae and adults) was 4.35 ± 1.27 taxa/host. Richness and diversity were higher in larval infracommunities than those composed by adults. This is the first ecological study of endoparasites in snakes from Argentina. New reports to parasite biodiversity in Dry Chaco were added, also, information about habitat use, diet, and position in trophic webs of host was generated.

Keywords. Community, endoparasitism, *Erythrolamprus poecilogyrus caesius*, metazoan.

INTRODUCTION

Parasitism is one of the most diverse life forms on Earth (Poulin and Morand, 2000). Among parasitic organisms, wildlife metazoan endoparasites, and helminths in particular, are often considered a 'hidden' and 'cryptic' biodiversity by several authors. 'Hidden', considering their life histories and the difficulty of note their

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presence related to their endoparasite living form, despite their important ecological, frequently undetected (Wood and Johnson, 2015). 'Cryptic' refers to the challenges in distinguishing them morphologically from other similar taxa within the same taxonomical group, or the necessity of using molecular biology techniques to identified them due to their high mutational rates and genetic diversity (Pérez-Ponce de León and Poulin, 2016; 2018).

Helminths parasites, as one of the most numerous and frequent components of this cryptic biodiversity, are far from being fully understood in certain vertebrate classes, including reptiles (Carlson et al., 2020 a). The decline of their populations, caused by anthropogenic interventions and their environmental consequences, could be one of the major obstacles preventing us from achieving this goal (Carlson et al., 2020 b).

In South America, 'Gran Chaco' region represents the second most extensive forest area following the Amazon rainforest (Eva et al., 2004). Sixty-two percent of this region is located in Argentina, representing 23% of the total extension of the country (TNC, 2005). Dry Chaco ecoregion comprise 489,000 km^2 of pastures and semi-deciduous forests that includes Chaco and Formosa provinces, two of the provinces with highest snake species richness (De Marzo et al., 2022; Williams and Vera, 2023). Nevertheless, only 9.1% of this surface is under a protected area regime (Nori et al., 2016).

Seasonal precipitation, vegetation, and soil structure of Dry Chaco landscapes allow the formation of temporal, semi-permanent and permanent ponds environments and microhabitats where several vertebrates species, especially herpetofauna, coexists (Lescano et al., 2015). Nevertheless, these landscapes and their endemic fauna are constantly threatened by intentional fires, deforestation, extensive pasture for cattle, and land uses changes. As a result of these activities, the loss of vegetation cover in the Dry Chaco has reached 200,000 hectares per year, highlighting the urgent need to establish conservation zones for snakes, which are highly threatened by these environmental changes and by climate change. A future biotic homogenization is predicted for this region (Andrade-Diaz et al., 2019; Cabral et al., 2024).

Erythrolamprus poecilogyrus (Wied-Neuwied, 1825) (Serpentes: Dipsadidae) is a species distributed in Argentina, Brazil, Bolivia, Paraguay, Peru, Uruguay, and Venezuela (Williams et al., 2021; Williams and Vera, 2023). *Erythrolamprus poecilogyrus caesius* (Cope, 1862) is one of the three subspecies present in Argentina with a wide distribution in the Argentine Chaco including provinces of Chaco, Corrientes, Formosa, Salta, Santa Fe, Santiago del Estero, and Tucumán (Williams et al., 2021; Williams and Vera, 2023). Quantitative studies on the diet of *E. poecilogyrus* in Argentina and Paraguay indicate that it is a specialist species with a predominantly batrachophagous diet, although it also consumes reptiles in a smaller proportion (Prieto et al., 2012; Bellini et al., 2015). In Brazil, some dietary analyses have identified additional prey items, including fish and mammals (Corrêa et al., 2016).

Erythrolamprus poecilogyrus caesius inhabits near to cricks, temporal ponds or humid pastures, where it could be seeing aggregated in groups until 10 individuals in different developmental stages together in a same pond, giving it reproductive advantages and protection against predators (Williams et al., 2021; Cabral et al., 2022; Williams and Vera, 2023).

At specific level, *E. poecilogyrus* is considered as of 'least concern' by Nogueira et al. (2020); at subspecific level, *E. p. caesius* is considered as 'not threatened' by Giraudo et al. (2012).

To date, three reports of digeneans and one of pentastomid were made for this species in three Argentinean provinces: *Opisthogonimus artigasi* Ruiz et Leão 1942 in Misiones (Caubisens Poumarau, 1968), *Travtrema stenocotyle* (Cohn, 1902) Goodman, 1951 and *Catadiscus uruguayensis* Freitas et Lent, 1939 both in Buenos Aires (Ostrowski de Núñez, 1979; Lunaschi and Drago, 2002), and *Kiricephalus* cf. *coarctatus* in Formosa (Montes et al., 2025).

On the other hand, comprehensive ecological studies in parasite fauna associated to reptiles, and specifically snakes, are non-existent in Argentina (Caraballo et al., 2024).

The aim of this study was to determine, for the first time, the metazoan endoparasites (from now referred as ME) community structure in *E. p. caesius* across its entire distribution range, based on specimens collected in Formosa province, located in the Argentine Dry Chaco. Additionally, the study explores possible relationships between the ME taxa reported and host biological characteristics.

MATERIAL AND METHODS

Site of study, host collection, and taxonomical ME identification

Ingeniero Juárez (23°54′S, 61°51′W) is a locality in Matacos Department of Formosa province, Argentina. It is located in the Dry Chaco ecoregion. In this area the forest is adapted to dry conditions (xerophytic deciduous forest), with predominance of small leaved deciduous and thorny species adapted to fluctuations in water availability, as well as to seasonal thermal variations. Woody vegetation (e.g., *Aspidosperma quebracho-blan-*

co, *Prosopis ruscifolia* and *Schinopsis lorentzii*) is sparse, and the herbaceous species are predominantly grasses (*Gouinia* sp., *Setaria argentina* and *Trichloris* sp.). The landscape is flat and the climate is featured by low rainfall (mean annual about <700 mm), and high temperatures, at times exceeding 47°C since this area comprises part of the South American Heat Pole (Maldonado et al., 2006). Twenty-one *E. p. caesius* specimens (15 females; 6 males) (Fig. 1) were collected by direct observation in microhabitats (temporal ponds at both sides of rural ways, surrounded by pastures and native forest patches) between February 2017 and March 2022. Hosts were euthanized using an intracoelomic injection of commercial anesthesia according to size of host (carticaine clorhydrate, 4%; L-adrenaline 1:100.000 units). Bioethical considerations and guidelines for host catching and euthanasia follow Beaupre et al. (2004). Each snake was sexed by direct observation of gonads or eggs in uterus. Specimens were weighted (in g), using an electronic scale, and the snout-vent length (SVL) was measured (in mm). Differences in mean weight and SVL between sexes were evaluated using a Mann-Whitney U test, and normality was assessed with an Anderson-Darling test. Snakes systematics, synonyms and distribution follow Williams et al. (2023). A snout-cloaca vent incision and body organs were isolated and surveyed (trachea, heart, lungs, esophagus, stomach, small and large intestines, kidneys, mesenteries, and coelomic cavity). All ME were fixed in hot formaldehyde 10% v/v and studied with standard techniques for helminths and pentastomids described in Amato and Amato (2010) and Barton and Riley (2004), respectively. Taxonomic identification followed Amin (1987, 2013) for acanthocephalans, Christoffersen and De Assis (2013) for pentastomids, Anderson et al. (2009) for nematodes, Khalil et al. (1994) for cestodes, Jones et al. (2005), Bray et al. (2008), and Fernandes and Kohn (2014) for digeneans. For larval stages of Platyhelminthes, identification followed Hamann et al. (2019, 2022 a). Voucher material from sampled hosts (12 specimens, 10 females, 2 males) were deposited in Colección Herpetológica of Instituto Nacional de Limnología (INALI) (accession numbers: INALI 7391, 7397, 7398, 7402, 7404, 7405, 7406, 7407, 7408, 7413, 7539, 7540). ME were deposited in the Helminthological Collection of Reference of Centro de Ecología Aplicada del Litoral (CECOAL-CONICET); accession numbers are provided in Supplementary Materials, Table S1.

Fig. 1. Lateral view of anterior region of *Erythrolamprus poecilogyrus caesius* (Cope, 1862) (Serpentes: Dipsadidae) from a locality of Dry Chaco, Argentina.

Description of component community

ME taxa were classified in function of their life cycle in geohelminths or biohelminths by consulting specialized literature for each genus (Table S1, Supplementary Materials). Prevalence (P), mean abundance (MA), and mean intensity (MI) were calculated following Bush et al. (1997). Dispersion index was calculated for each taxon through the variance/mean ratio in order to assign every taxon to their correspondent distribution ($\sigma^2/X << 1$ when a ME taxon followed a normal distribution; $\sigma^2/X = 1$ when a ME taxon followed a Poisson's distribution; $\sigma^2/X >> 1$ when a ME taxon followed a negative binomial distribution). We avoid to presenting standard deviations for ME taxa with negative binomial distribution; following Reiczigel et al. (2024) considerations (presenting standard deviations for mean values is not congruent with non-symmetrical distributions as negative binomial distribution). On the other hand, Bush and Holmes (1986) criterium (B&H), based on prevalence for the dominance of ME (>40% for core species, 10-20% for secondary species, and <10% for satellite species), were compared with the ecological roles assigned to each metazoan taxon. These roles were obtained by three different indices, which are defined and described as follows: (a) *Infection index* (ii= MA x P/100) with three ecological roles (dominants, subdominants, and accessories) based on logarithmic differences among values obtained by the index for each taxon [Janion (1967) *apud* Pence and Eason (1980) modified by Guerrero (1996)]; (b) *Specific importance index* [(SII= P + (MA x 100)] where no categories of importance are established, but, arithmetic differences between scores obtained by each taxon could be compared. We assign the following categories: high, medium and lesser importance (Bursey et al., 2001); (c) *Thul's index* (TI) with four categories (dominants, when TI ≥ 1.0; codominants, when 0.01 ≤ TI < 1.0; subordinates, when 0 < I < 0.01, and unsuccessful pioneer, when: TI = 0) (Thul et al., 1985). Ecological roles were represented graphically using Olmstead-Tukey diagrams. These representations are generated using means of P and ln (MA) + 1 of all taxa of ME of the component community. Representation of these in a cartesian plane allows the obtention of four quadrants, with a previous assignation of roles: I for dominant species; II for subdominants or constant species; III for accessory or rare species, and IV for indicator species.

Interspecific relations by site of infection, influence of host biological characteristics and structural attributes

Interspecific relations between pairs of ME taxa or the influence of host biological characteristics on the intensity of each taxon was analyzed in two levels: infrapopulations and component populations. An infrapopulation is a group of ME of the same taxon in an individual host. On the other hand, component population includes all stages of ME from the same species in a host population (Bush et al., 1997). Frequency (F) of each ME taxon component population by site of infection was calculated following Morales and Pino (1987) and plotted by bars graphs. In each pair of taxa collected in the same organ, a Spearman's rank correlation coefficient was obtained in order to analyze the covariation of their infrapopulation intensities and identify possible interactions among them. The influence of weight and SVL on the intensity of each ME infrapopulations was assessed using Spearman's rank correlation coefficient (p) between these variables. Hosts body condition was measured through Quetelet's index of body mass (defined as weight/SVL^2 and expressed in kg/m^2). Quetelet's index has been proven on other snakes' species successfully (Falk et al., 2017). The approach to structural attributes was performed in two levels, infracommunities and component community. An infracommunity includes all the infrapopulations of ME in an individual host. Consequently, component community includes all the component populations of ME in the host population (Bush et al., 1997). Infracommunity mean richness was estimated counting the number of ME taxa in each host (±1 standard deviation). Shannon-Weaver's diversity index (H'), and Simpson's dominance index (λ) were obtained at infracommunity and component community levels and compared with the ranges proposed by Margalef (1972) and Magurran (1988): (a) high dominance/low diversity (0 < H' < 1.35 / 0.01 < λ < 0.33); (b) medium dominance/medium diversity (1.36< H' < 3.5 / 0.34 < λ < 0.66) or (c) low dominance/high diversity (H' ≥ 3.6 / λ ≥ 0.67). For measuring the effect of the sample size in the estimation of component community richness and its precision, an accumulation species curve based in Chao-2 index was constructed using the "Mao's tau" method described in Colwell et al. (2004). All statistical analysis was performed at 95% confidence level using Paleontological Statistics (Past) in its version 4.16c (Hammer et al., 2001).

RESULTS

Description of component community

Eleven ME taxa from three phyla were identified: excluding *Kalicephalus appendiculatus*, all taxa identified were biohelminths. *Erythrolamprus poecilogyrus caesius* ME were generalist, parasitizing in larval stages other amphibians and reptiles or for their predators in the adult stage, excepting *Kalicephalus* and *Opisthogonimus* genera, which have strict parasitic specificity for snakes as definitive hosts. Detailed information about taxonomical classification of each ME and their intermediate, paratenic or definitive hosts is provided in Supplementary Material (Table S1).

Nine hundred sixty ME specimens were collected; 843 of them were found in larval stages and 117 were in adult stage. Of all host examined, 90.5% (n= 19) were infected at least by one ME. The most prevalent ME species in adult stage was *Ophiotaenia joanae*, meanwhile *K. appendiculatus* showed the highest mean abundance (Table 1). In larval stages, *Strigea* sp. 1 metacercariae and *Alaria* sp. mesocercariae showed the highest prevalence and mean abundance. With the exception of *O. joanae*, all ME component populations showed an aggregation pattern and overdispersion in the host population, proper of the negative binomial distribution (Table 1).

Considering only adult ME taxa, *K. appendiculatus* and *O. joanae* were not classified in the same ecologic role by the four indices applied. These species presented dominant and subdominant ecologic roles for ii, respectively, but were considered as secondary species by Bush and Holmes (1986) criterium, of medium and lesser importance by the SII and, codominant and subordinate species by TI. *Opisthogonimus lecithonotus*, *C. uruguayensis*, and *R. furcocerca* were considered as less importance ME by all indices compared (Table 2).

According to the TI index, all the larvae were classified as unsuccessful pioneers; no taxa were identified as dominant. All indices excepting TI consider *Alaria* sp. and *Stri-*

Table 1. Parasitological descriptors of the adult and larval taxa of ME collected in *E. p. caesius* from a locality of Dry Chaco, Argentina. SINT: Small intestine. LINT: Large intestine. ESO: Esophagus. TRA: Trachea. BCAV: Body cavity. STO: Stomach. MES: Mesenterium. G: Geohelminth. B: Biohelminth. N: Number of metazoans collected; P: prevalence (%), MA: mean abundance, MI: mean intensity, min-max: minimum and maximum value of abundance, σ^2/X: Dispersion index. BN: Binomial negative distribution. N: Normal distribution.

ME taxa	Site of infection	Life cycle/ Transmission of helminth	N	P	MA	MI	min-max	σ^2/X	Distribution
Adult stage									
Nematoda									
Kalicephalus appendiculatus	SINT	G	78	33.3	3.71	4.11	1–37	19.8	BN
Platyhelminthes									
Ophiotaenia joanae	SINT	B	9	38.1	0.43	0.47	1 – 2	0.83	N
Catadiscus uruguayensis	LINT	B	11	4.8	0.52	0.58	1 –11	11	BN
Opisthogonimus lecithonotus	ESO	B	9	19.1	0.43	0.47	1 – 4	3.8	BN
Pentastomida									
Raillietiella furcocerca	TRA	B	10	4.8	0.48	0.53	1 –10	10	BN
Larval stage									
Acanthocephala									
Oligacanthorhynchus sp.	BCAV	B	21	23.8	1.19	1.32	2 – 9	4.5	BN
Nematoda									
Physaloptera sp.	STO	B	25	19.1	1.00	1.11	1 – 15	13.6	BN
Platyhelminthes									
Alaria sp.	BCAV, TRA	B	241	42.9	11.48	12.68	1 – 116	64.2	BN
Strigea sp. 1	MES	B	508	42.9	24.19	26.74	1 – 216	164.7	BN
Strigea sp. 2	MES	B	15	14.3	0.71	0.79	1 – 9	6.7	BN
Strigea sp. 3	MES	B	33	23.8	1.57	1.74	1 – 16	12.8	BN

Table 2. Ecologic role for ME taxa in the community obtained by comparison of four different indices. D: Dominant. SD: Subdominant. A: Accessory. M: Medium. L: Lesser. H: High. C: Codominant. S: Subordinate. UP: Unsuccessful pioneer.

ME taxa	ii Janion (1967) *apud* Pence and Eason (1980)	B&H Bush and Holmes (1986)	SII Bursey et al. (2001)	TI Thul et al. (1985)
Adult stage				
Nematoda				
K. appendiculatus	1.24 (D)	Secondary	404.73 (M)	0.07 (C)
Platyhelminthes				
O. joanae	0.16 (SD)	Secondary	80.96 (L)	0.009 (S)
C. uruguayensis	0.03 (A)	Satellite	52.42 (L)	0.001 (S)
Op. Lecithonotus	0.07 (A)	Secondary	42.11 (L)	0.004 (S)
Pentastomida				
R. furcocerca	0.02 (A)	Satellite	52.42 (L)	0.001 (S)
Larval stage				
Acanthocephala				
Oligacanthorhynchus sp.	0.28 (SD)	Secondary	142.85 (M)	0 (UP)
Nematoda				
Physaloptera sp.	0.19 (SD)	Secondary	119.10 (M)	0 (UP)
Platyhelminthes				
Alaria sp.	4.92 (D)	Core	1190.52 (H)	0 (UP)
Strigea sp. 1	10.38 (D)	Core	2461.95 (H)	0 (UP)
Strigea sp. 2	0.17 (SD)	Secondary	95.23 (L)	0 (UP)
Strigea sp. 3	0.22 (SD)	Secondary	171.44 (M)	0 (UP)

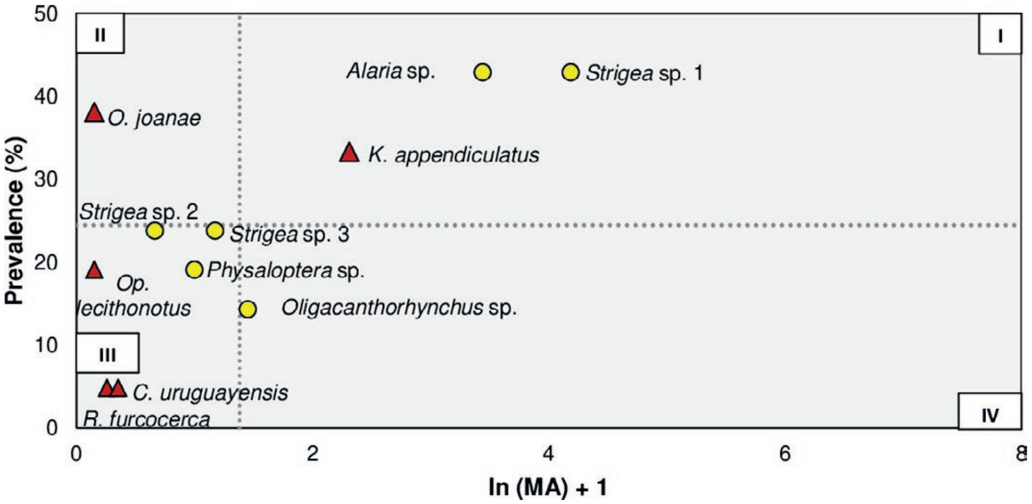

Fig. 2. Olmstead-Tukey diagram of ME in *E. p. caesius* from a locality of Dry Chaco, Argentina. Red triangles are adult species; yellow circles are larval species. Dashed lines represent mean values of ln (MA) + 1 (x axis) and prevalence (y axis). Legend: (I) dominants, (II) subdominants, (III) accessories, and (IV) indicators.

gea sp. 1 as core/dominant/high importance taxa. Plotting all identified taxa without considering their developmental stage in an Olmstead-Tukey diagram, 3 taxa (1 adult, 2 larval) were dominant, one taxon (*O. joanae*) was subdominant, 6 taxa (3 adults, 3 larval) were accessory, and *Oligacanthorhynchus* sp. was an indicator taxon (Fig. 2).

Interspecific relations by site of infection, influence of host biological characteristics and structural attributes

Erythrolamprus poecilogyrus caesius ME were site-specific. Most of the adult ME (4 taxa) were found at higher frequencies in the small intestine, while larval stages (6 taxa) were predominantly found in the mesenteries or free in the body cavity, most frequently associated with the trachea, as *Alaria* sp. (Fig. 3).

Covariation between abundances of ME parasitizing the same organ did not show a clear pattern, and the correlation coefficients were generally weak and not statistically significant (Fig. 4). Two pairs of larval mesenterial ME (*Oligacanthorhynchus* sp. – *Alaria* sp., *Strigea* sp. 1 – *Strigea* sp. 3), and two pairs of ME from the digestive system (*O. joanae* – *Physaloptera* sp., *K. appendiculatus* – *Physaloptera* sp.) had negative correlation coefficients. Contrastingly, in three pairs of larval mesenterial ME (*Oligacanthorhynchus* sp. – *Strigea* sp. 3, *Strigea* sp. 2 – *Strigea* sp. 3, *Alaria* sp. – *Strigea* sp. 1), and one pair of ME from the digestive system (*K. appendiculatus* – *O. joanae*), the correlation coefficients were positive.

The estimated number of taxa for our sample size was 11 taxa, with an expected richness of 11.95 taxa

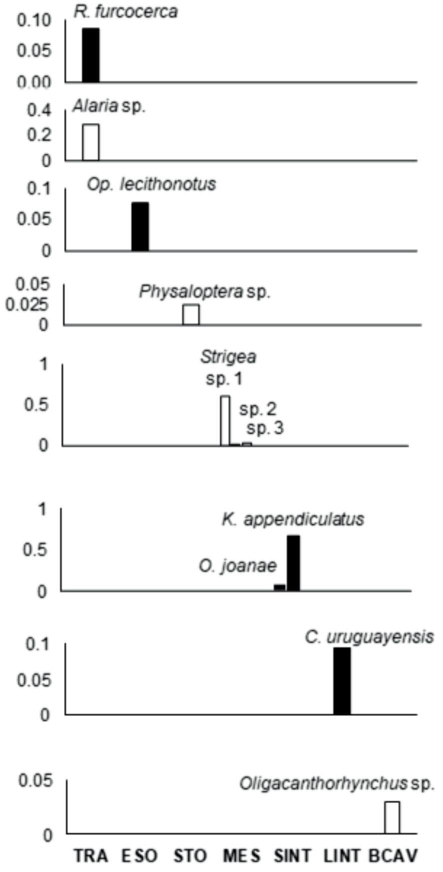

Fig. 3. Relative frequency of ME in adult (black bars) and larval stages (white bars) in different sites of infection. "y" axis for each species is different. Legend: TRA: trachea, ESO: esophagus, STO: stomach, MES: mesenteries, SINT: small intestine, LINT: large intestine, BCAV: body cavity.

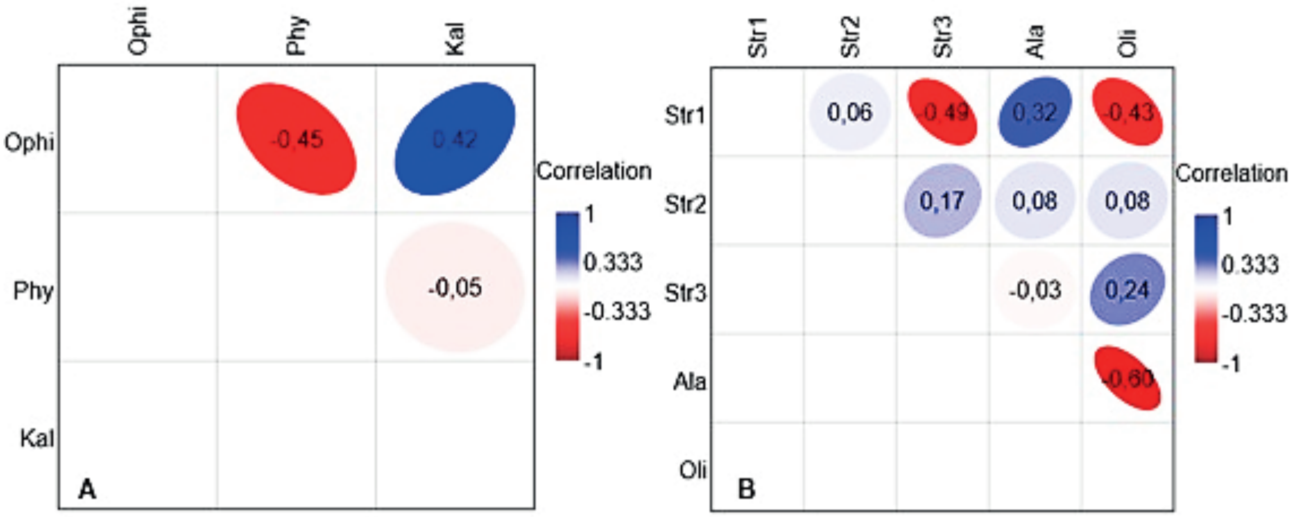

Fig. 4. Spearman's correlation coefficients between pairs of ME taxa parasitizing the same organs. A: Stomach, small and large intestines. B: Mesenteries and body cavity Legend: Kal: *K. appendiculatus*, Ophi: *O. joanae*, Phy: *Physaloptera* sp., Ala: *Alaria* sp., Str1: *Strigea* sp. 1, , Str2: *Strigea* sp. 2, Str3: *Strigea* sp. 3, Oli: *Oligacanthorhynchus* sp.

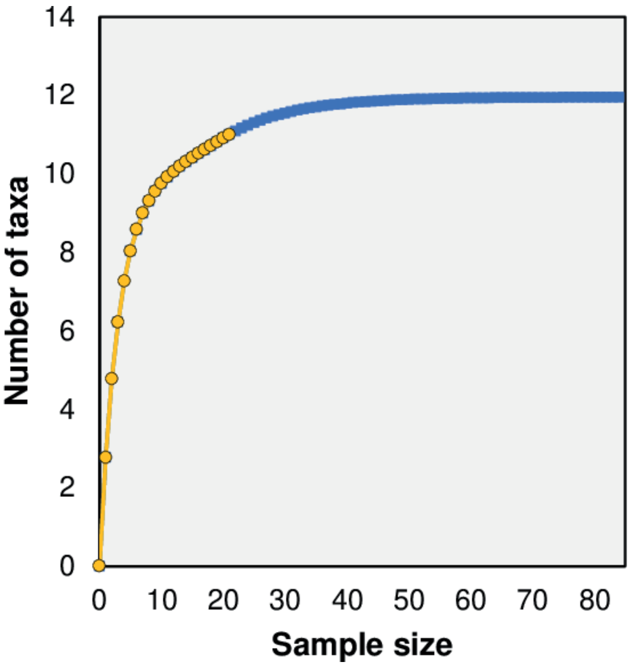

Fig. 5. Species accumulation curve by Mao's tau method. Yellow lines and dots represent observed number of taxa by sample size surveyed; blue lines and squares represent an extrapolation of estimated number of taxa.

based on the Chao-2 index (Fig. 5). This estimate reached 92.1% of the potential taxa that could be detected with a large sample size.

The infracommunity mean richness of snakes parasitized by both adults and larvae ME simultaneously was of 4.35 ± 1.27 taxa per host; in contrast, mean richness was higher in snakes infected solely by larval taxa compared to those infected only by adult taxa (Table 3).

Shannon-Weaver's diversity and Simpson's dominance indices showed that, their associated ME at infracommunity and component community levels are generally dominated by few species (*K. appendiculatus*, *Alaria* sp., and *Strigea* spp.), resulting in low diversity at this level. Diversity increased slightly and lower dominance were observed at infracommunities and component communities composed by adults and larvae at same time (Table 3).

Snakes showed slight variations in their weight (26.6±11.2) and SVL (43.8±6.9); SVL did not show statistically significant differences among sexes (U= 26.5; p = 0.16). On the other hand, weights show statistically significant differences among sexes (U= 6; p = 0.027). However, given the unbalanced sample – with more females (15) than males (6) – sex was not considered in subsequent analyses. All variables followed a normal distribution ($A_{weights} = 0.55$; $A_{SVL} = 0.23$; $p_{weights} = 0.14$; $p_{SVL} = 0.77$).

In general, host weight and the intensity of each ME infrapopulation showed weak positive correlations, with the exception of *Oligacanthorhynchus* sp., when SVL and intensity of infrapopulations were considered, only *O. joanae,* and *Physaloptera* sp. showed negative and weak correlation coefficients. Regarding of Quetelet's index of body mass vs. intensity, *O. joanae* and *Oligacanthorhynchus* sp. showed negative correlations and *Oligacanthorhynchus* sp. showed statistical significance, while the correlations coefficients for the other taxa were weak (Table 4).

Table 3. Ecological indices at infracommunity and community level for ME taxa parasitizing *E. p. caesius* from a locality of Dry Chaco, Argentina. MS: mean number of taxa, H': Shannon-Weaver's index of diversity, λ: Simpson's index of dominance, S: Number of taxa. SD: 1 standard deviation.

Stage	Infracommunity level			Community level		
	MS ± SD	H' ± SD	λ ± SD	S	H'	λ
Only adults	1.47 ± 0.83	0.15 ± 0.31	0.88 ± 0.21	5	1.10	0.45
Only larvae	2.92 ± 1.26	0.43 ± 0.31	0.75 ± 0.19	6	1.06	0.47
Adults + larvae	4.35 ± 1.27	0.61 ± 0.41	0.66 ± 0.24	11	1.43	0.35

Table 4. Spearman's rank correlation coefficients for intensity of each ME infrapopulation *vs.* host weight, snout-vent length (SVL) and Quetelet's index of body mass. Bold correlation coefficients indicate statistical significance for α = 0.05.

ME taxa	Weight *vs* ME intensity (ρ)	SVL *vs* ME intensity (ρ)	Quetelet's index of body mass vs ME intensity (ρ)
Adult stage			
Nematoda			
K. appendiculatus	0.48	-0.18	0.41
Platyhelminthes			
O. joanae	-0.32	-0.24	-0.25
Larval stage			
Acanthocephala			
Oligacanthorhynchus sp.	-0.51	-0.45	-0.78
Nematoda			
Physaloptera sp.	-0.71	-0.37	0.00
Platyhelminthes			
Alaria sp.	0.45	0.048	0.62
Strigea sp. 1	0.25	-0.098	0.12
Strigea sp. 2	0.50	1.00	0.50
Strigea sp. 3	0.45	-0.22	0.45

DISCUSSION

Studies on the ecology of helminths in snakes are extremely scarce in South American portion of Neotropics (Ávila et al., 2013; Mati et al., 2015; Carvalho et al., 2018; Matias et al., 2018, Oliveira et al., 2024). This study represents the first contribution to the knowledge of the ME ecology in snakes in Argentina, focusing on the genus *Erythrolamprus*.

Despite the lack of ecological studies in Argentina, several taxonomic studies, species distribution reports, new host records, and species listings have been conducted (see Caraballo et al., 2024). A compilation of parasitological studies from Argentina, Brazil, and Uruguay in *Erythrolamprus* species and subspecies reveal high parasite richness. Notably, acanthocephalans are the only helminth group with reported larval stages (Table S2, Supplementary Materials). With the exception of *C. uruguayensis*, all ME reported herein are being documented for first time in *E. p. caesius* as host in Argentina.

Description of component community

The taxonomic composition of ME component community in *E. p. caesius* could be indicative of semi-aquatic habitat uses of this subspecies, as well as, the amplitude of its diet. *Erythrolamprus poecilogyrus* has been described as a dietary generalist, preying on a broad range of taxa such as arthropods, fishes, anurans, amphisbaenas, lizards, birds, and rodents (Amaral, 1978; Corrêa et al., 2016; Di Pietro et al., 2020). Nevertheless, studies from Argentina and Paraguay suggest a degree of specialization, with a diet composed mainly by tadpoles, frogs, and toads (Prieto et al., 2012; Bellini et al., 2015; Cabral et al., 2017). In general, published data on trophic ecology studies in *E. poecilogyrus* did not present identification of snakes surveyed at subspecific level. In this sense, plausible differences in diet among subspecies in its distribution have to be considered, as well as its influence in catalogue them as generalist or specialist.

Despite the developmental stage of the ME reported herein, ecological conditions affect the spatial and temporal overlap of the host and parasite, while species-specific behaviors characteristics can modify these interactions. Adaptations that increase encounter with potential hosts will likely lead to higher infection probabilities (Combes, 2005; Bolek et al., 2024). Several potential routes for host infection were identified, including or not intermediate or paratenic hosts.

In regard of adult ME, *Kalicephalus* spp. could be considered as a geohelminth. Infections may occur when the host explores soil, introducing infective stages with tongue movement (Schad, 1956; Anderson, 2000). Values of parasitological descriptors of *K. appendiculatus* could represent parasitological evidence of the terrestrial habitat use in this snake. Nevertheless, the other four taxa were biohelminths with freshwater-associated intermediate hosts, together supporting the semi-aquatic habits of this subspecies.

The life cycle in *Ophiotaenia* species is not completely elucidated and studies about this topic are scarce (Scholz and De Chambrier, 2003). Two transmission pathways had been suggested: a three-host cycle including copepods as first intermediate hosts, fishes or amphibians as secondary intermediate hosts, and reptiles as definitive hosts, or a two-host cycle with a tissue phase of development in the final host, followed by migration to the intestine, without intermediate or paratenic vertebrate host (Biserkov and Kostadinova, 1997; De Chambrier et al., 2021). The first transmission route is considered more likely for *O. joanae*, given the semi-aquatic habits of *E. p. caesius* and its most common prey items (tadpoles and adult anurans).

For *Catadiscus* and *Opisthogonimus*, Planorbidae snails are obligatory intermediate hosts in their life cycle. *Catadiscus* cercariae encyst in vegetation, where it can be ingested by herbivorous tadpoles or during predation activity (Yamaguti, 1975; Hamann and Kehr, 2003). Metacercariae of *Opisthogonimus* have been found parasitizing the body cavity, muscle, pharyngeal region, and tegument of tadpoles (Hamann and González, 2009), and adult anurans (Hamann et al., 2009). Both amphibians life stages are plausible prey for *E. p. caesius*. Seasonal conditions in the Dry Chaco may limit water availability and shorter pond duration, limiting resources for anurans and explaining the low prevalence and mean abundances of these digeneans in adult stage.

Raillietiella furcocerca commonly infects lizards, where both nymphs and adults occur, using insect prey as intermediate hosts (Fain, 1966; Melhorn, 2004 *apud* Fredes and Raffo, 2005). Lizards are occasional prey for *E. p. caesius* (Andrade et al., 2020) and the low prevalence of this ME suggests they are minor component of its diet.

Parasite ecology studies in snakes from Nearctic and Neotropical do not report frequently the presence of larval helminths (see Detterline et al., 1984; Fontenot and Font, 1994; Jiménez-Ruiz et al., 2002; Ávila et al., 2013; Mati et al., 2015; Carvalho et al., 2018; Matias et al., 2018; Oliveira et al., 2024; Silva-Trejo et al., 2025). Interestingly, 81.87% of ME collected in our study were larval stages of biohelminths.

Oligacanthorhynchus sp. and *Physaloptera* sp. are biohelminths of broad host range that include arthropods (myriapods and several insect orders) as intermediate hosts. Their ocurrence in *E. p. caesius* suggests a paratenic role and potential predator-prey interactions with birds or mammals, as possible definitive hosts (Cawthorn and Anderson, 1976; Anderson, 2000; Kennedy, 2006; Richardson, 2006).

Pioneer studies in Argentine snakes reported high number of larval digeneans (mesocercariae and metacercariae) in mesenteries and epiploic folds (Caubisens Poumarau, 1968); in this particular case, from 960 digeneans collected, 797 were larval, indicating high abundance.

Alaria and *Strigea* include one obligatory intermediate host (Planorbidae), with snakes as secondary intermediate hosts, and reach adult stages in carnivorous mammals or birds of Accipritidae, Strigidae, and Falconidae families (Niewiadomska and Pojmańska, 2011). At Ingeniero Juárez and other localities from Dry Chaco, metacercariae of *Strigea* species and mesocercariae of Alariinae were found parasitizing anuran in previous research. These were reported as dominant and more abundant taxa in community of *Lepidobatrachus llanensis* Reig et Cei, 1963 (Hamann et al., 2019, 2022 a, b). We confirm the presence of larval stage of Digenea with this identity in the material collected from this snake species.

Considering the dynamics of microhabitats in Dry Chaco, where host as resource for ME are neither permanent nor abundant, and environmental conditions fluctuate drastically, ME as *Strigea* and *Alaria* may maximize their prevalence by enlarging their host range in secondary intermediate or paratenic roles in function of their temporal availability. The accumulation of stages in other suitable hosts, such an anurans or snakes, allows that predation could facilitate potential transmission to definitive hosts (birds for *Strigea* spp. or mammals for *Alaria* spp.).

Several *Strigea* species had been recorded in Formosa province, Argentina, parasitizing *Rupornis magnirostris* (Gmelin, 1788) (= *Buteo magnirostris*), *Buteogallus meridionalis* (Latham, 1790) (Accipritidae), and *Caracara plancus* (Miller, 1777) (Falconidae) (Lunaschi et al., 2007; Drago and Lunaschi, 2015). In Argentina and Brazil, predation on *Erythrolamprus* species and other dipsadids by birds of these and other families (de Souza et al., 2021; Fernández-Reinoso et al., 2022) and carnivores as *Procyon cancrivorus* (Cuvier, 1978) and *Cerdocyon thous* (Linnaeus, 1766) are reported (Quintela et al., 2014; Castellari-González et al., 2016). Another transmission route is the consumption of rodents that had been reported as prey as well as a paratenic host for *Strigea* and *Alaria* species in Holartic (Möhl et al., 2009; Batista et al., 2019). These could support acquisition routes of infective larval stages of these taxa by this snake and reaching maturity in predators in this site of study.

Species serving as intermediate hosts tend to lie in different trophic levels in food web to that for definitive host species. In these positions, they tend to have many different predators, mediating many food chains, acting as focal points of predation interactions involving species across various trophic levels, from higher to lower. Those mechanisms could promote frequent and stable predator-prey interactions, which are essential for the successful completion of parasite life cycles (Chen et al., 2008).

In regard of parasitological descriptors, in the limited studies on ME ecology in semi-aquatic to aquatic snakes in Neotropics and Holartic, it is observed that a single taxon or a few from one phylum often show high prevalence and mean abundances, while other taxa are less abundant or prevalent. In this sense, ME component populations prevalence in studies from Neotropical snakes, prevalence did not exceed 63% (see Jiménez-Ruiz et al., 2002; Ávila et al., 2013; Mati et al., 2015; Carvalho et al., 2018; Matias et al., 2018; Oliveira et al., 2024; Silva-Trejo et al., 2025), while in Holarctic snakes can reach 100% (see Fontenot and Font, 1994; Santoro et al., 2013). In both realms, mean abundances were between 0.02 and 262 parasites/host. Our results are similar to those obtained in other realms in terms of prevalence and mean abundance. Regarding ME proportion of phyla in taxonomic structure, in Neotropical studies, Nematoda taxa show higher richness, prevalence, and mean abundance, while other as Acanthocephala, Pentastomida, and Platyhelminthes (Digenea and Cestoda) appears occasionally with lower richness, prevalence, and mean abundance (Ávila et al., 2013; Mati et al., 2015; Carvalho et al., 2018; Matias et al., 2018; Oliveira et al., 2024). In contrast, North American studies on *Akgistrodon* Palisot de Beauvois, 1799, *Nerodia* Baird et Girard, 1853 and *Thamnophis* Fitzinger, 1843 showed high variability in the number of taxa and parasitological descriptors (Fontenot and Font, 1994; Jiménez-Ruiz et al., 2002; Silva-Trejo et al., 2025). Our results resemble with studies on colubrid snakes from Nearctic and Palearctic realms (Fontenot and Font, 1996; Santoro et al., 2013), where digeneans and larval helminths dominate the communities. However, contrast with finding in Neotropical Dipsadidae were geohelminths and Nematoda are often the most diverse (Table S3, Supplementary Materials)). These differences may reflect spatial and host-related variation in ME prevalence and mean abundances.

Despite their relevance, importance and dispersion indices are rarely included in Nearctic and Neotropical studies on snake parasite ecology (Fontenot and Font, 1994; Jiménez-Ruiz et al., 2002; Ávila et al., 2013; Mati et al., 2015; Carvalho et al., 2018; Matias et al., 2018). Their use can yield key insights into community structure, parasite transmission dynamics, and the selection of appropriate biomarkers for characterizing parasite assemblages (Poulin, 1993; 1999; McVinish and Lester, 2020; Lester and Blomberg, 2021).

Generally, parasite populations follow a negative–binomial distribution which reflects the common pattern of parasite dynamics in natural hosts' populations (many hosts with few parasites and few hosts with many parasites) (Reiczigel et al., 2024). According to metapopulations theory, this distribution support transmission dynamics by ensuring uninfected hosts serve as available 'patches' for parasites recruitment. In our results, only *O. joanae* showed a regular distribution, likely due to its constant abundance at infrapopulation level, making the mean and variance ratio close to 1.

In our study, the high abundance of larval forms, compared to adult, makes traditional importance indices less effective in assessing the true ecological role of these species. These indices tended to assign similar roles to species that are equally abundant and prevalent, or, in some cases, classify them into categories that exclude them from being considered 'important'. Therefore, it is crucial to analyze the helminth community considering both mature and immature stages, both simultaneously and separately. Additionally, it is necessary to employ other qualitative and quantitative techniques to prevent the exclusion of species categorized as 'unsuccessful pioneers', a label based solely on their maturation stage. This approach is essential, as it considers for the role of paratenic hosts in maintaining the life history of parasites and recognizes the potential of parasites as indicators of possible predators.

Interspecific relations by site of infection, influence of host biological characteristics and structural attributes

ME in adult stage exhibited high specificity for the site of infection. However, a high number of larval taxa, which lower specificity and wide host range, may influence interspecific interactions, aiding their survival until they reach full development in suitable hosts.

Some ME pairs of taxa showed positive correlation. Aho (1990) suggested that helminth communities in amphibians and reptiles tend to be isolationist and non-interactive. Nevertheless, recent studies on fish and mammal parasite communities supports that facilitation among parasite species is more common than negative interactions. Positive interactions contribute more significantly to increasing communities' richness (Dallas et al., 2019; Rodgers and Bolnick, 2024). The mechanisms are unclear, but may involve a pioneer species weakening the host's immune system, allowing secondary species to colonize. However, considering the indirect approach in testing these associations (Poulin, 2001), we suggest that a larger sample size could be needed to provide more statistically robust results.

Parasite community studies in aquatic snake in Nearctic and Palearctic region reported 11 to 13 taxa (Fontenot and Font, 1994; Santoro et al., 2013), similar to our finding, though, only Santoro et al. (2013) included larval stages. In contrast, the richness reported for Neotropical snake appears lower, which may be related to the absence or lack of reporting of larval stages.

Excluding larval stages underestimates infracommunity richness. Infracommunities that include larval stages, either exclusively or combined with adult, show greater richness than those composed only of adult. Larval and larval-adult communities show slightly higher diversity and dominance than adult-only communities. A similar pattern was observed by Santoro et al. (2013) in the habitat generalist snake *Hierophis viridiflavus carbonarius* (Bonaparte, 1833) (Colubridae) in southern Italy. The low diversity and dominance indices observed in our results are consistent with research on snakes with different habitat uses (Fontenot and Font, 1994; Jiménez-Ruiz et al., 2002; Santoro et al., 2013).

Weight and SVL are variables linked to host characteristics as body condition and age (Poulin, 1997). The most accepted hypothesis suggests that larger hosts may harbor more parasites due to their greater size which provides more resources and space. Additionally, age may influence parasite richness or abundance due to longer exposure to infections or changes in feeding preferences (Poulin, 1997). Studies on host size or mass as predictors of parasite richness or abundance in snakes are scarce (Lettoof et al., 2019; Oliveira et al., 2024), and no general pattern has been established. For example, while SVL predicts higher richness in Neotropical amphibians (Campião et al., 2015), no such relationship was found in lizards (Leung and Koprivnikar, 2019).

In snakes, a particularly relevant issue is the possibility that food availability early in life can have a disproportionate impact on later growth patterns and, ultimately, on maximum adult body size. Such an effect could generate substantial size variation among cohorts, even in the absence of genetic differences (Madsen and Shine, 2000; Brown et al., 2017).

Minor SVL and weight variation among specimens suggest they belong to the same cohort, consistent with Cabral et al. (2022), who observed newborn groups for this subspecies. These individuals were likely born in the pond and stayed nearby, using its water, shelter, and amphibian prey as a food source. Juveniles exhibit opportunistic feeding behavior – although not yet specifically reported for this subspecies – that could be associated with site fidelity, efficient use of resources, and the absence of alternative water bodies in the area (Cabral et al., 2017, 2022).

This may explain the observed pattern: snakes with greater body mass showed higher ME intensities, whereas those with longer SVL had lower ones. In this sense, we suggest that relationships between body weight or SVL in with snakes' age, as well as, associations of these host features with the intensity of some ME cannot be analyzed isolated, or as response of a univariate phenomenon.

In addition, reptiles have a strong innate immune response, a moderate adaptative component with a slower activation, no lymphatic nodes, and seasonal variations in T and B cell activity influenced by temperature (Rios and Zimmerman, 2015). We propose that unconsidered effects on the innate and adaptive immune system during host development may differentially affect the recruitment of ME taxa and the increase of its intensity over time.

Our results suggest that some in hosts with reduced body conditions (and possibly depressed immune system) could facilitate the colonization of some ME species could be facilitated over time. This pattern could not be necessarily only related to host age but could also occur before mating and reproduction seasons, hydric stress, prey availability and host characteristics do not hinder successful ME recruitment.

A non-significant positive correlation between larval ME intensity and host condition suggests that certain snake sizes may be more vulnerable to predation Predators could take advance of the temporal aggregation behavior reported in this species as prey, and consequently, this could increase the likelihood of ME reaching their definitive hosts.

Oligacanthorhynchus sp., was the only larval species showing a significant negative correlation, both with host condition and with other helminths sharing the mesenterial–body cavity site. In this sense, both experimental and meta-analytical approaches show that cystacanth infection has no effect on intermediate host size (Benesh and Tellervo Valtonen, 2007; Fayard et al., 2020). Even, a positive relationship is observed between helminth larvae (cystacanths and metacercariae) and intermediate host size in natural marine environments (Poulin et al., 2003). These patterns contrast with our results; cystacanths and other larval helminths sharing intermediate hosts had positive correlations among them, along with negative correlations with serotonin concentrations. This have been suggested as evidence of host manipulation and the removal of heavily infected hosts by predation (Poulin et al., 2003). However, this also contrasts with our results.

This could suggest that in this study area, larval stages could not present refined mechanisms of host exploitation, possibly due to a recent relation between hosts and parasites, in evolutionary terms. Warburton (2020) consider drylands as 'natural laboratories', where hypothesis on virulence evolution, host spectra, and life cycle strategies could be tested, because extreme conditions and low population density represent examples within extremes in understand parasite communities diversity, structure and the transmission dynamic.

Studies in amphibians and reptiles as intermediate or paratenic hosts in these environments are scarce. In this sense, our comparison with previous research has to be as conservative as possible. At the same time, we suggest the design of more field experimental studies in this area in order to tap the potential of these environments, unraveling the challenges and knowledge gaps in the subjacent mechanisms of these parasite-host relationships.

This study provides new insights into the parasitic fauna of snakes, contributing valuable data on the ecology of ME infracommunities and component community of *E. p. caesius*, as well as their relationship with the host's biological characteristics. Its helps fill the research gap in area, specifically within the Dipsadidae family in Neotropics. However, most Neotropical research remains largely descriptive, unlike the more quantitative Holarctic studies.

The sampling effort and sample size, combining scarce field material with museum specimens from different origins and times periods, have provide a valuable approach to parasite communities in areas where knowledge is still limited, as Neotropical zones (Carlson et al., 2020). Nevertheless, these methods have limitations that need to be addressed. The underestimation of certain factors may exclude measurable aspects that must be considered. It is important to account for the heterogeneity of each study area, including environmental conditions and intraspecific host differences.

Evidence has shown that ME in communities associated to amphibians and reptiles belong to generalist species with a broad host range and low specificity. Considering this, studies on the parasitic fauna of amphibian and reptiles species provide valuable insights into the parasitic communities of related species with similar habitat use and trophic ecology. This could be especially relevant for species that are more vulnerable or have restricted distributions due to population declines, often caused by environmental degradation, such as the one the American Chaco has been enduring for decades (Nori et al., 2016).

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SUPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at: https://oaj.fupress.net/index.php/ah/article/view/17983/14574.

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Men and Snakes: A long-term monitoring of wild caught snakes used in the Rito di San Domenico e dei Serpari (Cocullo, AQ, Italy)

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Abstract. In Cocullo, a small village in central Italy, the traditional religious rite of San Domenico involves the annual capture and temporary exhibition of wild non-venomous snakes, primarily *Elaphe quatuorlineata*, *Zamenis longissimus*, and *Hierophis viridiflavus*. In 2010 a citizen science project was launched to monitor the captured snakes and evaluate the sustainability of this practice and its potential conservation threats. Over 15 years, data on 1,505 individual snakes have been collected. This project also included PIT-tagging, improvements in temporary housing conditions, regular clinical checks and the release of the snakes at their original capture sites. The monitoring results suggest that based on the collected data, current practices are sustainable and underline the importance of continued survelliance. However, the need for comparative field studies has emerged. This study shows how local cultural traditions can be integrated with evidence-based conservation and long-term monitoring, providing a replicable model for managing human–wildlife interactions involving reptiles.

Keywords. Human-wildlife interaction, snake conservation, citizen science, *Elaphe quatuorlineata*, *Hierophis viridiflavus*, *Zamenis longissimus.*

INTRODUCTION

An ancient traditional religious rite, performed annually in the small mountainous village of Cocullo in Abruzzo, central Italy, involves the capture and temporary housing of local non-venomous snake species. Every year, on the first of May, this well-known religious rite (the Catholic cult of San Domenico, who lived in the area in the 11th century) attracts worldwide attention, draws thousands of visitors, including researchers, and international media (see for example Martinelli and Zavoli, 2023; Martinelli, 2024; Hall, 2025).

Dating back at least four centuries ago, this rite combines cultural, religious, and anthropological aspects (see, for example, Harrison, 1907; Haland, 2011) with important snake conservation implications. Indeed, the central figures are snakes, and the local snake catchers, known as *Serpari*. The ceremony has mostly remained unchanged for centuries. However, until the early 1900s it is reported that snakes were often killed at the end of the rite or sold (Harrison, 1907). In contrast, over the past decades, the local community has shown increased respect and protection towards snakes (Savoretti, 2016; Pellegrini et al., 2017; Zenoni, 2019), which is something unique in Italy, where snakes are generally among the most 'unpopular' animals and often persecuted and killed (Di Nicola et al., 2021).

Serpari are not professional herpetologists, but local inhabitants who preserve the tradition of snake-catch-

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ing in the weeks before the event (i.e., in the time span between March 19 and April 30).

The snake search by *Serpari* is carried out close to the Cocullo municipality. Snakes are captured either by hand or using a stick.

Various Colubridae species are used during the celebration. The main target species appears to be *Elaphe quatuorlineata* (Lacépède, 1789) one of the largest and most vulnerable snake species in central Mediterranean Italy (Filippi, 2003; Filippi et al., 2005; Filippi and Luiselli, 2006; Corti et al., 2011), as it is traditionally the only one destined to be placed on top on the statue of San Domenico (Bruno and Maugeri, 1990; Filippi and Luiselli, 2003; Pellegrini et al., 2017).

Other species, such as *Zamenis longissimus* (Laurenti, 1768) and *Hierophis viridiflavus* (Lacépède, 1789), both widespread throughout Italy (Filippi and Luiselli, 2000; Luiselli and Filippi, 2006; Corti et al., 2011) are also caught by *Serpari* to be shown during the rite of San Domenico. These three species are protected under the EU Directive habitats 92/43/CEE and by national law no. 357/1997. Since 2009, the Italian Ministry of the Environment, with the favorable opinion of the Italian Institute for Environmental Protection and Research (ISPRA), the Societas Herpetologica Italica (SHI) and Roma Tre University has authorized the capture and temporary possession of snakes on a three-year basis.

The first authorization required that the monitoring of the captured snakes included the collection of the following data: species, individual markings, and the names of *Serpari*. At the end of the ritual, the snakes must be released at their original point of capture.

Since 2010, we have carried out a citizen science project, with the support of the local administration, and the fundamental help of *Serpari*. This citizen science project has gone beyond the requested monitoring of snakes by collecting biometric parameters, assessing the health status of the snakes and improving housing conditions of snakes, as well as evaluating the sustainability of the number of snakes captured over the years. We have improved the temporary housing conditions of snakes by purchasing and distributing 22 professional terrariums to *Serpari*. We have provided them with detailed husbandry guidelines (i.e., regular disinfection of the terrarium, accessories such as hiding places and water bowls, differentiation between hot and cold areas). Before 2010, snakes were mainly kept in makeshift containers made of wood or plastic, often lacking the minimum welfare standards required for proper reptile housing.

The project has also involved promotional and scientific outreach activities: setting up a museum space, public meetings (e.g., various editions of the Herpethon by SHI since 2017), and the publication of outreach articles.

Summarizing, this study provides: i) monitoring data of the snakes (number of individuals captured and biometric parameters) collected since 2010, ii) an overview of *Serpari* community, iii) trends in capture numbers and morphometric parameters to indirectly assess the impact of the conservation measures implemented.

MATERIALS AND METHODS

All snakes captured by *Serpari* were monitored 2–3 days before May 1st. Data were collected between 2010 and 2024, resulting in a total sample of 1,505 snakes. For each snake the following information were recorded: species, sex, age class (juvenile, sub-adult, adult), biometric measurements (weight, snout-vent length, and tail length), and the specific capture location (since 2010, *Serpari* have recorded GPS coordinates of snake's capture locations).

Most snakes (n = 1,204) were implanted subcutaneously with a Passive Integrated Transponder (PIT tag) - a small injectable microchip containing a unique alphanumeric code readable by a scanner. Physical examination followed, and bacteriological swabs were collected for laboratory analysis. From 2010 to 2012, samples were processed by the Istituto Zooprofilattico Sperimentale (IZS) of Lazio and Toscana, while in 2013, analyses were conducted by the IZS of Abruzzo and Molise (e.g., Filippi et al., 2010). When necessary, veterinary staff administered appropriate treatments, including disinfection, wound care, hydration, antibiotics, and ectoparasite (e.g., mite) removal. After the event, or within few days, each snake was released at the same site by the *Serparo* who captured it.

To indirectly evaluate the impact of conservation measures, we have adopted methodologies similar to those used in studies of harvested snakes for the leather industry (Natusch et al., 2016; Natusch et al., 2019; Arida et al., 2024).

Statistical tests (univariate descriptive statistics, χ^2 test, one-way independent ANOVA several-sample test, Pearson's correlation coefficient) were calculated with Past 4.09 (Hammer et al., 2001). Statistical significance was accepted at $P < 0.05$.

RESULTS

From 2010 to 2024, a total of 1,505 snakes (including adults, subadults, and juveniles and recaptures) were

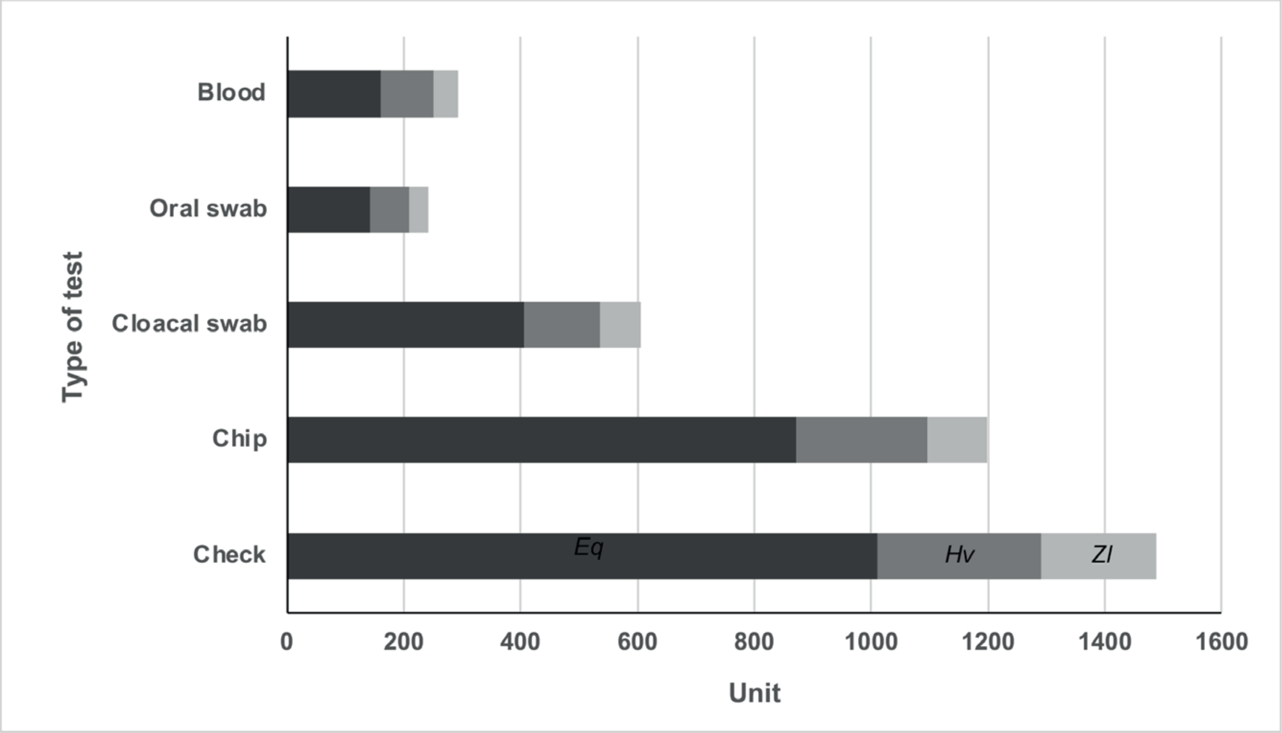

Fig. 1. Total check, oral and cloacal swabs, blood samples taken, and microchips inserted in the three most frequently caught snake species in Cocullo: *E. quatuorlineata (Eq - black), H. viridiflavus (Hv- dark grey), Z. longissimus (Zl – light grey)*.

brought to us by the Serpari (note that in 2020 and 2021 the ritual did not take place due to the Covid-19 pandemic).

A summary of snakes' health assessments, including physical examination, controls, oral and cloacal swabs, blood sampling, and microchips implantation, is provided in Fig. 1. Detailed results of clinical and laboratory analyses have been presented in other studies (e.g., Marini et al., 2023; Mendoza-Roldan et al., 2024; Ugochukwu et al., 2024; Alves et al., 2025; Fagundes-Moreira et al., 2025).

The snakes captured by *Serpari* were primarily *Elaphe quatuorlineata* (n = 1,011), with smaller numbers of *Hierophis viridiflavus* (n = 279) and *Zamenis longissimus* (n = 198). Other less captured species included, *Natrix helvetica* (Lacépède, 1789) (n = 10), *Coronella austriaca* Laurenti, 1768 (n = 5), and *Coronella girondica* (Daudin, 1803) (n = 2). Although *Vipera aspis* (Linnaeus, 1758) is syntopic, it is not permitted to capture it due to its venomous nature. Compared to the regional potentiality, Cocullo and the neighboring villages hosted 7 of 9 species (Di Tizio et al., 2024); only *Vipera ursinii* (Bonaparte, 1835) and *Natrix tessellata* (Laurenti, 1768) are absent.

The total number of individuals captured annually ranged from 90 to 186 (x = 115.77 ± 23.77) varied significantly among years (χ^2= 58.58, df = 12, P < 0.001).

Average morphometric data (snout-vent length - SVL, tail length and body weight) per year of the three most abundant species (*E. quatuorlineata*, *H. viridiflavus* and *Z. longissimus*) are reported in Table 1, 2, 3. Low number of *C. austriaca*, *C. girondica* and *N. helvetica* did not allow a statistical analysis.

The number of *Serpari* ranged from 19 to 35 (x = 24.5 ± 6.4) though this variation was not statistically significant (χ^2 = 20.13, df = 12, P = 0.07) (Fig. 2). The *Serpari* were mostly men, they ranged from 17 to 29 (x =21.3 ± 3.8), but there were also women (x = 3.2 ± 3.2) that ranged from 0 to 9. The number of *Serpari* was positively correlated with the number of snakes captured ($r_{(11)}$ = 0.67, P < 0.05).

The annual capture numbers of *E. quatuorlineata* ranged from 60 to 93 (x = 77.77 ± 10.78) and did not vary significantly over the study period (χ^2 = 17.93, df = 12, P = 0.12), unlike the number of the other two most frequently captured species, *H. viridiflavus* (range = 8-63, x = 21.46 ± 15.09, χ^2 = 47.89, df = 12, P > 0.001) and *Z. longissimus* (range = 7-26, x = 15.23 ± 5.96, χ^2 = 27.99, df = 12, P = 0.006). The captured numbers of *E. quatuorlineata* and the combined total of *H. viridiflavus* and *Z. longissimus* were negatively correlated ($r_{(10)}$ = -0.34, P > 0.05).

No significant trends were observed in the average number of snake captures per *Serparo* across years for the

Table 1. Morphometric adult males and females data (n = sample size) of *E. quatuorlineata*: values of snout-to-vent length (SVL), tail length (TL) and body weight report mean ± SD per year. Sample size (n) that differ from the reference column are highlighted with asterisks (*) and listed at the bottom of the table.

Year	Males (n)	SVL cm)	Tail (cm)	Weight (gr)	Females (n)	SVL (cm)	Tail (cm)	Weight (gr)
2010	36	129.01±9.23	30.44±2.60	758.17±168.40	17	134.44±10.70	25.65±3.95	719.65±188.24
2011	43	123.74±11.66	30.47±3.80	692.44±192.74*	18	127.28±14.41	26.19±2.64	704.78±263.93
2012	47	124.57±11.19	30.14±3.06	748.17±185.08	23	127.43±17.60	24.08±6.17	757.39±242.67
2013	41	123.32±10.61	29.17±3.18	738.96±214.88	24	127.54±12.07	27.15±3.18***	699.47±218.01***
2014	38	121.63±8.25	29.32±5.43	725.92±176.56	32	126.78±15.07	25.11±5.16	738.44±229.90
2015	43	125.84±9.25	29.89±4.07	777.02±176.01	26	130.44±8.57	26.67±4.61	772.23±169.56
2016	48	127.96±9.41	29.48±4.72	773.94±194.11	25	133.16±10.83	25.92±3.01	771.44±202.28
2017	64	126.48±10.02	29.91±3.50	736.68±211.68**	19	134.50±8.67	24.50±5.32	722.00±181.48
2018	52	126.93±11.60	30.27±4.11	690.12±210.13	26	135.63±10.38	25.12±4.74	711.08±197.18
2019	52	126.38±10.39	30.16±4.14	715.37±191.35**	23	134.22±10.82	25.80±3.50	708.17±187.10
2022	40	126.65±9.44	29.61±4.66	747.68±202.56	23	131.07±12.80	26.17±5.10	771.96±230.03
2023	44	126.93±14.26	29.83±4.78	705.80±269.06	19	134.58±10.36	27.00±3.64	728.11±148.02
2024	60	128.23±13.37	30.28±4.10	731.98±227.77	29	131.62±12.16	26.66±4.37	643.80±228.11*****
				*n =41			***n = 23	****n = 23
				**n = 51				*****n=28

Table 2. Morphometric adult males and females data (n = sample size) of *H. viridiflavus*: values of snout-to-vent length (SVL), tail length (TL) and body weight report mean ± SD per year. Sample size (n) that differ from the reference column are highlighted with asterisks (*) and listed at the bottom of the table.

Year	Males	SVL	Tail	Weight	Females	SVL	Tail	Weight
2010	8	87.5±12.30	28.81±8.61	178.00±83.31*	3	87.33±4.62	28.67±0.58	166±21.63
2011	7	88.79±4.69	31.5±1.04	199.43±44.30	5	84.50±8.72	26.00±3.52	198±89.25
2012	8	97.69±6.42	32.50±7.22	389.88±76.21	2	93.50±0.71	31.50±2.12	344±166.88
2013	9	94.78±8.21	27.44±6.26	243.78±76.06	2	94.50±2.12	30.00±1.14	188±5.66
2014	17	89.76±11.26	30.88±3.43	259.88±93.61	2	68.50±3.54	21.00±0.00	72.00±0.00
2015	10	94.60±5.42	29.65±5.86	320.80±52.02	1	84.00	29.50	132.00
2016	7	92.00±5.71	31.71±1.22	236.57±69.62	-	-	-	-
2017	11	91.23±9.16	31.13±3.52	226.73±67.89	1	84.00	27.00	122.00
2018	18	90.53±8.67	30.67±4.44	216.59±73.17**	3	87.00±4.58	26.50±4.27	136.67±30.62
2019	20	92.53±5.83	29.90±6.46	269.20±62.78	8	89.50±4.81	27.75±2.48	198.86±57.62*
2022	21	89.45±11.06	30.90±4.69	213.73±92.90	13	89.12±8.32	28.85±2.48	190.75±68.45
2023	21	92.95±8.90	30.71±5.88	228.95±70.68	5	79.00±7.97	26.60±2.61	120.80±48.90
2024	38	90.66±9.72	31.01±4.81	218.00±75.00	23	83.87±8.61	26.85±4.74	140.83±43.43
				*n=7				*n=7
				**n=17				

three main species: the average number of *E. quatuorlineata*, *H. viridiflavus*, and *Z. longissimus* caught per *Serparo* each year did not differ significantly ($F_{12,295} = 1.24$, P = 0.26; $F_{12,295} = 1.19$, P = 0.28; $F_{12,295} = 1.82$, P = 0.05). Capture trends are shown in Figure 3. A *post-hoc* power analysis, performed by G*Power (Faul et al., 2007), achieved sufficient statistical power (0.99 for the three species) to support the conclusion that average capture numbers of *E. quatuorlineata*, *H. viridiflavus*, and *Z. longissimus* captured per *Serparo* remained stable over time.

There were also no significant differences in the SVL mean between the years, including repeated snake captures, of *E. quatuorlineata* males ($F_{12,595} = 1.59$, P = 0.09), females ($F_{12,591} = 1.67$, P = 0.07), or in males of *H. vir-*

Table 3. Morphometric adult males and females data (n = sample size) of *Z. longissimus:* values of snout-to-vent length (SVL), tail length (TL) and body weight report mean ± SD per year. Sample size (n) that differ from the reference column are highlighted with asterisks (*) and listed at the bottom of the table.

Year	Males	SVL	Tail	Weight	Females	SVL	Tail	Weight
2010	12	98.54±21.49	21.00±5.31	260.00±156.37	7	84.57±8.81	18.21±2.55	163.43±54.69
2011	5	88.00±11.25	20.90±3.05	178.40±75.86	4	82.75±8.18	17.88±2.02	184.50±38.79
2012	7	96.79±11.46	23.64±3.47	282.29±99.30	2	79.00±16.97	12.00±0.00	212.00±84.85
2013	11	95.10±13.13	22.68±4.29	228.00±64.63	11	85.00±9.33	18.64±5.45	170.36±50.17
2014	9	95.72±11.23	22.56±3.57	248.00±94.87	5	86.50±4.56	19.10±1.85	188.40±44.71
2015	4	96.50±12.66	22.38±1.25	260.50±202.76	3	84.67±5.51	20.33±0.58	198.67±23.35
2016	8	105.86±14.13	23.00±4.87	324.50±131.39	7	83.86±12.06	17.58±2.11***	220.00±186.23
2017	1	140.00±0.00	32.50±0.00	972.00±0.00	5	91.00±11.20	18.50±6.06	210.00±68.69
2018	10	106.45±6.86	22.45±5.00	272.60±62.40	6	90.42±4.59	18.83±2.99	171.67±31.17
2019	5	102.80±8.50	24.00±2.35	272.00±94.45	8	88.63±4.73	17.63±3.65	191.50±49.13
2022	5	94.70±10.29	21.80±5.07	220.50±56.25*	1	96.00±0.00	21.00±0.00	228.00±0.00
2023	11	89.00±10.77	21.73±4.58	146.72±57.56	3	80.00±1.00	17.00±1.00	101.33±3.06
2024	17	96.26±25.11	28.65±21.30	221.06±134±66**	9	90.67±8.29	16.55±4.44	171.11±69.47
				*n=4			***n=6	
				**n=16				

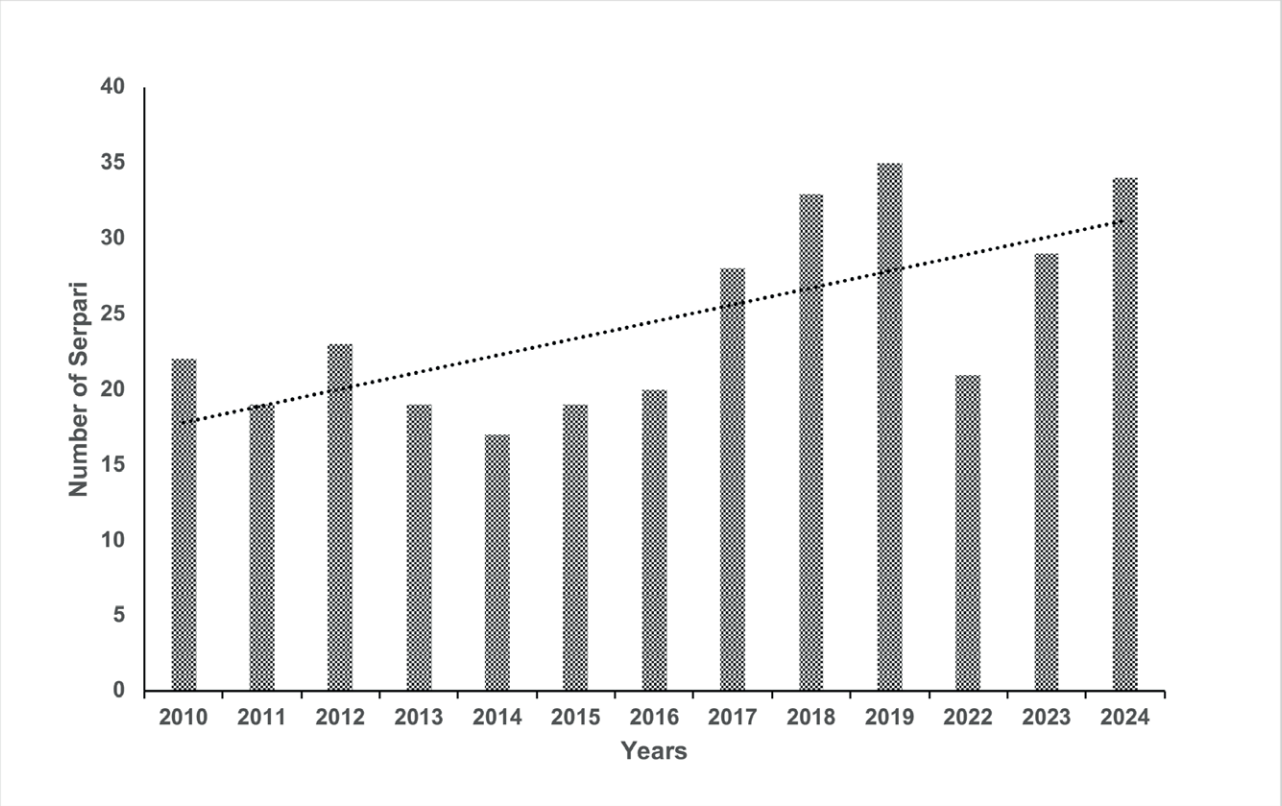

Fig. 2. Number of *Serpari* per year and trend line.

idiflavus ($F_{12,182}$ = 1.59, P = 0.50), or *Z. longissimus* males ($F_{11,92}$ = 1.50, P = 0.14). However, a significant difference was observed in the SVL of female *H. viridiflavus* ($F_{9,56}$ = 2.76, P = 0.01).

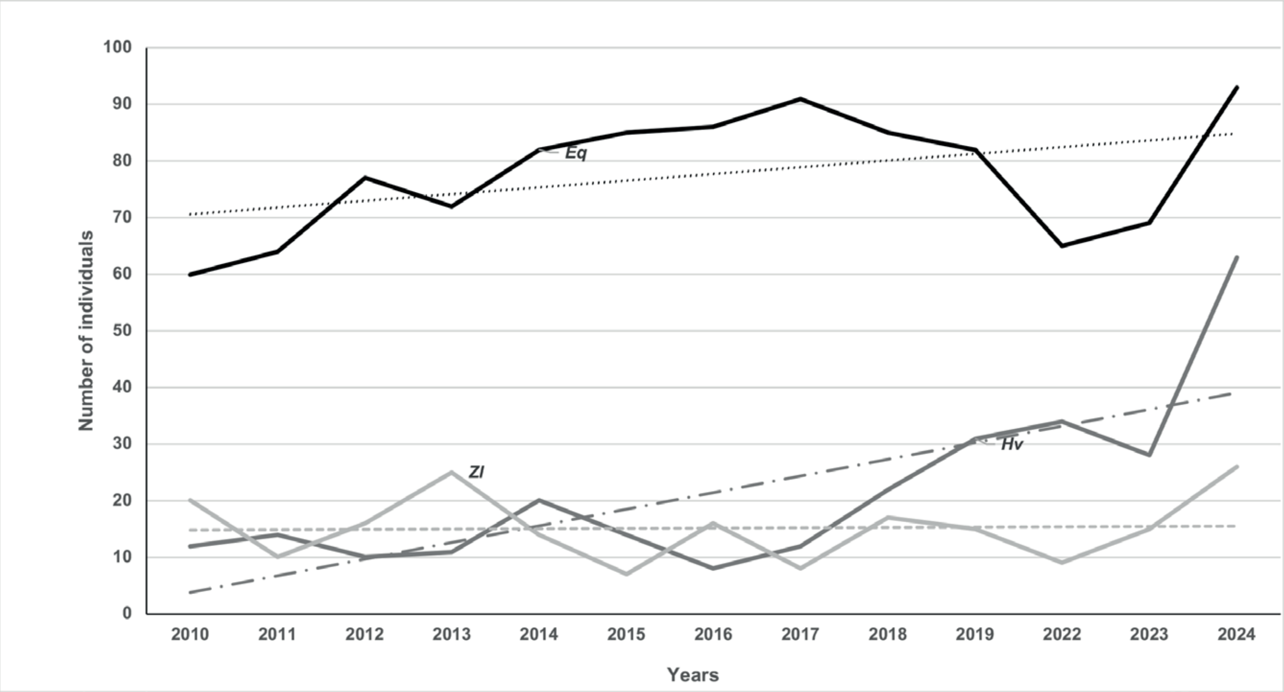

Fig. 3. Total of captures per year and trend of three most frequently caught snake species in Cocullo: *E. quatuorlineata* (Eq - black), *H. viridiflavus* (Hv- dark grey), *Z. longissimus* (Zl – light grey).

Table 4. Maximum total length (Tl), snout-to-vent length (SVL), tail length, body weight, and year of capture of the three most abundant species: *E. quatuorlineata* (Eq), *H. viridiflavus* (Hv) and *Z. longissimus* (Zl).

Species	Sex	SVL (cm)	Tail (cm)	Tl (cm)	Weight (gr)	Year
Eq	M	154.0	37.0	191.0	1156	2015
Eq	F	159.0	31.0	190.0	1064	2023
Zl	M	140.0	32.5	172.5	972	2017
Zl	F	106.0	22.0	128.0	288	2017
Hv	M	108.0	38.5	146.5	334	2022
Hv	F	105.0	25.0	130.0	368	2023

A χ^2 test on pooled data showed that sex ratio was similar between years for captured adult *E. quatuorlineata* (χ^2 = 10.85, df = 12, P = 0.54), *H. viridiflavus* (χ^2 = 19.75, df = 12, P = 0.07) and *Z. longissimus* (χ^2 = 112.79, df = 12, P = 0.38).

During the study period, no adverse effects were recorded from microchip implantation, even in individuals recaptured multiple times. For example, a male of *E. quatuorlineata* was captured for the first time in 2011 (SVL: 118,0 cm; tail: 31,0 cm; weight 678 g) and recaptured with increased measurements in 2014 (SVL: 123,5 cm; tail: 31,0 cm; weight 750 g), in 2016 (SVL: 128,0 cm; tail: 32,0 cm; weight 846 g) and 2023 (SVL: 143,0 cm; tail: 36,0 cm; weight 948 g) indicating healthy development and no adverse impact from tagging. A female of *E. quatuorlineata* was captured for the first time in 2013 (SVL: 125,0 cm; tail: 28,0 cm; weight 594 g) and recaptured in 2014 (SVL: 135,5 cm; tail: 28,0 cm; weight 896 g) and 2022 (SVL: 140,0 cm; tail: 29,0 cm; weight 1240 g).

The maximum lengths observed in our study are presented in Table 4.

DISCUSSION

This long-term citizen science project generated a substantial dataset and our data confirmed known morphometric patterns. In *E. quatuorlineata*, females were on average longer than males, supporting the presence of reversed sexual size dimorphism (RSD) in this species (see Rugiero and Luiselli, 1996; Capizzi and Luiselli, 1997; Filippi et al., 2005). Males had longer tail on average, while adult males and females exhibited comparable body weights. In *H. viridiflavus* and *Z. longissimus* males attained on average larger sizes and longer tails (see Scali and Montonati, 2000) and weight. Maximum length (record observed in Italy and published) was observed in *Elaphe quatuorlineata* and *Zamenis longissimus* (Corti et al, 2011).

We provided the first quantitative characterization of annual snake captures during this traditional ritual. Our results confirmed that the target species of *Serpari* is *E. quatuorlineata*, as qualitatively observed in the past (Filippi and Luiselli, 2003; Pellegrini et al 2017) with lower numbers of *H. viridiflavus* and *Z. longissimus* also being captured. This is likely due to the attractive appearance, larger size, and docile nature of *E. quatuorlineata*. Whereas *H. viridiflavus* and *Z. longissimus* and rarer species such as *N. helvetica*, *C. austriaca*, and *C. girondica* are typically captured as supplementary species when fewer *E. quatuorlineata* are caught by *Serpari*. This selective capture may introduce sampling biases, potentially misrepresenting the true composition and structure of the local snake community. For instance, *Elaphe quatuorlineata* is not recognized as the dominant species in other areas of central Italy (Filippi, 2003; Filippi and Luiselli, 2001, 2006; Luiselli and Filippi, 2006). Nonetheless, it remains possible that centuries of selective handling have influenced the current structure of the local snake populations.

Moreover, this study enabled a preliminary assessment of capture trends and morphometric stability over time: over this 15-year-old study, based on our results, the average number captured per *Serparo* of *E. quatuorlineata*, *H. viridiflavus*, and *Z. longissimus* and their morphometric parameters have remained stable. Our results confirmed the reliability and safety of microchip tagging for individual identification in wild colubrids (see Taggart et al., 2021).

Overall, the collected data can indirectly support the conclusion that the activities of capture, temporary detention, and release are currently sustainable for the local snake populations.

It is highly likely that accompanying conservation efforts – such as continuous health monitoring under a One-Health approach and improved temporary housing – have contributed to ensuring the sustainability of the practice (see Mendoza-Roldan et al., 2024; Ugochukwu et al., 2024; F Alves et al., 2025; agundes-Moreira et al. 2025). However, ongoing monitoring remains essential, both to detect potential issues and to guide future conservation or mitigation actions related to the rite and broader environmental changes. In this regard, it will be necessary to combine the current monitoring with standardized field studies, both to observe the emergence of any critical issues, to quantitatively assess the local snake community and to appropriate and/or eventual conservation and/or mitigation actions related to *Serpari* activities and/or related to the environment (see for example, Filippi and Luiselli, 2002; Edgar et al., 2010; Lelievre et al., 2010; Akresh et al., 2017; Filippi, 2019; Assmann, 2013).

In conclusion, the monitoring of snakes captured by the Serpari of Cocullo represents a unique convergence of traditional cultural practice, citizen science activities and scientific conservation effort. This case study offers a rare opportunity to assess species status and health under a One-Health framework, while promoting public awareness through citizen science and outreach. Continued research and engagement will be essential to maintain this balance and ensure the long-term well-being of both local wildlife and the human communities involved.

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Occupancy of artificial shelters by European tree frog (*Hyla arborea*) in Škocjanski zatok Nature Reserve: A non-invasive sampling method in ecologically sensitive habitats

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Abstract. Monitoring amphibians in non-breeding habitats is constrained by low detectability and potential disturbance to sensitive ecosystems. We tested the effectiveness of artificial shelters - PVC pipes - as a non-invasive method for monitoring the European tree frog (*Hyla arborea*) outside the breeding season in Škocjanski zatok Nature Reserve (Koper, Slovenia). Over three years (2022–2024), we installed 40- and 50-mm diameter shelter pipes at 24 sites and conducted regular inspections. A total of 338 encounters of the focal species were recorded, with frogs of all sizes using the shelters. Overall shelter occupancy declined across years, possibly reflecting a combination of factors including reduced recruitment, vegetation changes, demographic fluctuations, and weather conditions. A statistically significant trend was observed, with narrower (40-mm) shelter pipes having 1.6 to 1.7 times more captures than wider (50-mm) ones, despite no significant difference in the body size of frogs occupying each type. By using PVC artificial shelters, we greatly enhanced the detectability of *H. arborea* in the Škocjanski zatok Nature Reserve compared to classical monitoring methods such as acoustic surveys or dip-netting. Importantly, a non-invasive approach using artificial shelters minimized the risk of disturbance to breeding and migratory birds, which is essential in areas with high avifaunal conservation value. Our results support the broader use of artificial shelters for monitoring arboreal amphibians in ecologically sensitive areas and suggest future research should explore finer-scale shelter design to improve detection and ecological interpretation across life stages.

Keywords. *Hyla arborea*, artificial shelters, PVC pipes, amphibian monitoring, protected areas.

INTRODUCTION

In recent decades, multi-factorial human-induced changes have contributed to declines in amphibian populations worldwide through different mechanisms, such as habitat loss and fragmentation, emerging infectious disease, toxic substances, and climate change (Alford and Richards, 1999; Houlahan et al., 2000; Falaschi et al., 2019; Luedtke et al., 2023). As a result of their imperilled status, many amphibians are included in monitoring programmes, whereby a major challenge is the imperfect detection of species and the resulting underestimation of a species' distribution and abundance (Tanadini and Schmidt, 2011; Cruickshank et al., 2021). Most studies of amphibians focus on the reproductive season when they can be found at aquatic habitats in which the species breed, the use of which could be influenced by both landscape-related land-use patterns and site-specific factors. However, much less is known about the use of terrestrial habitats surrounding breeding habitats, these areas being crucial for the long-term survival of amphibian populations (Marsh and Trenham, 2001; Semlitsch and Bodie, 2003; Van Buskirk, 2005).

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One of the amphibian species experiencing population declines is the European tree frog *Hyla arborea* (Linnaeus 1758), which has shown population decreases in parts of its range, particularly in western Europe, despite being listed as Least Concern on the IUCN Red List (Agasyan et al., 2024). Nevertheless, *H. arborea* is listed as vulnerable and protected by Slovenian national legislation (Ministry of Environment, Spatial Planning and Energy, 2002). *Hyla arborea* has a prolonged breeding season that lasts from April to June. During the breeding cycle, adults stay in or near the spawning site or in riparian vegetation such as reeds (Ferreira et al., 2012; Pellet et al., 2006) where they can be readily sampled. In the post-breeding season, treefrogs can migrate a few hundred meters and live scattered about in terrestrial habitats (Pellet et al., 2006; Kovács et al., 2007). During this period, they are inconspicuous and very difficult to locate, so knowledge of microhabitat use outside the breeding season is very sparse, although good quality terrestrial microhabitat is crucial for the long-term persistence of this species (Pellet et al., 2004, 2006).

To address sampling this species during the non-breeding season, several approaches have been used. For example, Pellet et al. (2006) employed harmonic direction finders to track individual movements. Others, such as Kovács et al. (2007), searched directly for frogs in shrubs and trees within wetland areas to investigate diet and microhabitat use. However, such methods can be time-consuming or logistically demanding, limiting their applicability for regular monitoring. Artificial shelters – particularly polyvinyl chloride (PVC) or bamboo pipe shelters that mimic natural hiding places of hylids – have been proposed as an efficient and non-invasive alternative (Boughton et al., 2000; Bartareau, 2004; Myers et al., 2007; Johnson et al., 2008; Moulton et al., 2009; do Vale et al., 2018). This method has been proven useful in both ecological (Mahan and Johnson, 2007; Liner et al., 2008; Campbell et al., 2010; McGhee, 2020) and conservation contexts (Schurbon and Fauth, 2003; Wyatt and Forys, 2004; Suriyamongkol et al., 2021). Furthermore, it has also been explored as a tool for microhabitat creation or threat mitigation for amphibians and other vertebrates (Cowan et al., 2021; Suriyamongkol et al., 2021).

To our knowledge, the artificial shelter method has not yet been used in studies of *H. arborea*, but do Vale et al. (2018) have used this method to study another closely related hylid species of similar size, the Iberian tree frog *H. molleri* Bedriaga 1889. The purpose of the present study was to test a novel, non-invasive sampling method for *H. arborea* outside the breeding habitat within one of the last remaining strongholds of this species in Slovenian Istria (Veenvliet and Kus Veenvliet, 2014; Senič et al., 2024). The specific objectives of the study were to: (1) investigate the suitability of polyvinyl chloride (PVC) pipes as shelters for *H. arborea* monitoring; (2) evaluate the occupancy of PVC shelters of different pipe diameters in relation to the frog body size. Given that the design of the artificial shelters mimics natural hylid refuges, we hypothesized that *H. arborea* would readily occupy them.

MATERIAL AND METHODS

Study site and artificial shelters

Located in the municipality of Koper (Slovenian Istria), the Škocjanski zatok Nature Reserve is connected to the Adriatic Sea only via a narrow channel. The reserve comprises a shallow, tidally influenced brackish lagoon dominated by halophytic vegetation and a freshwater marsh (Fig. 1) characterised by open water, meadows, shrubland, and extensive reed stands dominated by common reed (*Phragmites australis)* (Mozetič and Lipej, 2014). *Hyla arborea* inhabits the freshwater section of the Nature Reserve, where it finds suitable spawning sites and abundant riparian and terrestrial vegetation (Veenvliet and Kus Veenvliet, 2014; Lipej B., 2022, pers. comm.). The population of *H. arborea* inhabiting the Natural Reserve is thought to be of both local and regional importance for the conservation and future viability of the species in Slovenian Istria (Senič et al., 2024). In 2014, the population of calling males was estimated to be about 50, despite the presence of alien fish species, such as *Gambusia holbrookii* Girard 1859 and *Lepomis gibbosus* (Linnaeus 1758) (Veenvliet and Kus Veenvliet, 2014). However, the interior of the freshwater part of the Nature reserve is closed for any non-essential visits. The reserve's primary focus is bird conservation and is considered as an important nesting, wintering, and migration site (Mozetič and Lipej, 2014). The peak of the nesting season coincides with the breeding season of *H. arborea*, so it is impossible to sample the frogs without significantly disturbing the nesting of the birds. Therefore, we tested the method with artificial shelters in the transition zone between the freshwater and terrestrial habitats along the naturalistic-educational trail (Fig. 1) to minimize the disturbance.

Artificial shelters were designed based on previous research on the genus *Hyla* (Boughton et al., 2000; Bartareau, 2004; Glorioso and Waddle, 2014; do Vale et al., 2018; Suriyamongkol et al., 2021). We used grey PVC pipes with a white interior, 60 cm in length, and with diameters of either 40 mm or 50 mm. The choice of 50-mm diameter shelters was based on the assumption that *H. arborea* is relatively large in size among the Hylidae (comparable to *Dryophytes cinereus* (Schneider,

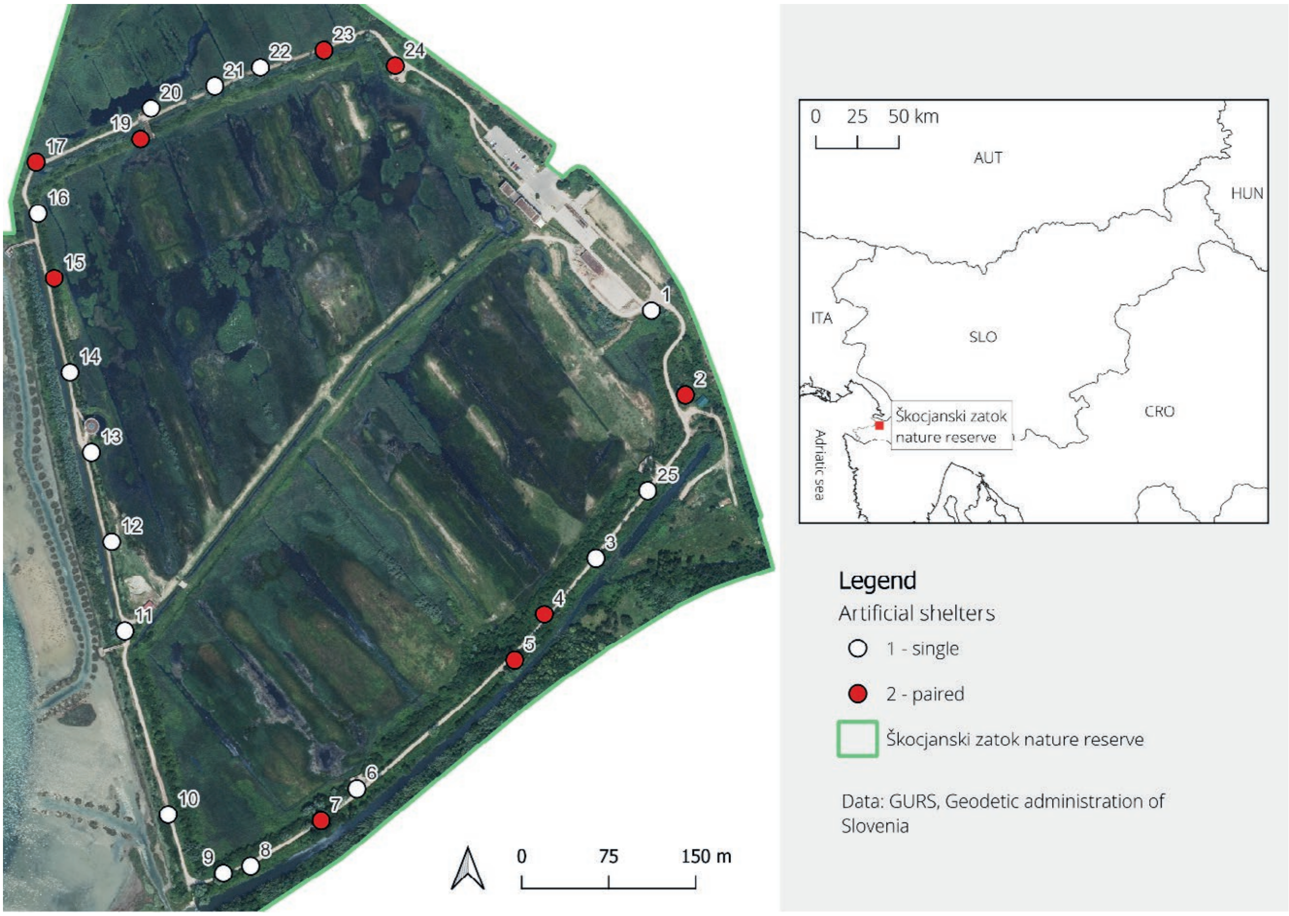

Fig. 1. Map showing 24 sites with artificial shelters along the naturalistic-educational trail in Škocjanski zatok Nature Reserve. Single shelter sites (50 mm) are marked with white circles; paired shelter sites (combined pipes with a diameter of 40 mm and 50 mm) are marked with red circles.

1799) and *H. molleri*); following designs of Zacharow et al. (2003) and do Vale et al. (2018). However, 40-mm shelters were also included, as some studies (Bartareau, 2004; Boughton et al., 2000) indicate that *Hyla* species may occupy narrower pipe diameters (i.e., 13–38 mm).

Shelters were installed at 24 permanent sites spaced 30 to 180 m apart (Fig. 1). They were mounted vertically on trees, in direct contact with the woody surface, with the opening facing upwards and positioned approximately 150 cm above ground level (Fig. 2). Each shelter was sealed at the bottom and filled with water up to a drainage hole located at 10 cm height to ensure high humidity and prevent overflow. At 15 sites, a single 50-mm shelter was installed (referred to as single-shelter sites; Fig. 2A), while at nine sites, two shelters were placed in close proximity (referred to as paired-shelter sites; Fig. 2B): one with a 40-mm and the other with a 50-mm diameter. This study design allowed us to independently assess the suitability of 50-mm shelters for capturing *H. arborea* as well as to examine shelter diameter occupancy under standardized conditions. Shelters were installed in May 2022 and, following an adaptation period, monitoring was conducted from July 2022 to December 2024. During each inspection, the presence and number of *H. arborea* individuals in each shelter were recorded (Fig. 2C) and each frog was photographed. Snout–vent length (SVL) of frogs found in paired shelters was measured using digital callipers to the nearest 0.1 mm except during occasional adverse weather conditions. After handling, frogs were returned to their respective shelters. For purpose of this study, frogs were not individually identified; therefore, numbers presented in results refer to frog captures, and not necessarily to individual frogs.

Statistical analysis

Capture counts of *H. arborea* were pooled regardless of sex or developmental stage; however, we per-

Fig. 2. Installation of (A) 50-mm (**) single shelter placed at site number 3 (March 2023); (B) paired shelter with 40-mm (*) and 50-mm (**) diameter pipes placed at site number 4 (June 2025); (C) *Hyla arborea* using artificial shelter (September 2022).

formed statistical analysis separately for 15 single shelter sites and nine paired shelter sites. We calculated the frequency of frog occurrence (FO) as i) the number of days with at least one frog present in the shelters divided by the number of monitored days, and ii) the number of shelters with frogs present divided by the number of all monitored shelters. For single shelter sites, we calculated the mean number of captures per shelter for all years combined and for each monitoring year separately. Corresponding 95% confidence intervals were derived using the Poisson distribution for count data while ensuring the lower confidence limit did not fall below zero. Given the sufficient sample size (N > 100), the normal approximation method was applied using the formula: $\lambda \pm 1,96 * \sqrt[2]{(\lambda/\mathrm{n})}$ (Eq. 1), where λ is the expected value (mean) of the sample Poisson distribution and n is the number of monitored shelters (Bégaud et al., 2005).

For shelter diameter occupancy analysis, we used only the days when at least one frog was present in order to reduce the effect of double-zero observations in the comparison (i.e., no frogs captured in any of the 18 shelters; Zuur et al., 2007). We calculated the mean number of captures in each shelter diameter and corresponding 95% confidence intervals. Confidence intervals were derived using the Poisson distribution for count data using the normal approximation method (Eq. 1; Bégaud et al., 2005). We tested whether the number of frogs captured per shelter and their measured body size follow the normal distribution using Shapiro–Wilk test, with the significance level set at $P < 0.05$. Both data sets violated normality assumptions, and non-parametric tests were applied (Dytham, 2011). The difference in the number of captures between shelter types was assessed using Wilcoxon matched-pairs signed-rank test due to the parallel placement and simultaneous monitoring of shelter pairs. Due to an extensive period of missing data for 2022, which was covered from July to December, a paired Wilcoxon test was additionally applied to the 2023–2024 data. Furthermore, we assessed shelter selection in relation to frog's body size using a Wilcoxon signed-rank test. All statistical analyses were performed in R (version 4.4.2; R Core Team, 2024).

RESULTS

A total of 44 monitoring days of all 24 sites were conducted, of which six days (13.6%) were between July and December 2022, 13 (29.5%) between January and

Table 1. Summary results of single and paired shelter site captures. Diam. = shelter diameter; N = number of captures; SD = Standard deviation; CI = Confidence interval; FO (%) = frequency of occurrence calculated as the number of shelters with frogs present divided by the number of all monitored shelters.

Period	Diam. (mm)	Monitoring occasions	Captures			FO (%)
			Total (N)	Mean ± SD per shelter	95% CI	
Single shelters						
2022	50	6	71	0.789 ± 1.590	0.605–0.972	27.8%
2023	50	13	47	0.241 ± 0.805	0.172–0.310	12.8%
2024	50	25	52	0.139 ± 0.459	0.101–0.176	9.9%
All years	50	44	170	0.258 ± 0.836	0.219–0.296	13.2%
Paired shelters						
All years	40	44 (25*)	104	0.462 ± 1.246	0.373–0.551	22.2%
	50	44 (25*)	64	0.284 ± 0.737	0.215–0.354**	17.3%
2023-24	40	38 (19*)	83	0.485 ± 1.087	0.381–0.590	24.0%
	50	38 (19*)	49	0.287 ± 0.673	0.206–0.367**	19.3%

* sample size after removal of double-zero occasions, on which the Mean ± SD, 95% CI and FO (%) were calculated (see Materials and methods).

** statistical significance at $p < 0.05$.

December 2023 and 25 (56.8%) between January and December 2024 (Table 1). The total number of captures was 338, with the number of captures exceeding 100 in each year for all shelters combined (Table 1).

At single shelter sites, frogs were detected on 27 of 44 monitoring days (61.4%) and in 87 of 660 monitored shelters overall (13.2%). Of the 87 occupied shelters, single captures were most numerous (Table 2), that is, in 49 instances (56.3%) only one frog resided in a shelter and in 16 instances (18.4%) two frogs occupied the shelter. Occasions with three or four frogs residing in a single shelter represented 19.5% (17 out of 87), while in the remaining occasions (5 out 87; 5.7%) there were five, six, or seven frogs found in one shelter. Across all years the mean number of frog captures in single shelter sites was 0.258 per shelter with 95 % confidence interval of 0.219 – 0.296 (Table 1). The mean value differed between years, with the 2022 mean frogs captured per shelter (0.789) being 3.2- to 5.7-fold greater than in 2023 (0.241) and 2024 (0.129), respectively (Table 1). Similarly, the frequency of occurrence per shelter was greater in 2022 compared to 2023 and 2024 (Table 1).

The overall frequency of occurrence in paired shelter sites was 56.8% (25 out of 44 days, Table 1). There were 19 occasions (43.2%) with no frogs present in any of the paired shelters, i.e., double-zero occasions, that were removed from further analysis. The frequency of occurrence per shelter was 22.2% (50 out of 225) in 40-mm and 17.3% (39 out of 225) in 50-mm shelters (Table 1). Both shelter types commonly captured one or two individuals (76.0% in 40-mm, 84.6% in 50-mm shelters), followed by three, four or five (22.0% in 40-mm, 15.4% in 50-mm shelters). Additionally, on one occasion (2.0%) in 2022, there were 12 individuals captured in a 40-mm shelter. The number of frogs captured per shelter deviated from normality both for pooled paired shelter sites ($W = 0.410$, $P < 0.001$), as well as separated by the shelter diameter (40-mm: $W = 0.417$, $P < 0.001$; 50-mm: $W = 0.446$, $P < 0.001$). Overall, the total number of frog captures was 1.6-fold higher in 40-mm shelters ($N = 104$) compared to the 50-mm shelters ($N = 64$). A similar pattern was observed for the 2023–2024 only data, with the difference amounting to 1.7-fold ($N = 83$ in 40-mm, $N = 49$ in 50-mm shelters). This difference is statistically significant across all years ($V = 1223.5$, $P < 0.05$) and for 2023–2024 data ($V = 870.5$, $P < 0.05$) and is reflected in mean values and corresponding 95% confidence intervals (Table 1).

Snout–vent length (SVL) was measured for a total of 142 frog captures at paired shelter sites, while 26 captures were not measured (see Materials and methods). In 40-mm diameter shelters ($N = 87$), SVL ranged from 24.1 mm to 52.1 mm (mean ± SD = 39.6 ± 5.8 mm; median SVL 39.5 mm; Fig. 3). In 50-mm diameter shelters ($N = 55$), SVL ranged from 22.4 mm to 51.9 mm (mean ± SD = 37.3 ± 8.3 mm; median = 38.7 mm; Fig. 3). Snout-vent length data deviated from a normal distribution (Shapiro–Wilk test: $W = 0.951$, $P < 0.001$), and no significant difference in body size was found between shelter diameters (Wilcoxon rank-sum test: $W = 2652$, $P = 0.278$).

Table 2. Number of individuals captured per shelter for single and paired shelter sites. Ind = individuals; Freq = frequency.

Ind / shelter (N)	50-mm single		40-mm paired		50-mm paired	
	Freq (N)	%	Freq (N)	%	Freq (N)	%
0	573	86.8	346 (175*)	87.4 (77.8*)	357 (186*)	90.2 (82.7*)
1	49	7.4	28	7.1 (12.4*)	23	5.8 (10.2*)
2	16	2.4	10	2.5 (4.4*)	10	2.5 (4.4*)
3	9	1.4	4	1.0 (1.8*)	4	1.0 (1.8*)
4	8	1.2	3	0.8 (1.3*)	1	0.3 (0.4*)
5	1	0.2	4	1.0 (1.8*)	1	0.3 (0.4*)
6	3	0.5				
7	1	0.2				
12			1	0.3 (0.4*)		
Total	660	100	396 (225*)	100	396 (225*)	100

* results after removal of double-zero occasions (see Materials and methods).

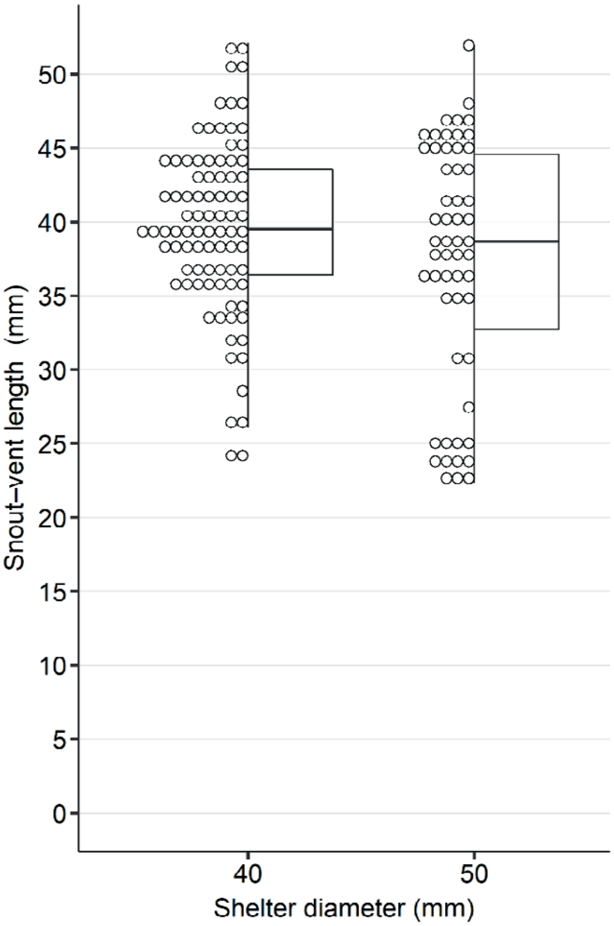

Fig. 3. Snout-vent length (SVL) distribution of *Hyla arborea* individuals captured in paired shelter sites (40 mm and 50 mm).

DISCUSSION

This study is the first in which we test the use of artificial shelters for occupancy by *H. arborea*. So far, in Europe artificial shelters have been used only in one study of the closely related *H. molleri* in north-western Portugal (do Vale et al., 2018). Other known studies using similar shelters for capturing arboreal hylid species were from North America (for species such as *Dryophytes cinereus* (Schneider, 1799), *D. squirellus* (Bosc, 1800), *D. femoralis* (Bosc, 1800), *D. gratiosus* (LeConte, 1856), and *Osteopilus septentrionalis* (Duméril et Bibron, 1841); see Boughton et al., 2000; Campbell et al., 2010; Suriyamongkol et al., 2021) and from South America for genera such as *Osteocephalus*, *Scinax, Trachycephalus*, and *Aparasphenodon* (Ferreira et al., 2012; Pereira-Ribeiro et al., 2017). Trimble and van Aarde (2014) reported the usefulness of this method for capturing arboreal species of the family Hyperoliidae (such as *Hyperolius* sp. and *Afrixalus* spp.) from South Africa.

The size of the *H. arborea* population in Škocjanski zatok Nature Reserve is currently unknown, although it was estimated to be around 50 calling males indicating the presence of a population multiple times larger (Veenvliet and Kus Veenvliet, 2014). In our study, the frequency of frog occurrence per shelter varied between years in single shelters (see Table 1). We hypothesize several reasons underlying this distribution.

Availability of natural shelters provided by the surrounding vegetation may reduce the attractiveness of artificial refuges and thereby influence occupancy (Pittman et al., 2008; Hoffmann et al., 2009; Trimble and van Aarde, 2014). Although we did not quantify vegetation density in different years of the study period, vegetation management, such as cutting along the trails within the Nature Reserve, may have influenced habitat structure and consequently affected the presence or behaviour of the species. There are several anecdotal reports of sightings of *H. arborea* in the vegetation surrounding the trails, especially in leaf axils of teasel plants *Dipsacus fullonum* (Lipej B., 2022, pers. comm.).

Notably, a high number of small individuals were recorded in July 2022, suggesting that newly metamorphosed frogs readily occupied the artificial shelters. These individuals made up more than half of all frogs detected in that year (Lužnik, in preparation), indicating a likely successful breeding season and recruitment. In contrast, no such individuals were observed in 2023 and only one in 2024, when mostly larger, adult-sized frogs were recorded. However, it is important to note that there is no clear-cut size threshold distinguishing juveniles from adults in *H. arborea*. For example, Moravec (1990) reported juveniles reaching up to 33 mm in the season of metamorphosis, but that sexually active males were also of a similar size at breeding sites. In our study, individuals ranged from 22.4 to 52.1 mm in snout–vent length (see below). Frogs captured after the breeding season with sizes between 30.0 and 35.0 mm could not always be reliably assigned to a specific age class. Despite this uncertainty, our findings indicate that the method is suitable for monitoring both juvenile and adult *H. arborea*. With further development, such as incorporating a mark–recapture approach with regular sampling, it could potentially allow estimation of interannual variation in population structure and abundance (Carlson and Edenhamn, 2000), which remains to be tested in the studied population. One approach to consider is mark-recapture with photo identification, as used in Lukanov et al. (2024). Some of the juveniles recorded early on (in 2022) may have continued using the shelters in subsequent seasons as adults, suggesting a potential habituation to these artificial refuges. Such behavioural adjustment could explain continued but reduced occupancy over time. However, this remains speculative, as we currently lack direct data to confirm long-term individual shelter use; further analyses on this topic are in preparation.

The last possible reason is the interference of researchers and handling of animals (getting them out of the shelter), which could have affected the use of artificial shelters by the species and reduced their numbers (Perry et al., 2011). Frogs tend not to return to the same refuge following disturbances caused by researchers, such as toe-clipping, measuring, or removal from shelter (Boughton et al., 2000). Researchers should consider the varying degrees of disturbance associated with these procedures. Toe-clipping, for example, constitutes a substantial disturbance due to the physical injury it inflicts (Waddle et al., 2008). In contrast, we argue that brief and careful handling for the purpose of photographing and measuring SVL is considerably less invasive. Therefore, we do not consider this type of handling to be a likely explanation for the observed decrease in shelter use (Pittman et al., 2008, do Vale et al., 2018; Hutton et al., 2024).

For artificial shelters, several parameters have been suggested to influence occupancy, such as pipe colour (Ferreira et al., 2012), pipe design (Granatosky and Krysko, 2011), pipe placement (Pittman et al., 2008), pipe orientation (Bartareau, 2004; Ferreira et al., 2012), and pipe diameter (Bartareau, 2004). For this study we used grey PVC pipes with white interior because Ferreira et al. (2012) reported that shelter colour (grey, white or black) did not result in statistical differences in occupancy between pipe shelters. Among environmental conditions, moisture retention and temperature are one of the main factors influencing habitat selection by the study species (Goin, 1958; Boughton et al., 2000; Granatosky and Krysko, 2011). With caps at the bottom of pipes and providing starting level of water to 10 cm height in our pipe design we enabled more suitable environment as suggested by Granatosky and Krysko (2011), while the seasonal variability of ambient/air temperature may have contributed to the observed occupancy patterns and should be examined in future studies.

In addition to shelter characteristics and environmental conditions, several studies have suggested that characteristics of study animals such as sex, life stage (e.g., adult versus juvenile) and body size may influence the use of shelters by tree frogs (Zacharow et al., 2003; Myers et al., 2007; Johnson et al., 2008; Hoffmann et al., 2009). In our study, we did not differentiate individuals by sex or life stage in the analysis. While the presence of vocal sacs is a reliable external trait distinguishing adult males from females in *H. arborea*, it is not possible to confidently classify all captured individuals, particularly those within the intermediate size range (30.0–35.0 mm), which may include both small adult females and large juveniles (Moravec, 1990; Gvoždík et al., 2008). To eliminate this uncertainty, we would have had to exclude a significant portion of our dataset, which would have further reduced the already limited sample size. However, our results demonstrate that individuals of all sizes used the shelters (see above). We therefore opted to retain the full dataset to better reflect the overall size distribution of shelter occupants.

We acknowledge that capture success varies across studies using different pipe designs or placements (Zacharow et al., 2003; Bartareau, 2004;), suggesting that tree frogs exhibit selectivity in shelter use. Bartareau (2004), who tested three pipe diameters (13 mm / 25 mm / 38 mm), found that juveniles and smaller adults preferred the narrower shelters (13 mm /25 mm). Similarly, Zacharow et al. (2003) tested 19-mm, 51-mm, and 77-mm pipes and observed that smaller frogs (*D. squirellus*, mean SVL: 28.7 mm; range: 24.0 mm–35.0 mm) favoured 19-mm pipes, while larger individuals (*D. cinereus*, mean SVL: 45.0 mm; range: 37.0 mm–66.0 mm) also used 51-mm pipes, but only half as often as 19-mm pipes. Interestingly, in Bartareau's (2004) study, *D. cinereus* did not use 38-mm pipes at all. do Vale et al. (2018) reported *H. molleri* occupying 44-mm pipes, with most individuals measuring 35.0 mm–45.0 mm. *Hyla arborea* is comparable in body size to *H. molleri* and *D. cinereus*, and in our study, frogs showed a consistent and statistically significant preference for narrower shelters (40 mm) over wider ones (50 mm), with 1.6 times greater use across three years (2022–2024), and 1.7 times greater use in 2023–2024 when monitoring was conducted year-round. However, no statistically significant relationship was found between body size and shelter diameter occupancy, as SVL ranges were similar between the two pipe types (24.1 mm–52.1 mm in 40-mm shelters vs. 22.4 mm–51.9 mm in 50-mm shelters). We hypothesise that the observed occupancy in narrow shelters could reflect a general tendency toward microhabitats with more favourable microclimatic conditions, particularly improved moisture retention (Granatosky and Krysko, 2011). Additionally, narrower shelters may offer more enclosed space, potentially reducing visibility and access to predators (Johnson, 2005). Forks (2015) suggested that PVC shelters may serve as effective refuges for juveniles shortly after metamorphosis, which presents an interesting direction for future research. Based on our observations, we also propose testing whether *H. arborea* would use artificial shelters with even smaller pipe diameters, as this could further clarify microhabitat preferences across developmental stages.

CONCLUSIONS

Our results demonstrate that PVC shelters offer a practical and effective non-invasive method for monitoring *H. arborea* in sensitive habitats such as wetlands with high conservation value, for both juvenile and adult individuals. Compared to traditional more invasive techniques, this passive sampling approach significantly improves detectability – especially outside the breeding season – and minimizes disturbance to non-target species, such as protected bird populations or sensitive vegetation. These advantages make it particularly suitable for long-term monitoring in ecologically sensitive or physically less accessible areas.

Throughout three consecutive years, we observed consistent occupancy in narrower (40-mm) shelters over wider (50-mm) ones, although frog body size did not differ significantly between shelter types. The observed pattern may reflect opportunistic use by juveniles and a potential preference for more enclosed microhabitats among adults – possibly due to favourable microclimatic conditions or lower predation risk. Despite an overall decrease in occupancy over time, this trend may be influenced by a combination of factors, including sampling biases, vegetation dynamics, demographic fluctuations typical of *H. arborea* populations, and possibly habituation or disturbance tolerance. Notably, artificial shelters provided refuge to both juvenile and adult individuals, allowing us to detect shifts in population structure among years. Future research should explore shelter use across a wider range of pipe diameters to clarify age- or size-based preferences, and integrate demographic and environmental data to improve the ecological interpretation of occupancy trends. Given the success and low invasiveness of this method, we recommend its application in other protected or sensitive amphibian habitats where traditional methods may be impractical or undesirable.

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Oxyrhopus in Bahia, Brazil: Pholidosis, maturity, sexual dimorphism, and reproduction

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Abstract. Research on snake reproduction in the Neotropics is limited by a lack of data, necessitating further studies to better understand their reproductive biology. The genus *Oxyrhopus* has received increasing interest in studying its natural history, although several aspects remain unknown. This investigation aims to shed light on *Oxyrhopus* morphometry, maturity, and sexual dimorphism in the state of Bahia, Brazil. An analysis was conducted on 421 snake specimens from the Museum of Zoology at the State University of Santa Cruz to determine their external characteristics and reproductive conditions through gonadal inspection. Species identification was performed via pholidosis, and various measurements such as snout-vent length, tail length, head length, and width were meticulously recorded. Sexual maturity was estimated, and sexual dimorphism was assessed through statistical analysis, including regressions and seasonal comparisons, to study reproductive cycles and testicular variations in these species. The species of the genus *Oxyrhopus* studied included *O. trigeminus, O. petolarius, O. guibei*, and *O. rhombifer*. The investigation delineated the minimum size required for sexual maturity in each species and clarified the disparities in length between males and females. Reproductive males were found throughout the year in *O. trigeminus* and *O. petolarius*, with no significant seasonal differences in testicular volume. Females of both species exhibited annual reproductive activity. Similar patterns were observed in *O. guibei* and *O. rhombifer*, albeit with fewer data available. A relationship between female body size and fecundity was found, but further research is needed on how climatic factors may influence the reproductive cycle.

Keywords. Reproductive biology, morphometry, snakes, sexual dimorphism and maturity, testicular variations.

INTRODUCTION

Given the remarkable diversity of snakes in the Neotropics, comprising over 880 species, the study of their ecology and natural history remains a field with gaps requiring further exploration (Reed and Shine, 2002; Pizzatto, 2005). However, in recent years there has been an increase in research focused on the natural history of the genus *Oxyrhopus,* commonly referred to as false coral snakes. These studies have addressed various aspects, including diet, distribution, and certain reproductive traits (Andrade and Silvano, 1996; Pizzatto and Marques, 2002; Alencar et al., 2012; Cabral and Scott, 2014; Coelho et al., 2019). This information on natural history and reproductive ecology is important for making appropriate comparisons between species and determining patterns (Pizzatto and Marques, 2002).

Reproductive dynamics constitute a fundamental aspect of an organism's life history, allowing for reproduction and species continuity (Pizzatto and Marques,

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2002; Pizzatto, 2005). However, crucial reproductive parameters such as sexual maturity (both minimum and average size) and sexual dimorphism can exhibit substantial variation among species and populations due to multifaceted influences, including abiotic, ecological, environmental, phylogenetic, and geographical factors (Vitt and Vangilder, 1983; Di Bernardo et al., 1998; Barron and Andraso, 2001). These factors can also affect reproductive cycles, fecundity, reproductive frequency, and reproductive potential (Di Bernardo et al., 1998; Barron and Andraso, 2001; Pizzatto et al., 2006).

In most snake species, females are longer than males (Shine, 1994; Cox et al., 2007). This sexual size dimorphism (SSD) in snakes is related to fecundity selection, favoring longer females to produce more offspring (Ford and Seigel, 1989; Shine, 2000; Cox et al., 2007). Males mature earlier and are smaller than females, likely due to different selective pressures. Enhanced chemosensory abilities are presumed to be favored by sexual selection, as they help males more effectively locate and choose mates (Madsen and Shine, 1993; Rivas and Burghardt, 2001; Shine et al., 2003). Overall, there is still much unknown about the reproductive processes of tropical snakes (Pizzatto and Marques, 2002; Pizzatto et al., 2006). Available information often comes from observations of captive snakes, sporadic encounters in the wild, or community studies (Pizzatto, 2005; Sawaya et al., 2008; Maia and Travaglia-Cardoso, 2017; Coelho et al., 2019). However, studies conducted with snakes preserved in scientific collections are still scarce, yet, these studies could provide valuable insights into the reproductive biology of tropical snakes (Pizzatto and Marques, 2002; Bizerra et al., 2005; De Aguiar and Di Bernardo, 2005). Such research could lead to a better understanding of snake reproductive biology across various regions, as well as the identification of trends and patterns in species reproduction (Pizzatto and Marques, 2002; Pizzatto, 2005).

The genus *Oxyrhopus* belongs to the tribe Pseudoboini, a monophyletic group composed of species that are closely related to each other. (Zaher et al., 2009; Gaiarsa et al., 2013). With 15 primarily terrestrial species distributed throughout the Neotropical region, they inhabit a wide range of habitats, from tropical forests to oceanic islands, from southern Mexico to northern Argentina (Zaher et al., 2009; Alencar et al., 2012; Gonzales et al., 2020). In the state of Bahia, Brazil, six species of *Oxyrhopus* have been recorded, some of which coexist in the same distribution area, with up to three or four species occurring together (Lira da Silva, 2011; Nogueira et al., 2019).

A study conducted by Pizzatto and Marques (2002) in the state of São Paulo determined that *O. guibei* females have longer body sizes, which gives them a considerable selective advantage, as fecundity depends on this trait. Females appear to adopt two possible reproductive strategies: producing many small eggs or only a few large ones. This trade-off aligns with classical r- and K-selection strategies in animals, where r-selected species maximize reproductive output with numerous offspring and minimal parental investment per offspring, while K-selected species invest more in fewer offspring, improving their survival probability (Pianka, 1970). Additionally, *O. guibei*'s reproductive cycle is continuous, producing a single clutch per year, although this can occur at any time within the annual cycle, while males exhibit spermatogenesis throughout the year. Given this information, it is reasonable to assume that other *Oxyrhopus* species may exhibit characteristics similar to *O. guibei.*

This research delves into the reproductive characteristics of *Oxyrhopus* species in the state of Bahia, Brazil. We examine physical traits, minimum size for sexual maturity, and attributes that potentially contribute differences between males and females. In addition, we provide information on reproductive aspects, such as to estimation of fecundity, reproductive frequency, and reproductive potential in females, and determination of the periods of reproductive activity in males, through gonadal analysis.

MATERIAL AND METHODS

A total of 421 samples preserved at the Museum of Zoology of the State University of Santa Cruz (MZUESC), from various parts of the state of Bahia, were analyzed. The museum's collection is combined with that of the Gregorio Bondar Zoological Collection, held by the Cacao Research Center (CEPLAC/CZGB). All individuals were preserved in 70% ethanol. The initial identification of species followed dichotomous keys (Peters and Orejas-Miranda, 1970; Gonzales et al., 2020) that involved determining the scale count of each snake (dorsal, ventral, subcaudal, infralabial, and supralabial), followed by recording various morphometric data for each specimen. These included snout–vent length (SVL; from the rostral scale to the cloaca), Total length (TL; from rostral scale to the tip of the tail) and tail length (LC; from the cloaca to the tip of the tail, using only complete tails to avoid underestimation), which were measured using a measuring tape. Head length (HL; from the rostral scale to the quadrate bone), head height (HH; the highest part of the head) and head width (HW; measured at the level of the quadrate bones) were measured using a digital caliper with an accuracy of 0.01 millimeters (Boretto and Ibargüengoytía, 2006). The capture times of the individuals were determined based on museum records from 1990 to 2019, which did not include exact dates, so the data were classified into the wet season (October to May) or the dry season (June to September). The dataset supporting this study is available at Zenodo: https://doi.org/10.5281/zenodo.14861556.

Maturity and determination of reproductive status

The sex of each individual was determined by examining the gonads, following the methodology described by Alencar et al. (2012), which involved making an incision in the midsection of the ventral scales to determine the presence of testes or ovaries. The reproductive conditions and sexual maturity of each species were determined through gonadal inspection. For females, uterine characterization was categorized into two groups: juvenile uterus, characterized by the absence of folds or folds that do not extend throughout the uterus; and adult uterus, characterized by folds extending throughout the uterus, indicative of distended oviducts, generalized enlargement of the oviducts associated with vitellogenic recrudescence, or markedly stretched oviducts resembling a post-reproductive uterus, indicating recent parturition (Boretto and Ibargüengoytía, 2006). Additionally, females with oviductal eggs or vitellogenic follicles ≥10 mm were also considered adult and reproductive (De Aguiar and Di Bernardo, 2005; Pizzatto, 2005; Boretto and Ibargüengoytía, 2006; Coelho et al., 2019; Fig. 1).

Males were considered adult and reproductively active if they had turgid and opaque testes, and if they presented coiled epididymides and deferent ducts, indicative of the presence of spermatozoa typical of adults (Pizzatto and Marques, 2002; Pinto and Fernandes, 2004; De Aguiar and Di Bernardo, 2005; López et al., 2009). The minimum maturity size was estimated as the size of the smallest reproductive individual for each sex (Alencar et al., 2012), taking the minimum snout-vent length (SVL) at sexual maturity for each species.

Morphometry and sexual dimorphism

Comparisons regarding general characteristics of age and sex among the studied species were conducted. Before performing any statistical analyses, the collected data were subjected to normal distribution and homogeneity of variances assessments using the Shapiro-Wilk test and Levene's test, respectively. A significance threshold of $P < 0.05$ was used to determine significant results and all statistical analyses were performed using R software (version 4.3.1; R Core Team, 2024).

To evaluate sexual dimorphism, two methods were used. i) Quantifying the degree of sexual size dimorphism (SSD): this involved calculating the SSD index by

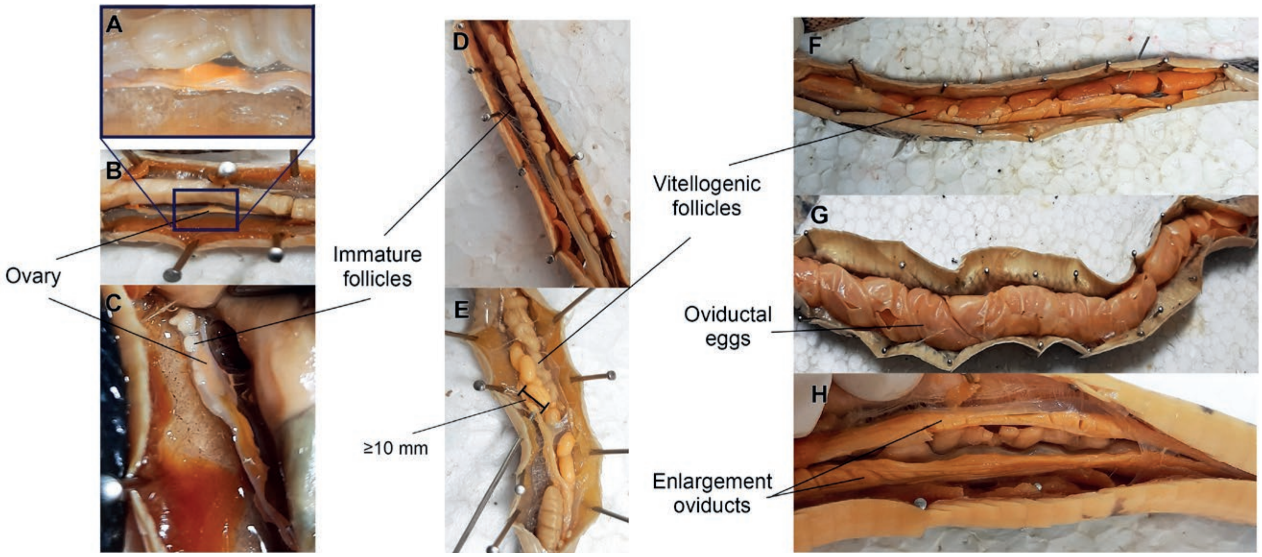

Fig. 1. Stages of female sexual maturity in *Oxyrhopus* spp. Figures A to D represent juvenile stages. A) Close-up of the ovary without visible follicles (overview in B). C) Ovary with small developing follicles, and D) Immature follicles smaller than 10 mm in diameter. Figures E to H represent adult stages E) Vitellogenic follicles ≥10 mm, indicating sexual maturity. F) Larger vitellogenic follicles are observed in advanced maturation. G) Oviductal eggs near the time of oviposition, and H) illustrates enlarged and folded oviducts, characteristic of females that have already undergone oviposition.

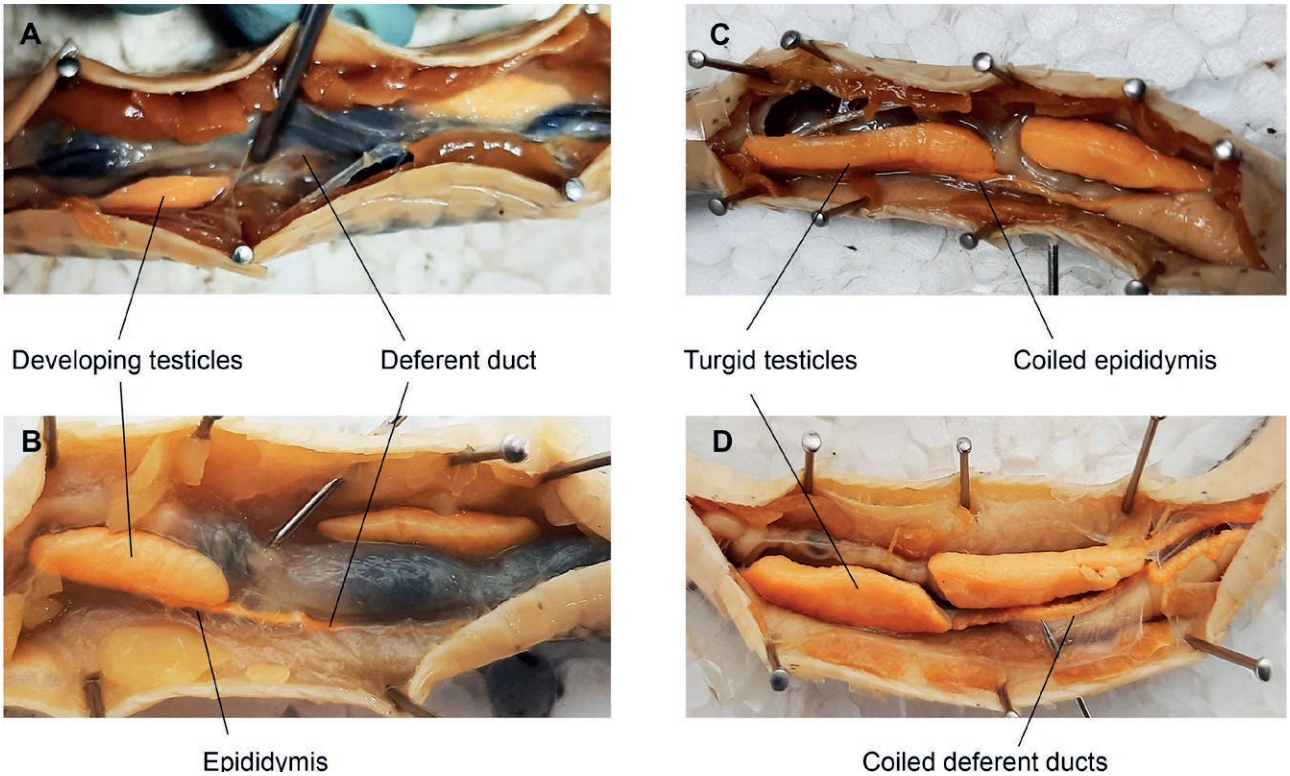

Fig. 2. Stages of male sexual maturity in *Oxyrhopus* spp. A) Immature testes with elongated, transparent deferent ducts. B) The testes and epididymides are more developed but still lack macroscopic signs of spermatogenesis. C) Turgid testes and coiled epididymides, consistent with active spermatogenesis. D) The testes appear reduced in volume and non-turgid, while the deferent ducts remain coiled, suggesting the presence of stored sperm after a recent reproductive phase.

dividing the mean snout-vent length (SVL) of the longer sex by that of the smaller sex, then subtracting 1 from the result. This index was considered arbitrarily positive when females were the longer sex and negative when males were longer (Gibbons and Lovich, 1990; Shine, 1994). This index was compared with other studies from Brazil. ii) Two-factor analysis of variance (ANOVA): this method was employed with sex and species as factors, and number of ventral scales, number of subcaudal scales, SVL, and tail length (LC) as dependent variables (Pinto and Fernandes, 2004; Pizzatto, 2005). Juveniles were excluded from the morphometric study based on SVL. Additionally, a covariance analysis (ANCOVA) was performed for tail length (LC), head length (HL), and head width (HW), with SVL as the covariate. Finally, as an exploratory analysis, we conducted a principal component analysis (PCA), separated by sex for each species.

Analysis of reproductive conditions

Measurements of the gonads were taken with a digital caliper with an accuracy of 0.01 millimeters, recording the length and width of the testes, as well as the length of vitellogenic follicles and eggs (Pizzatto, 2005; Bellini, 2013). To calculate the testicular volume for both the right and left testes, reflecting reproductive activity, the ellipsoid formula ($4/3\pi$ abc) was used, where a = length/2, b = width/2, and c = height/2 (Pizzatto and Marques, 2002; Pizzatto, 2005). A simple regression was performed between testicle size and the snout-vent length (SVL) of all males. Male reproductive cycles were studied by comparing testicular variation and deferent duct condition between seasons, only in reproductively active adult males, using SVL as a covariate through an ANCOVA (Bellini, 2013).

To estimate clutch size or female fecundity, the number of oviductal eggs was used. A simple regression was performed between the SVL of adult females and clutch size, as well as another simple regression between clutch size and the size of the largest oviductal egg (Pizzatto, 2005; López et al., 2009). In cases where fecundity could not be determined with oviductal eggs, data from follicles larger than 10 mm were used (Almeida-Santos et al., 2014). Reproductive frequency was estimated by the

Table 1. Morphological characteristics of the *Oxyrhopus* species, separated by species, sex and ages (Adults and Juvenils), with the values of the ventral count (V), subcaudals (SC); the average, maximum, minimum and standard deviation (SD) of the following characteristics are presented: snout-vent length (SVL), total length (TL), tail length (LC), percentage of tail size according to total length (%LC), percentage of head size according to SVL (% HL) and head width (HW). For some characteristics, adult individuals were separated from juveniles.

Specie	Sex	Age	n	V	SC	TL	SVL	LC	% LC	HL	% HL	HW
Oxyrhopus clathratus	♀	J	1	189	60	29.6	24.8	4.8	16.21	11.1	4.5	4.44
	♂	J	1	184	74	32.4	26	6.4	19.75	-	-	-
Oxyrhopus formosus	♀	A	2	199 (197-203)	69 (66-75)	(70.8-90.5)	(56.5-74)	(56.5-74)	(18.23-20.20)	21.35	2.89	12.56
		J	2			(33.4-48.8)	(27-40.1)	(6.4-8.7)	(17.83-19.16)	(10.61-14.7)	(3.67-3.93)	(6.55-7.79)
	♂	A	2	190 (189-191)	86 (83-87)	(63.3-64.7)	(48.6-51)	(13.7-14.7)	(21.17-23.22)	(17.49-17.56)	(3.44-3.6)	(8.47-8.95)
		J	1			53.3	41.2	12.1	22.7	14.24	3.46	8.23
Oxyhropus guibei	♀	A	9	196 (192-212)	75 (70-82)	85.64 (54.9-109.6) SD 15.954	69.27 (44-93.6) SD 14.082	16.42 (10.9-20.2) SD 2.719	19.89 (19.13-21.45) SD 0.762	22.84 (19.98-26.67) SD 2.501	3.17 (2.85-3.47) SD 0.209	12.61 (8.92-18.73) SD 3.198
		J	8			46.96 (20.3-64) SD 14.374	38.1 (16.4-51.8) SD 11.517	9.52 (3.9-12.3) SD 3.226	20.13 (19.06-21.59) SD 0.943	14.61 (9.92-18.24) SD 2.834	4.16 (3.51-6.05) SD 0.893	7.32 (5.29-9.66) SD 1.387
	♂	A	11	181 (172-185)	82 (80-85)	67.01 (57.5-75.3) SD 7.071	51.62 (44.3-58.6) SD 5.399	15.38 (12.9-18) SD 1.838	22.94 (20.98-24.41) SD 1.034	17.56 (9.36-20.91) SD 3.108	3.42 (1.65-3.78) SD 0.603	9.36 (8.31-10.39) SD 0.668
		J	7			35.6 (23.9-41.1) SD 6.396	27.84 (18.9-31.8) SD 4.916	7.76 (5-9.3) SD 1.494	21.74 (20.92-22.63) SD 0.595	12.12 (9.74-13.91) SD 1.464	4.51 (4.16-5.17) SD 0.437	6.15 (4.75-6.92) SD 0.766
Oxyrhopus petolarius	♀	A	42	204 (199-215)	90 (81-97)	102.08 (85.2-127.5) SD 11.461	80.93 (65.6-100.8) SD 9.556	21.92 (18.6-27.5) SD 2.53	21.46 (20.2-23.51) SD 0.877	25.83 (20.97-35-49) SD 3.081	3.17 (2.66-4.22) SD 0.237	12.4 (9.66-16.23) SD 1.449
		J	33			48.9 (24.5-77.5) SD 14.075	38.27 (18.7-61.1) SD 11.026	10.64 (5.6-16.5) SD 3.081	21.78 (20-23.98) SD 0.913	14.94 (11.3-21.95) SD 2.343	4.19 (3.57-6.15) SD 0.697	7.63 (6-10.46) SD 1.352
	♂	A	44	196 (191-200)	100 (95-107)	82.72 (55.2-107.3) SD 13.066	62.73 (42.1-82.2) SD 9.872	20.1 (13.1-26.1) SD 3.610	24.72 (23.14-26.96) SD 0.983	21.08 (12.27-26.4) SD 2.892	3.35 (1.98-4.18) SD 0.325	10.46 (7.49-13.11) SD 1.192
		J	23			40.26 (25.3-56.4) SD 7.863	30.77 (19.3-42.6) SD 5.965	9.67 (6-13.8) SD 1.989	23.88 (22.67-25.66) SD 0.758	13.17 (10.73-16.89) SD 1.489	4.42 (3.82-5.56) SD 0.531	6.92 (5.28-8.37) SD 0.792

(Continued)

Table 1. (Continued).

Specie	Sex	Age	n	V	SC	TL	SVL	LC	% LC	HL	% HL	HW
Oxyhropus rhombifer	♀	A	10	210 (204-217)	77 (70-85)	75.53 (59.8-87.8) SD 8.837	60.39 (49.8-70.1) SD6.229	16 (12.4-17.7) SD 1.572	20.71 (19.5-22.24) SD 0.891	19.54 (18.1-21.91) SD 1.32	3.21 (3-3.63) SD 0.194	9.9 (8.82-12.38) SD 1.507
		J	3			65 (51.3-75.2) SD 12.328	50.93 (40.1-59.5) SD 9.897	14.1 (11.2-15.7) SD 2.491	21.68 (20.88-22.34) SD 0.687	14.97 (12.38-17.56) SD 3.663	3.19 (3.09-3.3) SD 0.148	8.42 (8.35-8.49) SD 0.099
	♂	A	3	198 (194-200)	86 (82-90)	58.35 (48.9-67.8) SD 13.36	44.55 (37.7-51.4) SD 6.22	13.8 (11.2-16.4) SD 3.677	23.55 (22.9-24.19) SD 0.908	17.11 (14.16-20.06) SD 4.172	3.83 (3.76-3.9) SD 0.099	9.2 (8.81-9.59) SD 1.25
		J	2			34.4 (32.7-36.1) SD 20.404	27 (25.7-28.3) SD 1.838	7.4 (7-7.8) SD 0.566	21.51 (21.41-21.61) SD 0.141	12.53 (12.04-13.02) SD 0.693	4.6 (4.6-4.68) SD 0.057	6.85 (6.74-6.96) SD 0.156
Oxyrhopus trigeminus	♀	A	85	199 (191-211)	61 (54-70)	78.07 (53.4-103.7) SD 11.58	65.26 (43.4-86.9) SD 9.97	12.88 (9.1-17.4) SD 1.77	16.5 (14-19.7) SD0.96	22.4 (16.47-27.71) SD 2.51	3.46 (2.85-3.95) SD 0.23	11.09 (7.9-13.77) SD 1.55
		J	38			39.12 (19.8-68.5) SD 11.62	32.59 (18-59.4) SD 9.82	6.61 (3.3-9.8) SD 1.74	17.07 (14.04-20.42) SD 1.36	13.9 (10.31-20.36) SD 2.62	4.5 (3.43-5.95) SD 0.67	7.27 (4.75-10.82) SD 1.45
	♂	A	63	187 (173-197)	69 (61-81)	59.31 (39.2-77.7) SD 8.24	47.28 (31.8-63.2) SD 6.53	12.18 (6.9-15.9) SD 1.87	20.14 (17.2-24.6) SD 1.33	18.28 (13.78-23.16) SD 1.99	3.98 (3.26-5.63) SD 0.37	9.34 (6.98-12.71) SD 1.3
		J	31			33.46 (18.9-50.3) SD 8.34	27.16 (15.7-39.7) SD 6.53	6.85 (4-10.6) SD 1.69	19.45 (17.62-21.58) SD 1.11	12.49 (9.63-17.18) SD 1.84	4.8 (3.85-6.38) SD 0.62	6.46 (4.25-8.2) SD 1.09

Table 2. Comparison of sexual size dimorphism (SSD) indices among different *Oxyrhopus* species by analyzing the average snout-vent length (SVL) sizes of adult individuals, categorized by sex. Additionally, we considered the SSD indices from published studies on *Oxyrhopus* species in Brazil.

Species	n		SVL mean (cm)		SSD	Location	Reference
	Female	Male	Female	Male			
O. formosus	2	2	65.25	49.8	0.31	Bahia/Br	This study
O. guibei	9	11	69.27	51.62	0.34	Bahia/Br	This study
"	15	15	73.28	49.79	0.47	São Paulo/Br	Rocha-Barbosa et al., 2000
"	9	9	65.61	53.94	0.22	Minas Gerais/Br	Vaz, 1999
"	211	160	81.9	59.9	0.37	São Paulo/Br	Pizzatto and Marques, 2002
O. petolarius	42	44	80.93	62.73	0.29	Bahia/Br	This study
"	6	12	97.0	70.3	0.41	Minas Gerais/Br	Mendonça et al., 2024
O. rhombifer	10	2	60.39	44.55	0.36	Bahia/Br	This study
O. trigeminus	85	63	65.26	47.28	0.38	Bahia/Br	This study
"	3	4	67.17	54.25	0.24	Minas Gerais/Br	Vaz 1999
"	16	13	71.77	55.34	0.30	Minas Gerais/Br	Alencar et al., 2012
"	42	25	46.71	38.94	0.20	Pernambuco/Br	Coelho et al., 2019

percentage of reproductively active mature females in the sample (with vitellogenic follicles >10 mm or eggs), where a result below 50% indicates a biannual or multiannual reproductive cycle, and more than 50% indicates an annual cycle, this analysis was conducted per year, and the final frequency was averaged across years with sufficient data (Pizzatto, 2005; Bellini et al., 2013 2018). Reproductive potential was calculated, showing the potential number of neonates per female per year, following the formula: (mean clutch size) × (reproductive frequency) (Bellini, 2013).

RESULTS

Morphometric data of 217 *Oxyrhopus trigeminus* individuals (148 Adults, 69 Juvenils), 142 *O. petolarius* (86 A, 56 J), 35 *O. guibei* individuals (20 A, 15 J), 18 O. *rhombifer* individuals (13 A, 5 J), seven *O. formosus* (4 A, 3 J), and two juvenils of *O. clathratus* were obtained. The latter two species were excluded from the statistical analyses due to the limited amount of data, and their information is presented for comparison purposes (Table 1). We determined the minimum size (SVL) at which each species reaches sexual maturity. For *O. trigeminus*, females mature at 43.4 cm and males at 31.8 cm; for *O. petolarius*, females mature at 65.6 cm and males at 42.1 cm; for *O. guibei*, females mature at 44 cm and males at 44.3 cm; for *O. rhombifer*, females mature at 49.8 cm and males at 37.7 cm; and for O. *formosus*, females mature at 70.8 cm and males at 63.3 cm.

Morphometry and sexual dimorphism

The SSD was calculated for five species, including *O. formosus*, despite the limited data. Across all species, females consistently displayed longer sizes than males, with the indices closely to each other (Table 2), a trend that is statistically supported for the species in our study. We observed that adult females of all species were significantly longer (SVL) than adult males ($F_{sex} = 37.002$; $P < 0.001$), and there was a significant difference in body size among species ($F_{species} = 46.885$; $P < 0.001$), with *O. petolarius* exhibiting the largest body size. However, the interaction between sex and species did not show a significant difference ($F_{interaction} = 0.035$; $P = 0.998$), indicating that males of one species could be as large as females of another species.

Through comparisons of the number of ventral scales, significant differences were evident between species ($F_{species} = 63.326$; $P < 0.001$), notably with *O. rhombifer* exhibiting the highest number of ventrals among the species examined. Furthermore, in examining sexes, a significant distinction emerged ($F_{sexes} = 54.077$; $P < 0.001$), with females possessing more ventral scales than males. The interaction between species and sexes also yielded a significant difference ($F_{interaction} = 2.454$; $P = 0.033$).

Similarly, the analysis of subcaudal scales revealed a comparable trend, with *O. petolarius* exhibiting the highest count of these scales compared to other species ($F_{species} = 959.592$; $P < 0.001$). Additionally, males across all studied species displayed a greater number of subcaudal scales than females ($F_{sexes} = 82.045$; $P < 0.001$; $F_{interaction} = 3.617$; $P = 0.003$).

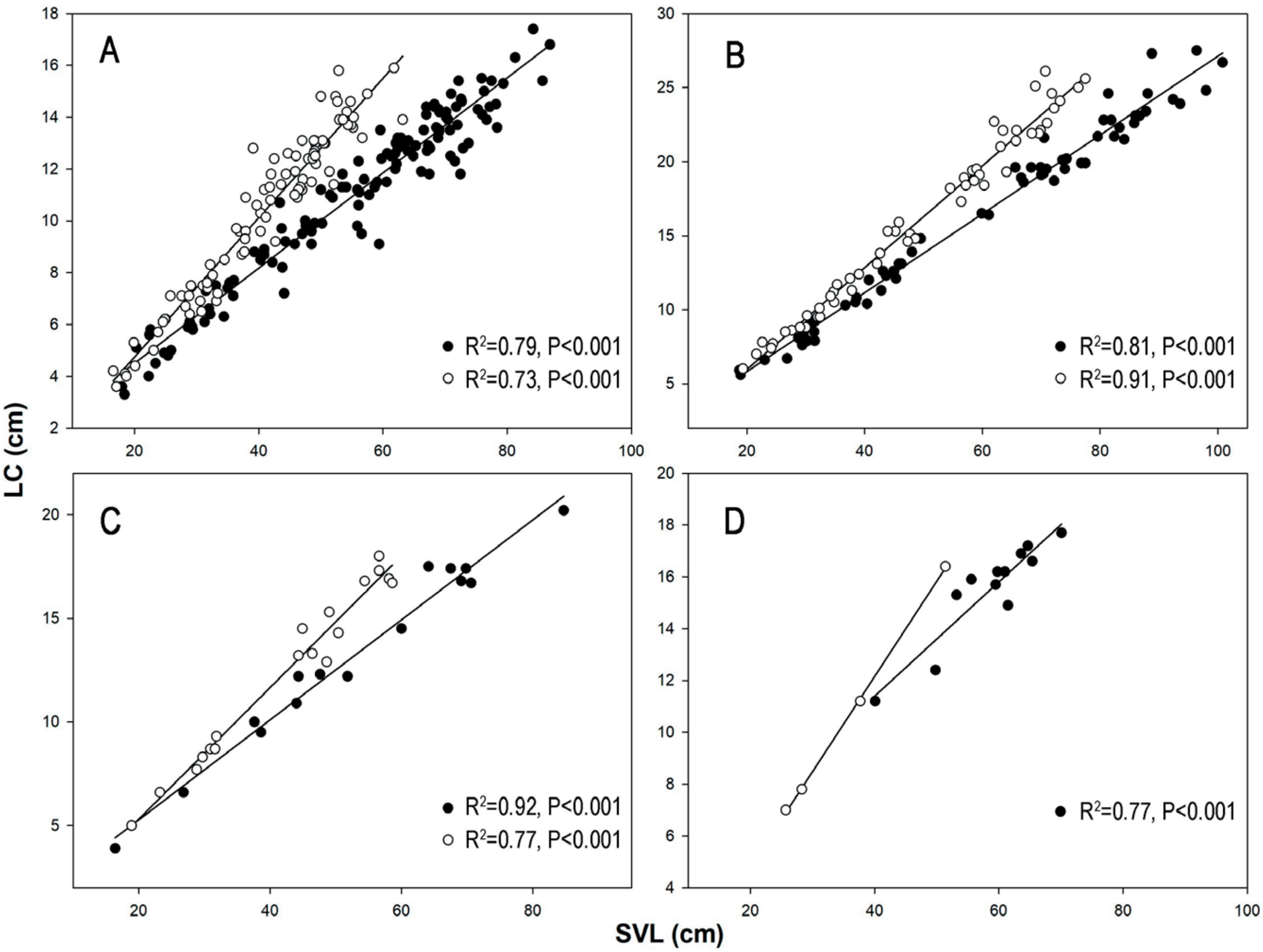

Fig. 3. Linear regressions of tail lenght (LC) in relation to SVL, data separated by sex, females with black circles and males with white circles, A) of *O. trigeminus* (females: n=125; males: n=90). B) *O. petolarius* (females: n=65; males: n= 54). C) *O. guibei* (females: n= 15; males: n= 18). D) *O. rhombifer* (females: n= 12; males: n=4).

The relative tail length (LC) in males across all species surpassed that of females (F_{sex} = 7.558; P = 0.006; $F_{species}$ = 212.570; P < 0.001), with *O. petolarius* exhibiting the largest tail size. However, the interaction between sex and species did not yield a significant difference ($F_{interaction}$ = 0.962; P = 0.411) indicating that tail length variation is influenced more by interspecific body size differences than by sex alone. As a result, females of larger species may have tails as long as, males of smaller species. Notably, significant differences were observed in *O. trigeminus*, where adult males' tails averaged 20.14% of the total body length compared to 16.5% in females. Linear regression analysis between tail length and SVL depicted positive relationships, indicating males tended to have longer tails than females (Fig. 3A).

Similarly, *O. petolarius* displayed differences in tail size between sexes, with males' tails accounting for 24.72% and females' tails for 21.46% of total length. The regressions depicted positive relationships, clearly illustrating the difference between the sexes (Fig. 3B). Likewise, *O. guibei* exhibited distinct percentages of tail length relative to total body size, with males at 22.94% and females at 19.89%. The regressions for both males and females showed positive relationships, highlighting their differences (Fig. 3C). Although limited by sample size, regression analysis for *O. rhombifer* showed differences between sexes in the regressions (Fig. 3D), with males' tails occupying 23.55% of their total length compared to 20.71% in females. Conversely, head length (HL) and head width (HW) did not show significant differences among species (F(HL) = 2.274; P = 0.132; F(WH) = 1.495; P = 0.222).

The PCA analysis revealed partial morphological differentiation among the four *Oxyrhopus* species (Fig.

4). All species showed considerable overlap, particularly along the first principal component. When males and females were plotted together (Fig. 4A), partial separation between sexes was observed within some species, most notably in *O. petolarius* and *O. trigeminus*, suggesting the presence of sexual dimorphism. These trends were further explored in separate PCAs for females and males (Fig. 4B and 4C), where similar variable loadings were observed, though slight differences in group distribution and vector orientation reinforce sex-based morphological patterns. Overall, the patterns suggest moderate interspecific differentiation and variable degrees of sexual dimorphism depending on the species.

Male reproductive cycle and testicular variation

Among the examined individuals of *O. trigeminus* and *O. petolarius*, it was observed that the reproductive cycles of males can occur in both seasons of the year, as ANCOVA analyses showed no significant variations in testicular volume (F = 0.029, P = 0.866 for *O. trigeminus*; F = 0.172, P = 0.684 for *O. petolarius*) or deferent duct (F = 0.085, P = 0.774 for *O. trigeminus*; F = 0.157, P = 0.6973 for *O. petolarius*) between seasons. In *O. trigeminus*, 54 adult males were analyzed, and a positive regression was found between testicular volume and snout-vent length (SVL), indicating a gradual increase in testicular size with sexual maturity and body growth (Fig. 5A). Similarly, in *O. petolarius*, 37 adult males were analyzed, and a positive relationship between testicular size and SVL was observed, indicating that testicular size remains stable until reaching approximately 40 cm SVL, at which point it begins to increase (Fig. 5B).

For the species *O. guibei* and *O. rhombifer*, it was not possible to perform ANCOVA analyses comparing testicular volume and deferent ducts between seasons due to a lack of data for both species. In the case of *O. guibei*, out of 18 reported males, only nine were reproductive. Simple regressions between testicular volume and SVL showed a positive relationship between these variables.

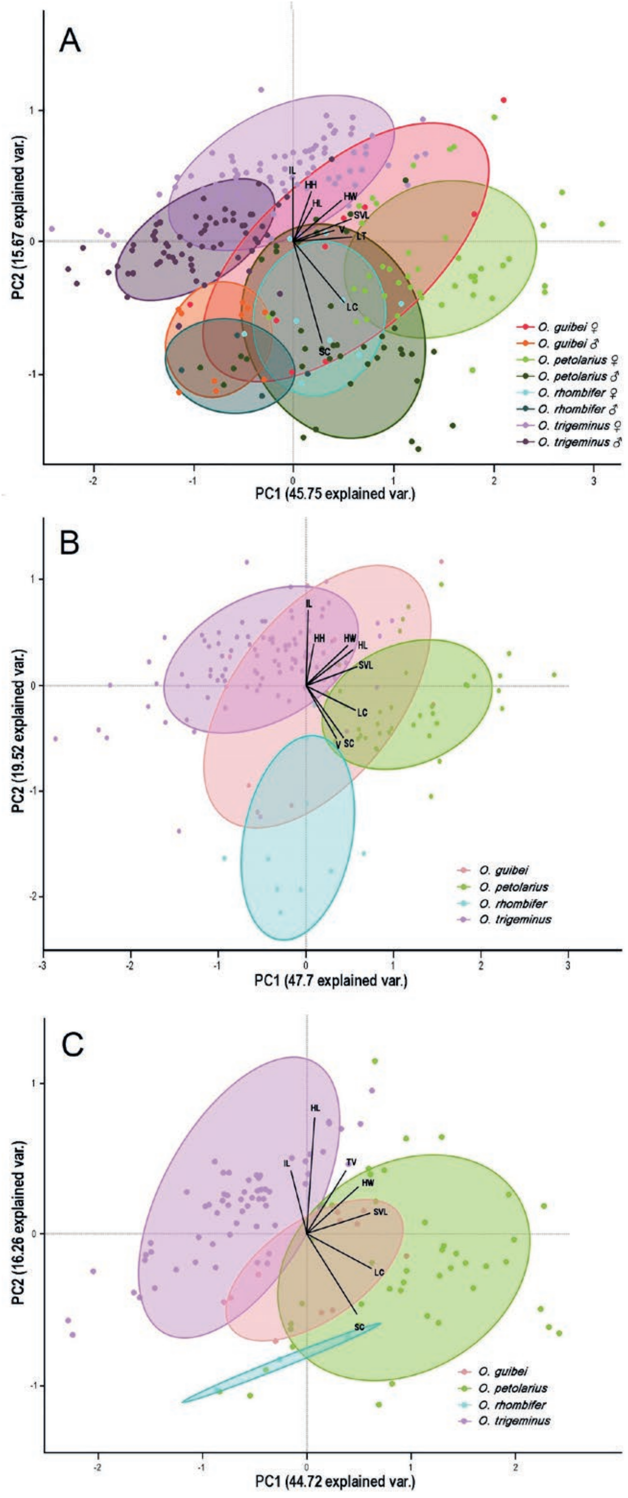

Fig. 4. Principal Component Analysis (PCA) of morphometric traits in four species of Oxyrhopus. (A) All individuals combined, grouped by species and sex; (B) females only; (C) males only. Each point represents one individual, and ellipses correspond to 95% confidence intervals for each group. Colors represent species: *O. guibei* (red), *O. petolarius* (green), *O. rhombifer* (cyan), and *O. trigeminus* (purple). In panel A, male and female individuals are represented separately. The first two principal components explain 61.43% of the total variance in the combined analysis (PC1: 45.75%, PC2: 15.67%), 67.22% in females (PC1: 47.7%, PC2: 19.52%), and 60.98% in males (PC1: 44.72%, PC2: 16.26%). Vectors indicate the direction and contribution of each morphometric variable: snout-vent length (SVL), head width (HW), head length (HL), head height (HH), infralabial scale count (IL), total length (TL), tail length (LC), ventral scale count (V), and subcaudal scale count (SC).

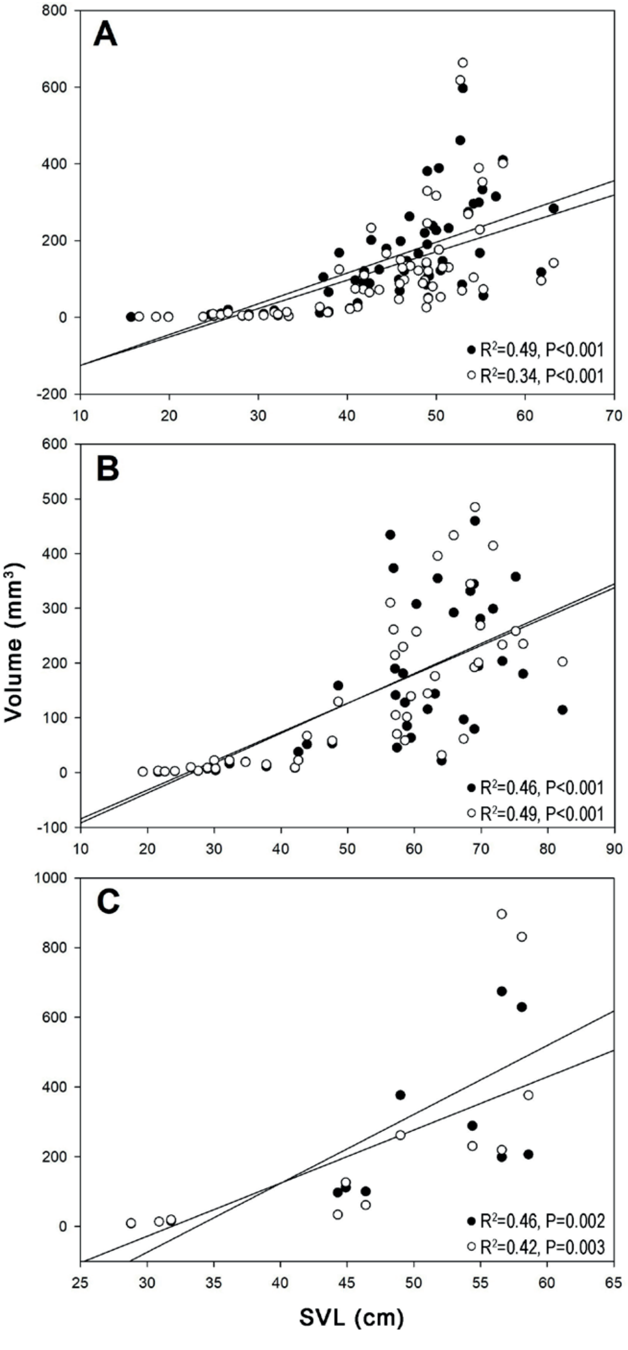

Fig. 5. Linear regression of testicular volume and body size of all males. Black circles represent the right testicle, and white circles represent the left testicle. A) *Oxyrhopus trigeminus*, showing slight variation in the regressions; however, both are significant (right: n= 67; left: n= 62). B) *Oxyrhopus petolarius*, regressions with higher slope and almost identical between right (n= 53) and left (n= 52). C) *Oxyrhopus guibei*, shows a different regression between the right testicles (n= 18) and the left testicles (n= 18).

A similar pattern to those previously mentioned was observed, with stability in testicular volume until sexual maturity was reached (Fig. 5C). For *O. rhombifer*, of the five males analyzed, only three were reproductive. Linear regression analyses could not be performed, although it was observed that the size of juvenile testes was smaller than that of adults, as expected.

Fecundity and reproductive cycles of females

Females of *O. trigeminus, O. petolarius*, and *O. guibei* can be found reproductive in both the wet and dry seasons, as vitellogenic follicles and oviductal eggs of various sizes were observed throughout the year (Table 3). Two females of *O. trigeminus* did not present follicles but had lax uteri, suggesting recent oviposition. The reproductive frequency of these species was determined to be annual. These three species showed an estimated reproductive potential of approximately five offspring per female per year. Regarding *O. rhombifer*, three studied females were considered reproductive, although the capture period of the individuals could not be determined.

For the females of *O. trigeminus*, a positive correlation was observed between clutch size and SVL (Fig. 6A), as well as a negative relationship between clutch size and egg size (Fig. 6B). For *O. petolarius*, statistically significant positive correlations were observed between clutch size and snout-vent length (Fig. 6C), and between clutch size and oviductal egg size (Fig. 6D). Similarly, in *O. guibei*, positive relationships were determined between clutch size with snout-vent length and vitellogenic follicles (Fig. 6E and F), Although none of the relationships were statistically significant, it is important to highlight that fecundity in *O. guibei* was estimated based on the number of vitellogenic follicles, unlike the other species, for which oviductal eggs were used. This methodological difference should be considered when comparing reproductive parameters across species. For *O. rhombifer*, regression analyses were not performed due to the limited amount of data.

DISCUSSION

Our results align with previous studies on *Oxyrhopus* species and other snake groups, highlighting consistent patterns of sexual dimorphism (SVL, LC, ventrals, and subcaudals) (Shine, 1994; Pizzatto and Marques, 2002; Cox et al., 2007; Alencar et al., 2012; Coelho et al., 2019). This pattern has been observed across various regions of Brazil, including *O. trigeminus* (Alencar et al., 2012; Coelho et al., 2019), *O. guibei* (Pizzatto and Marques,

Table 3. Reproductive aspects of female *Oxyrhopus* species, including the number of reproductive females (NFR) for each species, the number of vitellogenic follicles (VF) and eggs, fecundity calculated with eggs or vitellogenic follicles, reproductive frequency (RF), and reproductive potential (RP), indicating the number of neonates per female per year.

	NFR	VF (mm)	Eggs (mm)	Fecundity	RF	RP
O. trigeminus	44	14.43 (10.2-19.4; n=43)	26.99 (22.01-37.88; n=11)	9.61 (5-13 E)	0.55	5.28
O. petolarius	29	13.85 (10.16-16.55; n=7)	25.9 (18.21-31.89; n=22)	8.58 (4-15 E)	0.66	5.72
O. guibei	6	15.02 (12.6 -17.36; n=4)	22.46-23.07; n=2	6.83 (3-11 FV)	0.66	4.55
O. rhombifer	3	12.9 -16.05; n=2	17.3; n=1	5.33 (3-8 FV)	0.3	1.59

2002; Pires de Toledo, 2020) and *O. melanogenys* (Pires de Toledo, 2020). Notably, males of all studied *Oxyrhopus* species exhibited longer tails than females, potentially to accommodate the hemipenis and associated muscles (Cox et al., 2007). Conversely, the longer SVL of females likely relates to reproductive aspects, providing space for larger or more numerous eggs (Rivas and Burghardt, 2001; Cox et al., 2007; Alencar et al., 2012).

Snakes can exhibit territorial behavior and male combat (Rivas and Burghardt, 2001, Senter et al., 2014). However, this behavior has been recorded in only a few Dipsadidae species and none from the Pseudoboini tribe. Following Pizzato et al. (2002) and considering the reported SSD values in *Oxyrhopus*, it is unlikely that false coral snakes exhibit this behavior, reducing selective pressure for longer males. Our findings support this, showing that males reach sexual maturity at smaller sizes than females, and could be allocating more energy towards reproduction than growth. This contrasts with females, which attain maturity at longer sizes, allowing for extended growth before reproduction.

In several snake species, it has been observed that males mature at a smaller size than females (Shine, 2000). This pattern was also confirmed in the genus *Oxyrhopus* by the study of Pizzatto and Marques (2002) with *O. guibei*, where they detected that the minimum size for adult males was 38.8 cm SVL, while for females, it was 63.2 cm, using data from the state of Sao Paulo. When comparing these findings with the results of our study, we observed a similar pattern in *O. petolarius* and *O. trigeminus*. Even with the limited amount of data for *O. rhombifer*, we also found this difference in maturation sizes. However, only *O. guibei* seems not to present such a marked difference, as both males and females had a minimum adult size with an SVL of approximately 44 cm. This apparent discrepancy could be due to the limited amount of data analyzed for this species (only 20 records), and it is possible that males maturing at even smaller sizes, as observed in the study of Pizzatto and Marques (2002) or even lower, may be found. Nevertheless, in all studied species, females exhibited longer maximum sizes than males.

In adult males, spermatogenesis appears to occur year-round in all the species we studied, which is consistent with previous studies conducted on *O. guibei* (Pizzatto and Marques, 2002). However, defining the male reproductive cycle is more complex than in females, as it cannot be directly assessed through gonad observation but rather through the analysis of testicular volume (Bellini, 2013) in addition histological analysis of seminiferous tubules and spermatozoa presence, which allow more precise determination of reproductive activity (Mendonça et al., 2024). In the present study, we focused on macroscopic indicators, such as testicular enlargement and the presence of coiled deferent ducts, both of which have been associated with sexual maturity and active spermatogenesis (Pizzatto and Marques, 2002; López et al., 2009; Almeida-Santos et al., 2014). It is also worth noting that the use of preserved specimens may introduce certain biases, as dehydration or compression can alter gonad size, potentially affecting assessments of reproductive seasonality (Almeida-Santos et al., 2014). Therefore, evaluating the condition of the deferent ducts and/or epididymis, particularly whether they are coiled, serves as an important complementary indicator of sperm presence. While the absence of histological data may limit more detailed interpretations, the external and internal morphological traits used here offer reliable evidence of reproductive condition in males.

Given that the production of male gametes is less energetically costly than the production of female gametes, it is expected that males in tropical areas have continuous reproductive cycles and remain active throughout the year (Pizzatto, 2005). Additionally, for the *Oxyrhopus* species studied here, it makes sense that males are active year-round, given that females exhibit a continu-

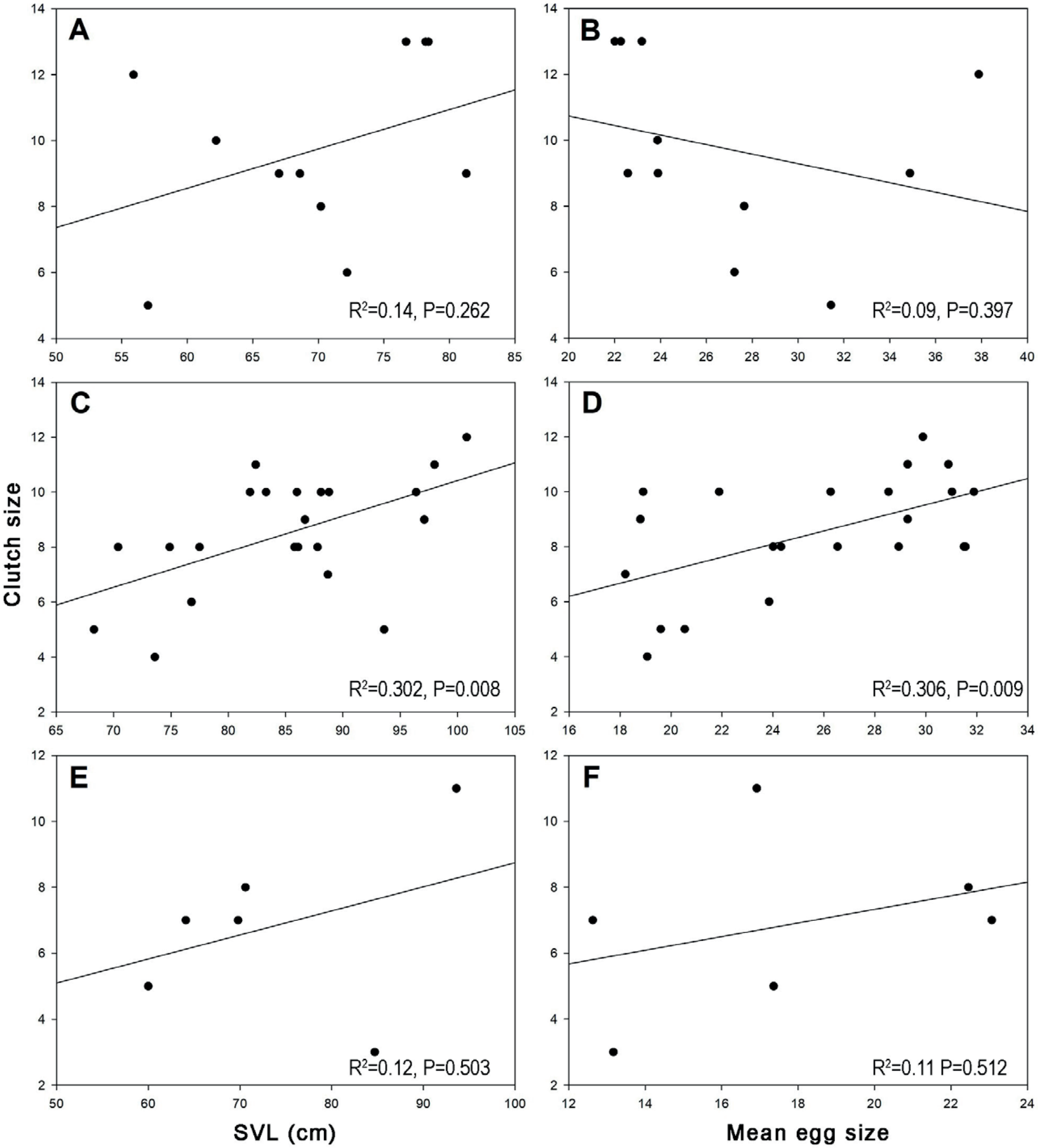

Fig. 6. Linear regressions for *Oxyrhopus trigeminus*: A) Clutch size (number of eggs) and body size of females (n= 11), B) Clutch size and average egg size (n= 11). Linear regression for *Oxyrhopus petolarius:* C) clutch size (number of eggs) and body size of females (n=21), D) clutch size and average egg size (n= 21). Linear regression for *Oxyrhopus guibei:* E) Clutch size (number of vitellogenic follicles) and body size of females (n= 6), F) Clutch size and average egg size (n= 6).

ous reproductive cycle, which could stimulate males to produce gametes constantly.

The longer body size of females compared to males provides a selective advantage, as fecundity is correlated with body size. In other words, longer females have the capacity to produce a greater number of eggs or longer offspring (Shine, 2000). In this study, although we did not measure egg volume or width, and therefore cannot infer offspring size or shape, we observed that larger females tended to have either more eggs or eggs with greater length. According to previous studies, *O. guibei* exhibits two possible reproductive strategies (similar to r and K strategies): the production of many small eggs or few large eggs (Pizzatto and Marques, 2002). Although these strategies were not clearly distinguishable in our data, the variation observed in egg length and clutch size suggests that female body size may influence reproductive investment. Nonetheless, future studies incorporating egg volume and hatchling data would allow for a more detailed understanding of reproductive strategies in these species.

In the case of *O. trigeminus, O. petolarius*, and *O. guibei*, positive relationships were found between the size of the females and the number of eggs they could produce. However, only *O. trigeminus* showed a negative relationship between the number of eggs and their size, suggesting a reduction in egg size as their number increases, as one reproductive strategy proposes. On the other hand, both *O. petolarius* and *O. guibei* did not show this negative relationship; instead, they exhibited a positive relationship, suggesting that in addition to having a greater number of eggs due to their longer size, these eggs also tend to be longer, which could result in longer offspring. However, it is important to note that this has its limits, as the eggs cannot occupy more space than is available within the body of the snakes. Therefore, we might think that these two species are balancing the two strategies proposed by Pizzatto and Marques (2002), having the largest possible number of eggs without compromising their size, thus ensuring a higher number of offspring with better chances of survival by being longer.

Fecundity is a crucial aspect in the study of snake reproduction (Almeida-Santos et al., 2014), commonly assessed by counting eggs in the oviducts of females. However, some studies also consider the number of vitellogenic follicles (Bizerra et al., 2005; Pizzatto, 2005, López et al., 2009; Bellini, 2013; Braz et al., 2014; Coelho et al., 2019), which can lead to overestimation, and should be specified clearly specified the method used to avoid it (Almeida-Santos et al., 2014; Braz et al., 2014). Our data on the fecundity of *O. trigeminus* and *O. petolarius* were based on egg counts, showing similar fecundities. However, for *O. guibei*, fecundity was estimated based on vitellogenic follicles rather than oviductal eggs due to the limited number of females with oviductal eggs available. It was observed that the fecundity of *O. guibei* was slightly lower than that of the other species. This suggests that considering only follicles larger than 10 mm could provide an accurate estimate of the actual fecundity for this species.

The study of fecundity in *Oxyrhopus* has been conducted in different regions of Brazil, with variable results. For example, a fecundity of 7.8 eggs has been determined for *O. trigeminus* in the state of Pernambuco (Coelho et al., 2019), and 7.5 in the state of Minas Gerais (Alencar et al., 2012). In the case of *O. guibei*, a fecundity of 10.9 was estimated in the state of Sao Paulo (Pizzatto and Marques, 2002). Comparing our data with these studies, we observed that *O. trigeminus* showed higher fecundity, while *O. guibei* exhibited a lower fecundity than reported. However, fecundity can vary within the same species depending on its distribution area and be related to climate, food availability, or even behavioral characteristics (Pizzatto et al., 2006). Some authors suggest that pregnant snakes may refrain from feeding (Pizzatto and Marques, 2002; Shine, 2003; Bellini, 2013); however, we observed pregnant snakes with stomach contents, which could affect both the available body space to develop the eggs and the level of energy that snakes can invest in reproduction, leading to variations in species fecundity.

The reproductive cycle of many Neotropical snakes is continuous, implying that they have the capacity to reproduce at any time of the year, although this does not necessarily mean they have multiple clutches (Shine, 2000; Pizzatto and Marques, 2002). It has been observed that oviparous species generally present annual cycles, or at least shorter ones than viviparous species, and longer females tend to reproduce more frequently. However, if females reach excessively large sizes, they may cease to reproduce as frequently due to senescence (Pizzatto et al., 2006). This pattern has been documented in species such as *Boiruna maculata, Clelia pumblea*, and *O. guibei* (Pizzatto, 2005), all belonging to the monophyletic tribe Pseudoboini. Therefore, it is reasonable to expect that other members of this group, including species of the genus *Oxyrhopus*, exhibit a similar reproductive system. Our data confirm that *O. trigeminus*, *O. petolarius*, and *O. guibei* display this type of reproduction, as we found eggs during both the wet and dry seasons. Furthermore, the reproductive cycle of these species appears to be annual, as expected. In contrast, *O. rhombifer* might exhibit a biannial reproductive cycle; however, due to the limited data available, this cannot be confirmed with certainty. It would be necessary to investigate whether females that reproduce during the dry season in one year also do so in the following year's dry season, or if they might instead reproduce during the subsequent wet season. This could indicate if their reproductive cycle is shorter than 12 months. Since reproductive cycles can be affected by climate, even if the same species exhibits a continuous cycle in warm areas, it may show seasonal patterns in colder regions (Pinto and Fernandes, 2004). It would be interesting to study the effect of climatic parameters on the reproduction of this genus, considering its wide distribution in the American continent.

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Spiny treasures of Gorgona Island: Unveiling the natural history secrets of the lizard *Enyalioides heterolepis* (Squamata: Hoplocercidae) in Colombia

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Abstract. Novel insights into the natural history of the spiny woodlizard or spiny dwarf iguana, *Enyalioides heterolepis* (Bocourt, 1874), are presented based on a distinctive population from Gorgona Island, Cauca department, Colombia. Between July 2023 and August 2024, diurnal and nocturnal field surveys were conducted, and some ecological and morphological data were recorded. *E. heterolepis* is a diurnal, terrestrial species that primarily uses leaf litter. However, it shifts to arboreal microhabitats at night for sleeping, perching on trunks and leaves of similar heights and diameters across sexes. The species exhibits sexual dimorphism in body size and ventral sexual dichromatism, as well as a low frequency of caudal autotomy in individuals. These findings contrast with patterns observed in other neotropical rainforest iguanian lizards (*Anolis*, *Plica*, *Uracentron*) and partially align with previous reports for this and other *Enyalioides* species. Additionally, results are discussed in the context of island-mainland differences in lizard population densities. Predation pressure, social behavior, and the ecological and evolutionary bases of sexual dimorphism and dichromatism are proposed as key factors influencing the ecological traits of this species.

Keywords. Density compensation, Chocó biogeographic region, hoplocercid lizards, sleeping site fidelity.

INTRODUCTION

There is a particular phenomenon within island biogeography known as “density compensation” (MacArthur et al., 1972), which postulates that, especially on small or isolated islands, a reduction in the number of species lowers the intensity in limiting factors such as predation, parasitism, or competition, thereby allowing some species – typically less abundant on the mainland – to reach higher population densities (MacArthur et al., 1972; Rodda and Dean-Bradley, 2002; Buckley and Jetz, 2007). This discrepancy provides an opportunity to better understand various biological and ecological aspects of these species by studying island populations, in order to extrapolate findings to the continent or compare them with those found in mainland populations.

One possible case of density compensation is the spiny woodlizard or spiny dwarf iguana *Enyalioides heterolepis* (Bocourt, 1874; Fig. 1), a medium-sized lizard (182 mm maximum snout-vent length) that inhabits the lower stratum of primary and secondary tropical humid forests of the Chocó biogeographic region, ranging from 0 to 1150 meters in elevation from Panama to Ecuador, passing through Colombia (Páez et al., 2002; Eisenberg and Pantchev, 2010; MECN, 2010; Rios et al., 2011; Castro-Herrera et al., 2012; Cardona-Botero et al., 2013; Medina-Rangel et al., 2017; Pinto-Erazo et al., 2020). Its abundances are typically low, with most studies reporting

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Fig. 1. A male individual of *Enyalioides heterolepis* (Squamata: Hoplocercidae) from San Cipriano, Valle del Cauca department, Colombia

findings on the mainland (Eisenberg and Pantchev, 2010; MECN, 2010; Rios et al., 2011; Pinto-Erazo et al., 2020). However, populations of this species are particularly numerous on Gorgona Island, in Colombia (Urbina-Cardona and Londoño-M, 2003; Castro-Herrera et al., 2012).

Some natural history traits of *E. heterolepis* have been mentioned in the literature, but these reports are often descriptive and provide either insufficient data or involve a low number of individuals (Páez et al., 2002; Eisenberg and Pantchev, 2010; MECN, 2010; Castro-Herrera et al., 2012; Medina-Rangel et al., 2017). This raises several questions: Is this species primarily terrestrial (Castro-Herrera et al., 2012; Medina-Rangel et al., 2017), arboreal in the lower stratum (Páez et al., 2002), or both? What is the range of body size, and what heights do nocturnal perches reach for this species (Eisenberg and Pantchev, 2010)? Does it prefer only fallen branches and trunks for sleeping sites (Páez et al., 2002), or does it also use leaves (MECN, 2010) or other types of perches?

In the present study, I am to clarify and expand the knowledge on some natural history traits of E. heterolepis using data from populations on Gorgona Island, Colombia, including a preliminary quantification of overall and sex-related prevalence of tail loss.

MATERIALS AND METHODS

Study site

Gorgona National Natural Park, or PNN Gorgona, is located in the Pacific region of Colombia, on Gorgona Island and Gorgonilla islet (2°9' N, 78°2' W, 0-338 m a.s.l.), Cauca Department, Guapi Municipality (Fig. 2). The island measures 8.5 km in length and 2.5 km in width, with a maximum elevation of 338 meters above sea level. It is part of the National Natural Parks of Colombia (PNN) system, designated as a protected area. The island is primarily covered by primary and secondary tropical rainforest with some palm groves (*Cocos nucifera* L.; Sedano-Cruz et al., 2024). The average annual temperature is 26°C, and the relative humidity is 90% (Blanco, 2009), with both showing an almost negligible variation throughout the year, reflecting the highly stable climatic conditions of the island (Rangel-Ch and Rudas-LL, 1990). The protected area covers 617 km^2, underscoring its ecological significance and conservation value.

Sampling and data collection

Data were collected on three different sampling dates: July 2023, January-February 2024, and August 2024 (Table 1). Three observers searched for lizards in the tropical rainforest vegetation cover using the time-constrained Visual Encounter Survey (VES) transect method (Crump and Scott, 1994; Lips, 2001) during two different sampling periods: daytime (06:00 – 18:00) and nighttime (20:00 – 24:00) hours.

During diurnal samplings, sighting time, microhabitat type (leaf litter, rock, soil, leaf/leaves, branch, fallen trunk, vine, trunk or root; Fig. 2), and, where applicable, perch height (in cm) for each individual were recorded without capturing them. During nocturnal samplings, individuals were captured along the same routes surveyed during the day, although not necessarily covering the exact same distance or duration. For each captured individual, microhabitat type, perch height and perch diameter (in cm), snout-vent length (SVL; in mm), total tail length (TL, in mm), regenerate tail length (regTL; in mm), non-regenerate tail length (no.regTL; in mm), and in case of adult individuals, sex (determined by femoral pores present only in males; Eisenberg and Pantchev, 2010) were recorded. Perch height and perch diameter were measured using a measuring tape, while SVL and TL were measured with a precision analog caliper (precision = 0.05 mm).

To avoid pseudoreplication, diurnal samplings were conducted in different locations for each sampling period, while nocturnal samplings were conducted in distinct locations throughout the entire study.

Statistical analysis

The encounter rate (relative abundance) was calculated as the total number of lizards observed divided by the total observation hours and the number of observers (individuals h^{-1} observer^{-1}; Lips, 2001). For microhabitat use, I used the Chi-square test of independence to test differences between diurnal and nocturnal use of terrestrial/ground (leaf litter, rock, soil and root) and arbo-

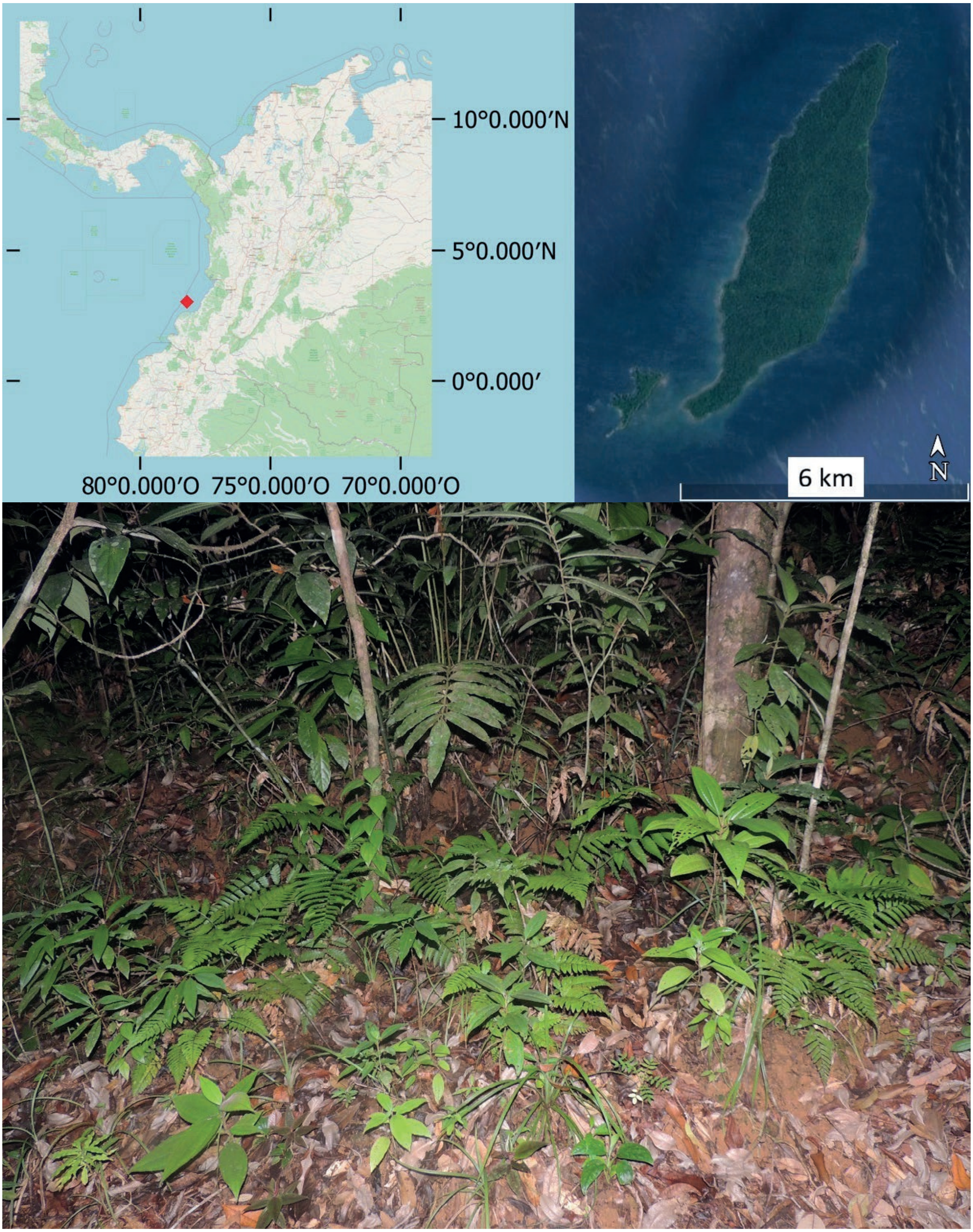

Fig. 2. Top: Location (red diamond) of Gorgona Island and Gorgonilla islet (Gorgona National Natural Park; Gorgona PNN), Guapí municipality, Cauca department, in Colombia, and Landsat image of the Island on Google Earth Pro 7.3.6. Bottom: Representative microhabitats of *Enyalioides heterolepis*, including leaf litter and soil at ground level, and arboreal perches such as leaves, branches, trunks, and vines.

real/perch (leaves, branch, fallen trunk, tree trunk and vine) microhabitats. I assessed differences between sexes in snout–vent length (SVL), intact tail length (TL), and perch diameter using an ANOVA or Kruskal-Wallis test. Posteriorly, differences between sexes in perch height were evaluated using a linear model, with perch height as the response variable, sex as a fixed factor, and snout–vent length (SVL) as a covariate to account for potential effects of body size. Prior to analyses, data were inspected for linearity, normality, and homoscedasticity assumptions.

On the other hand, the Chi-square goodness-of-fit test was conducted to determine whether there was an equal number of individuals with regenerated tails compared to those with intact tails. Additionally, I employed the Fisher's exact test to assess whether there was a significant difference in the proportion of regenerated tails between sexes only in adult individuals (see Results for age class criteria). Finally, the ANOVA or the Kruskal-Wallis test were used to determine if lizards with intact tails exhibited similar tail lengths compared to individuals with regenerated tails. Statistical tests and graphics were conducted using RStudio software v1.3.959 (RStudio Team, 2020).

RESULTS

Over four days, a total of 12 hours of diurnal sampling were conducted, distributed between 06:00 to 18:00 h, amounting to a total of 36 observer hours, and 21 lizards were recorded (Table 1). Similarly, over six days, from 20:00 to 24:00 h, I completed 15.5 hours of nocturnal sampling, totaling 46.5 observer hours and recording 25 sleeping lizards (Table 1). As a result, the lizard encounter rate was 0.58 individuals h^{-1} observer^{-1} for diurnal sampling and 0.54 individuals h^{-1} observer^{-1} for nocturnal sampling.

Table 1. Sampling days, sampling periods, sampling intervals (total hours) and number of lizards observed or captured in this study.

Sampling day	Sampling period	Sampling interval (total hours)	Number of lizards
5 July 2023	Day	11:00-14:00 (3)	4
8 July 2023	Day	09:00-11:00 (2)	4
4 February 2024	Day	14:00-18:00 (4)	11
5 February 2024	Day	06:00-09:00 (3)	2
5 July 2023	Night	20:30-23:30 (3)	4
7 July 2023	Night	21:00-23:00 (2)	7
9 July 2023	Night	21:00-23:00 (2)	6
28 January 2024	Night	20:00-24:00 (4)	1
26 August 2024	Night	20:00-23:00 (3)	5
30 August 2024	Night	21:00-22:30 (1.5)	2

The spiny dwarf iguana *Enyalioides heterolepis* from Gorgona Island was found exclusively in the lowland tropical forest (mostly secondary) of the island. In general, lizards were observed on the ground, generally under shade or filtered sunlight. However, particularly at 6:00 h and 15:00 to 18:00 h, a few lizards were recorded using perches such as fallen trunks, leaves and branches, positioned between 23 and 127 cm above the ground (Fig. 3).

Eight additional diurnal lizard observations were

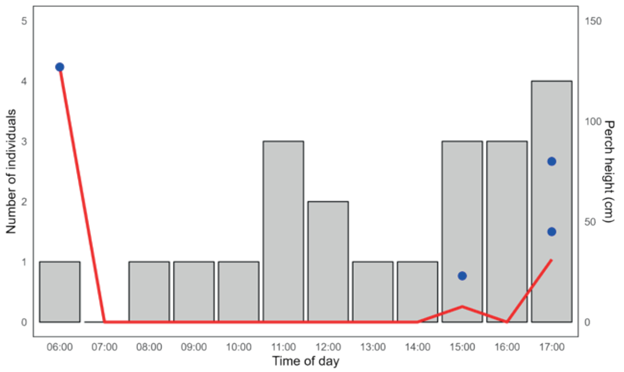

Fig. 3. Hourly number of individuals of *Enyalioides heterolepis* observed on Gorgona Island, Colombia. The bars represent the number of individuals observed during each hour of the day, from 06:00 to 18:00 h. The blue dots indicate the height of perched individuals. The red line shows the average perch height of observed individuals in each hour.

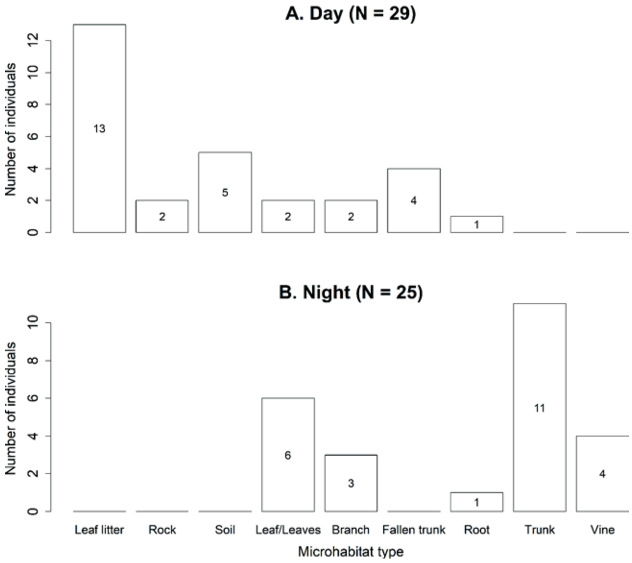

Fig. 4. Microhabitat use of *Enyalioides heterolepis* on Gorgona Island, Colombia, during the day (A) and at night (B). The bars represent the number of individuals observed in each microhabitat category, with the value of each bar indicated.

Fig. 5. Sleeping individuals of *Enyalioides heterolepis* showing variation in nocturnal perch use: from left to right, two on tree trunks, one on a branch, and one on leaves.

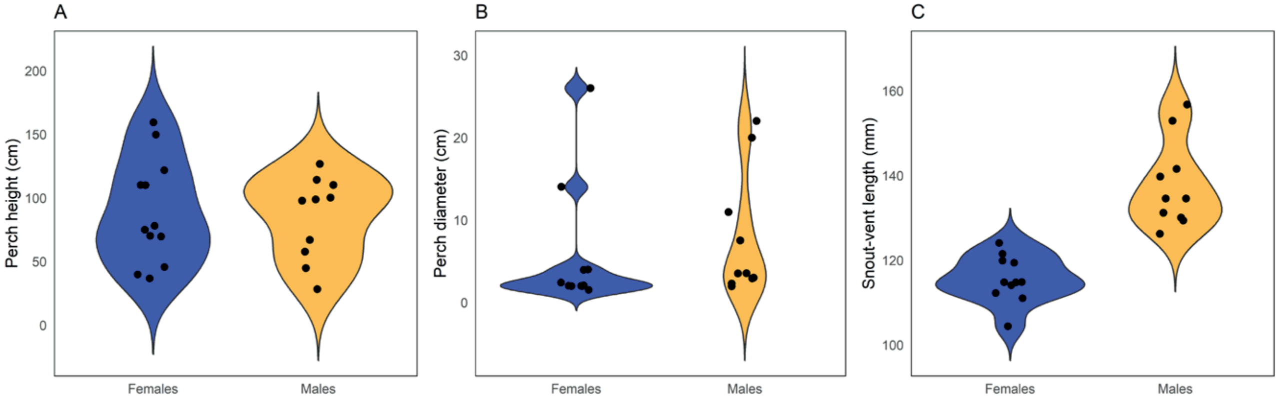

Fig. 6. Violin-jitter plots of perch height (A), perch diameter (B) and snout-vent length (SVL) (C) of adult male (N = 10) and female (N = 11-12) individuals of *Enyalioides heterolepis* on Gorgona Island, Colombia. Each point represents a measured individual.

recorded from occasional encounters or brief samplings, completed a total of 29 lizard observations for microhabitat data. During the day, *E. heterolepis* inhabits principally terrestrial/ground microhabitats (N = 21; 72.4%), mostly on leaf litter (44.8%), followed by bare soil (17.2%) and fallen trunks (13.8%; Fig. 4A). Some of the individuals using fallen trunks, leaves and branches correspond to the arboreal/perch microhabitat observations in Fig. 3 (in blue dots). In contrast, during the night, of the 25 observed lizards, 44% were found sleeping perching on tree trunks, followed by leaves (24%), vines (16%) and branches (12%) (Fig. 4B), with a total of 24 lizards (96%) using arboreal/perch microhabitats to sleep (Fig. 5). Thus, terrestrial and arboreal microhabitat use change significantly between day and night ($\chi^2 = 26.028$, df = 1, $P < 0.05$).

Adult males were found to be significantly larger than adult females in terms of the snout-vent length (SVL) ($F_{1,19} = 39.5$, $P < 0.05$; Fig. 6C; Table 2) and intact tail length (TL) ($F_{1,12} = 24.1$, $P < 0.05$; Table 2). During nocturnal sampling, only two juveniles were captured, while a third juvenile was occasionally captured during diurnal sampling. Although there is no available evidence about the sexual maturation size for males or females in this species, the largest juvenile reached 84.4 mm SVL, while the shortest adult, a female, measured 104.4 mm SVL, with a gap of 20 mm between the two sizes. This difference is larger than any SVL variation among adult measurements (Fig. 6C), suggesting that this gap could serve as an alternative criterion to differentiate between adults from juveniles in this dataset. Additionally, the smallest gravid female found had an

Table 2. Ecological and morphological measurements of *Enyalioides heterolepis* individuals on Gorgona Island, Colombia. The measurements correspond to adult males, females, juveniles, and the total number of individuals. For each variable in each group the following is shown: Mean + SD (Min-Max) N = sample size.

	Perch height (cm)	Perch diameter (cm)	Snout-vent length (SVL) (mm)	Tail length (TL) (mm)	Non-regenerate tail portion (no. regTL) (mm)	Regenerate tail portion (regTL) (mm)	regTL + no.regTL (mm)
Males	84.6 ± 32.9 (28-127) N = 10	7.7 ± 7.5 (2-22) N = 10	137.6 ± 10.3 (126-156.8) N = 10	199.2 ± 13.1 (179.5-214.1) N = 8	40.5 ± 13 (31.25-49.7) N = 2	70.8 ± 14.8 (60.4-81.3) N = 2	111.3 ± 1.7 (110.1-112.5) N = 2
Females	88.9 ± 41.1 (37-159) N = 12	5.4 ± 7.3 (1.5-26) N = 12	115.5 ± 5.5 (104.4-124.1) N = 11	159.9 ± 16.9 (134.8-175.7) N = 6	57.7 ± 37.7 (21.2-121) N = 5	39.8 ± 16 (19.3-58) N = 5	87.4 ± 39.2 (44-140.3) N = 5
Juveniles	45.5 ± 16 (34-57) N = 2	12.2 ± 2.5 (10.5-14) N = 2	62.2 ± 23 (38.5-84.4) N = 3	87.65 ± 38.5 (59.5 - 131.6) N = 3	-	-	-
Total	83.5 ± 37.1 (28-159) N = 24	6.9 ± 7.2 (1.5-26) N = 24	118.1 ± 26 (38.5-156.8) N = 24	165.6 ± 45.4 (59.5-214.1) N = 17	52.8 ± 32.3 (21.2-49.7) N = 7	48.7 ± 20.9 (19.3-81.3) N = 7	94.2 ± 34.1 (44-140.3) N = 7

SVL of 111 mm, providing additional support for the established adult size threshold.

Perch diameter (H = 2.14, df = 1, P = 0.14) of nocturnal arboreal microhabitats was quite similar between adult males and females (Fig. 6A-B; Table 2). However, it is necessary to highlight the presence of four outliers above 12 cm (two for each sex), which corresponds to perches used in leaf microhabitats (Fig. 6B). Excluding these data points revealed a marginally tendency (H = 3.69, df = 1, P = 0.055) for males to use slightly broader perches (Mean ± SD (Min-Max) N: 4.5 ± 3.1 (2-11) N = 8) compared to females (Mean ± SD (Min-Max) N: 2.5 ± 0.8 (2-4) N = 9). On the other hand, the linear regression model including sex and snout–vent length (SVL) as predictors indicated no significant effects on perch height (overall model: $F_{2,18} = 1.46$, P = 0.26, $R^2 = 0.14$). Although males tended to use slightly lower perches than females (β = −44.76 ± 27.36, P = 0.12) and perch height tended to increase with body size (β = 1.65 ± 1.02, P = 0.12), neither effect was substantial.

A total of 17 individuals were found to have intact tails, while 7 individuals (representing 29% of the total individuals) exhibited regenerated tails (Table 2). This discrepancy in the proportions between the two groups was statistically significant ($\chi^2 = 4.167$, df = 1, P < 0.05). All captured juveniles had intact tails. Among adults, 8 males and 6 females had intact tails while 2 males and 5 females had regenerated tails. There was no significant association between tail condition and sex (Fisher's exact test, P = 0.36).

Among the 7 individuals with regenerated tails, the average proportion of the regenerated section was 0.5 ± 0.19, ranging from 0.14 to 0.72. Additionally, these individuals had shorter total tail lengths (regTL + no.regTL) compared to the total tail lengths (TL) of the 14 individuals with intact tails ($F_{1,19} = 48.26$, P < 0.05).

DISCUSSION

Island vs. mainland abundance

Unlike previous studies on the mainland (Eisenberg and Pantchev, 2010; MECN, 2010; Rios et al., 2011; Pinto-Erazo et al., 2020), a substantial abundance of the lizard *Enyalioides heterolepis* on Gorgona Island was found, observing 21 diurnal, 25 nocturnal, and 8 occasional encounters, with a total 54 lizard observations. Moreover, this value is similar to the 46 individuals found by Urbina-Cardona and Londoño-M (2003) with a sampling effort of 32 transects of 200 m each, and contrast with the 17 individuals recorded by Rios et al. (2011) in 448 h^{-1} $observer^{-1}$ (0.038 individuals h^{-1} $observer^{-1}$) or the two 2 individuals reported by Pinto-Erazo et al. (2020) in 1604 hours.person-1 (0.001 individuals h^{-1} $observer^{-1}$) at other continental sites in the Pacific region of Colombia. This mainland-island difference in abundance of *E. heterolepis* agrees with the "density compensation" phenomenon (MacArthur et al., 1972) of higher populations densities of species in islands vs. continent, which also has robust evidence in lizards (Novosolov et al., 2015).

These authors suggest that lizard population densities are higher in snake-free islands. In this regard, our findings are even more notable given the large snake species richness of Gorgona Island, hence the name of the place (Urbina-Cardona and Londoño-M, 2003; Urbina-

Cardona et al., 2008; Castro-Herrera et al., 2012). However, only a better comparison of the richness, abundance and composition of snake diversity between *E. heterolepis* continental vs. Gorgona Island populations could outperform our understanding of island-mainland snake predatory pressure on this species.

Microhabitat use

Studies on natural history and ecology of hoplocercid lizards are scarce (but also see Eisenberg and Pantchev, 2010 and Thomas et al., 2021), compared with studies of other neotropical rainforest diurnal lizards such as *Anolis* (Vitt et al., 2003 a, c), tropidurids (Vitt et al., 1997; Ellinger et al., 2001), teiids (Biázquez, 1996; Mesquita et al., 2006), and microteiids (Vitt and Avila-Pires, 1998; Vitt et al., 1998, 2003 b, 2007). Specifically, *E. heterolepis* is a terrestrial species that uses principally leaf litter during the day, contrasting to arboreal habits of other iguanian lizards such as *Anolis*, *Plica*, and *Uracentron* (Vitt et al., 1997; 2003 a, c; Ellinger et al., 2001), and showing some ecological resemblance to microteiids such as *Potamites*, *Cercosaura* and *Alopoglossus*, although these lizards are more cryptic and live under the leaf litter (Vitt and Avila-Pires, 1998; Vitt et al., 1998, 2003 b, 2007). Terrestriality was also observed twice in the hoplocercid *E. groi* (Corredor et al., 1985; Vásquez-Restrepo, 2021). On the other hand, teiids prefer the open ground (Biázquez, 1996; Mesquita et al., 2006), with species of the genus *Crocodilurus* associated with water streams, while *Dracaena guianensis* is found perched on shrubs (Mesquita et al., 2006). Microteiids of the genus *Potamites* are also associated with water streams, in contrast to *E. heterolepis*.

Microhabitat use switches completely between day and night in *E. heterolepis*, changing from a leaf litter terrestrial habit to arboreal perches, particularly trunks and leaves. Sleeping perches have been previously reported in this species (Eisenberg and Pantchev, 2010) as well as in *E. laticeps*, although *E. laticeps* is mainly arboreal during its diurnal activity period (Thomas et al., 2021). The number of observed individuals and perch height agree with this circadian change of microhabitat use, with an afternoon-crepuscular peak of observations between 15:00 and 18:00 h and three individuals with perch heights of 23, 45, and 80 cm within this period, suggesting active searching and climbing to sleeping perches. In contrast, Vásquez-Restrepo (2021) found a female of *E. groi* at 19 h in a burrow, suggesting a different sleep microhabitat for this *Enyalioides* species.

Thomas et al. (2021) noted that sleeping site selection could play an anti-predation role, provide greater stability (also related to predation) when sleep, or offer thermoregulatory advantages in the morning. This could also explain the abundance of *E. heterolepis* on Gorgona Island despite its richness of snake species. In addition, a midday peak of observations between 11:00 to 13:00 h occurs, which could be more related with thermoregulatory activity of diurnal lizard species (Ellinger et al., 2001; Biázquez, 1996; Mesquita et al., 2006; Vitt and Avila-Pires, 1998; Vitt et al., 1997, 1998, 2003 a, b, c, 2007), in which case predation or stability seems to be better explanations of sleeping site selection. However, additional studies on predation risk and thermoregulation are necessary to test these hypotheses.

Sexual dimorphism

Both males and females used sleeping perches of similar heights and diameters. In contrast, there was evident sexual dimorphism in SVL, with males being larger than females. This pattern differs from other studies on neotropical rainforest diurnal lizards, where there is no sexual dimorphism (Vitt et al., 1997, 2003 b, 2007, Mesquita et al., 2006, Ellinger et al., 2001) or when it exist, females are larger than males (Vitt and Avila-Pires, 1998; Vitt et al., 1998, 2003 c). Additionally, not only size, but also coloration differed between sexes. In all captured adults, distinct blackish blotches were observed only in males, a color trait previously noted by Eisenberg and Pantchev (2010). This type of ventral sexual dichromatism has been documented in other lizard genera, such as *Sceloporus* (Putman et al., 2025) and *Platysaurus* (Whiting 1999; Whiting et al., 2003), and is associated with conspecific interactions, for example, during male agonistic ventral displays in the latter.

Sexual dimorphism is commonly attributed to sexual selection, in terms of male competition or female choice, or natural selection related to male-female resource partitioning, for instance, in *Anolis* lizards (Rodríguez-Rodríguez and Calderón-Espinosa, 2024). Based on the above, it seems that habitat source does not play a role in the sexual dimorphism and dichromatism observed in *E. heterolepis*, and other processes such as sexual selection, male status signaling, territorial defense, or individual recognition may be more plausible explanations. However, further studies are needed to clarify the evolutionary basis of sexual difference of these traits, as well as additional animal behavior research on this species.

Caudal autotomy

The proportion of individuals exhibiting caudal autotomy was moderate to low (29%), which may reflect low predation pressure or efficiency, or a generally low susceptibility to predator attacks (Bateman and Fleming, 2009). Alternatively, this pattern may result from the use of other effective antipredator strategies, such as crypsis or early flight initiation (Vitt and Caldwell, 2014). For instance, individuals usually flew away from observers, some with a flight initiation distance greater than ten meters. Likewise, juveniles with intact tails may be more cryptic than adults, which could also explain their low abundance in this study, but the small sample size makes it difficult to assert this. Intraspecific aggression has also been proposed as an explanation for caudal autotomy rates, which are related to sex differences, with males having a higher rate than females due to their agonistic behavior (Bateman and Fleming, 2009). However, this is not the case for *E. heterolepis*, where these differences were not found. In fact, I found more adult females than males with caudal autotomy. Regardless, shorter tails in *E. heterolepis* individuals with caudal autotomy vs. individuals with intact tails seem to reflect the energetic cost of tail regeneration, but the time of regeneration is needed to confirm this (Bateman and Fleming, 2009).

Additional natural history notes

Similar to *E. laticeps* (Thomas et al., 2021), sleeping site fidelity in further surveys was observed: three adult individuals, one male and two females, were found 1.5, 4, and 7 meters away, respectively, from their original sleeping perches three nights after their first detection on 28 January 2024. Other natural history observations regarding sleeping sites include: (1) on 8 June 2023, a pair was observed sharing the same trunk perch, with the male and female positioned at perch heights of 98 cm and 159 cm, respectively; (2) on 28 January 2024, an adult female was found sleeping on a trunk perch that was subsequently used by an adult male three and eight nights later; and (3) homing behavior was documented on 27 August 2024, when an adult female translocated 50 meters from its original perch site returned within two days to a location approximately 4 meters away from it. Male-female interactions also occurred during diurnal activity, on 8 July 2023, when a pair was observed on the leaf litter at 11:00 h.

Conclusions

Gorgona Island provided an opportunity to study key ecological traits of the spiny woodlizard or spiny dwarf iguana (*Enyalioides heterolepis*). Present results indicate that this species is diurnal and terrestrial, primarily using leaf litter as its main substrate. At night, it shifts to arboreal microhabitats for sleeping, perching on trunks and leaves of similar heights and diameters in both sexes. I also found clear sexual dimorphism in body size and ventral sexual dichromatism, as well as low rates of caudal autotomy. Further research on predation pressures in island versus mainland populations, thermal ecophysiology, social behavior, and ecological and evolutionary bases of sexual differences is needed to better understand the patterns observed in this study.

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Characterization of suitable breeding sites for Asiatic toads (*Bufo gargarizans* Cantor, 1842) (Anura, Bufonidae) using ecological modeling

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Abstract. Efforts to protect amphibian habitats and breeding sites are increasing, with ecological modeling playing a key role in predicting suitable habitats and informing conservation strategies. The Asiatic toad (*Bufo gargarizans*) is widely distributed across South Korea and is ideal for establishing comprehensive conservation measures. However, it is increasingly vulnerable to human activities and climate change. In this study, we investigated the breeding site characteristics of *B. gargarizans* using a generalized linear model (GLM). Field surveys were conducted at 124 reservoirs across three study sites at similar longitudes but different latitudes (Paju, Cheongju, and Gwangyang). To develop 30 candidate GLMs, using model selection based on the Akaike Information Criterion (AIC), 17 variables were collected from field measurements and geographic data. The results identified six factors influencing the suitability of *B. gargarizans* breeding sites: water temperature, dissolved oxygen, the average water depth 1 m from the shoreline, reservoir area, percentage of barren land within a 1km buffer, and percentage of water within a 1km buffer. The model indicated that areas with a shoreline depth of around 48 cm, low surrounding barren ratios (3%), and more than 3% water bodies are suitable for *B. gargarizans* breeding. These findings provide valuable insights into the conservation of *B. gargarizans* and can support the development of effective habitat protection measures.

Keywords. GLM, AIC, conservation management, shoreline depth, barren, water body.

INTRODUCTION

Amphibians are sensitive to subtle habitat changes (Beebee, 1997; Alford and Richards, 1999; Stebbins and Cohen, 2021), and thus face significant threats to their survival, such as habitat fragmentation and alteration (Dixo et al., 2009; Decena et al., 2020). Most amphibians breed and develop in water, undergoing an aquatic larval stage before metamorphosing and moving onto land. Therefore, the quality and stability of their habitats are crucial for survival (Evans et al., 1996) and protecting key breeding habitats (wetlands and reservoirs) is essential for amphibian conservation.

The suitable selection of breeding sites by amphibians significantly impacts their survival and breeding success (Ra et al., 2010; Borzée et al., 2018). Previous studies have identified factors that differentiate breeding sites based on adult responses to different variables such as water-holding capacity (Lin et al., 2008), reproduction avoidance in response to predators (Murphy, 2003 a; Jowers and Dowine, 2005), and negative relationships between conspecific density and breeding site prefer-

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ence (Resetarits and Wilbur, 1989; Crump, 1991; Spieler and Linsenmair, 1997; Murphy, 2003 a). Additionally, for amphibians that breed primarily in permanent water sources, the disappearance of breeding sites due to drying can be a major cause of mortality for the hatched tadpoles (Smith, 1983; Newman, 1988), highlighting the importance of maintaining breeding sites for stable larval growth (Edgerly et al., 1998; Murphy, 2003 b; Rudolf and Rödel, 2005).

Amphibians select breeding sites based on various environmental factors, including water temperature, dissolved oxygen, water depth, aquatic vegetation, and the presence of predators (Skelly et al., 1999; Semlitsch, 2000). The anuran family Bufonidae, commonly known as toads, tend to prefer larger bodies of water with stable environmental conditions, as seen in studies on the *Rhinella marina*, which selects breeding sites consisting of shallow pools and unvegetated muddy banks (Semeniuk et al., 2007), and *Epidalea calamita* whose site preference is also influenced by water temperature, chemistry, and the presence of competitor species (Banks and Beebee, 1987). Understanding these factors provides valuable insights into amphibian breeding and conservation efforts. Among the various research methods for identifying these key factors, ecological modeling has been widely used to predict suitable breeding sites by analyzing combinations of variables. Studies have applied habitat suitability models to assess amphibian breeding habitats based on environmental predictors, such as land cover, climate, and topography (Cunningham et al., 2007; Ra et al., 2010; Blank and Blaustein, 2012). These models have proven effective in identifying relationships between species and their environments and predicting species distributions, contributing to conservation planning (Guisan and Zimmermann, 2000; Lunghi et al., 2015; Su et al., 2020).

This study focused on the Asiatic toad (*Bufo gargarizans* Cantor, 1842). *Bufo gargarizans* is a species of the family Bufonidae that inhabits the inland areas of the Korean Peninsula and parts of mainland East Asia and breeds in flat water (Lee et al., 2011). A recent taxonomic review of this species was conducted by Matushkina et al. (2022), and based on molecular analyses, proposed reclassifying populations from the Korean Peninsula, northeastern China, and Russia Primorye region as *Bufo sachalinensis* (Othman et al., 2022). However, the International Union for Conservation of Nature (IUCN) and South Korea's National Species List has not adopted this classification; therefore, this study will use *Bufo gargarizans*. According to the IUCN, the *B. gargarizans* classified as a "Least Concern (LC)" species (IUCN 2022). However, the population of *B. gargarizans* has been declining due to human activities and climate change, highlighting the need for conservation efforts (Sung et al., 2007; Yang et al., 2020). Identifying suitable habitats is essential to conserve the populations of *B. gargarizans* (Yang et al., 2023). Additionally, since *B. gargarizans* is widely distributed across South Korea (Lee et al., 2011), analyzing the key environmental factors of its breeding sites can contribute to broader conservation strategies for amphibians. Therefore, this study aimed to characterize the conditions of suitable breeding sites for *B. gargarizans* using Generalized Linear Models (GLM) to identify critical variables and their influence on the selection of breeding sites.

MATERIALS AND METHODS

Study area

Since *B. gargarizans* are known to breed in stable water bodies (Lee et al., 2011), only reservoirs were selected as study sites where the water is stable. Data on *B. gargarizans* breeding sites nationwide were collected through preliminary research and surveys (NIE 2018) to determine the criteria for selecting reservoirs. Based on this data, regions located in the northern, central, and southern parts of the country and situated at 127° longitude were selected (Paju in the North, Cheongju in the Center, and Gwangyang in the South; Fig. 1). After selecting the study sites, a preliminary survey was conducted in January and February to observe the breeding activities and identify breeding and non-breeding sites.

Field survey

Considering the breeding period of *B. gargarizans* (Lee et al., 2011), a field survey was conducted from March to May. The variables used for the model were referenced from previous studies (Evans et al., 1996; Ra et al., 2010). In previous studies, the variables were categorized into micro, biological, and non-biological categories. However, based on the toxicity of the eggs and tadpoles from the family Bufonidae (Crossland and Alford, 1998; Lim et al., 2005) and the fact that fish tend to avoid or do not prey on Bufonidae (Kruse and Stone, 1984; Kiesecker et al., 1996), this study assumes there is no correlation between Bufonidae larvae and the biological variables. Consequently, biological variables were not collected. Additionally, data collection was conducted exclusively through field observations and did not involve animal capture; therefore, no animal ethics approval was needed.

A total of 17 variables, consisting of 7 micro and 10 macro variables, were collected (Table 1). The micro vari-

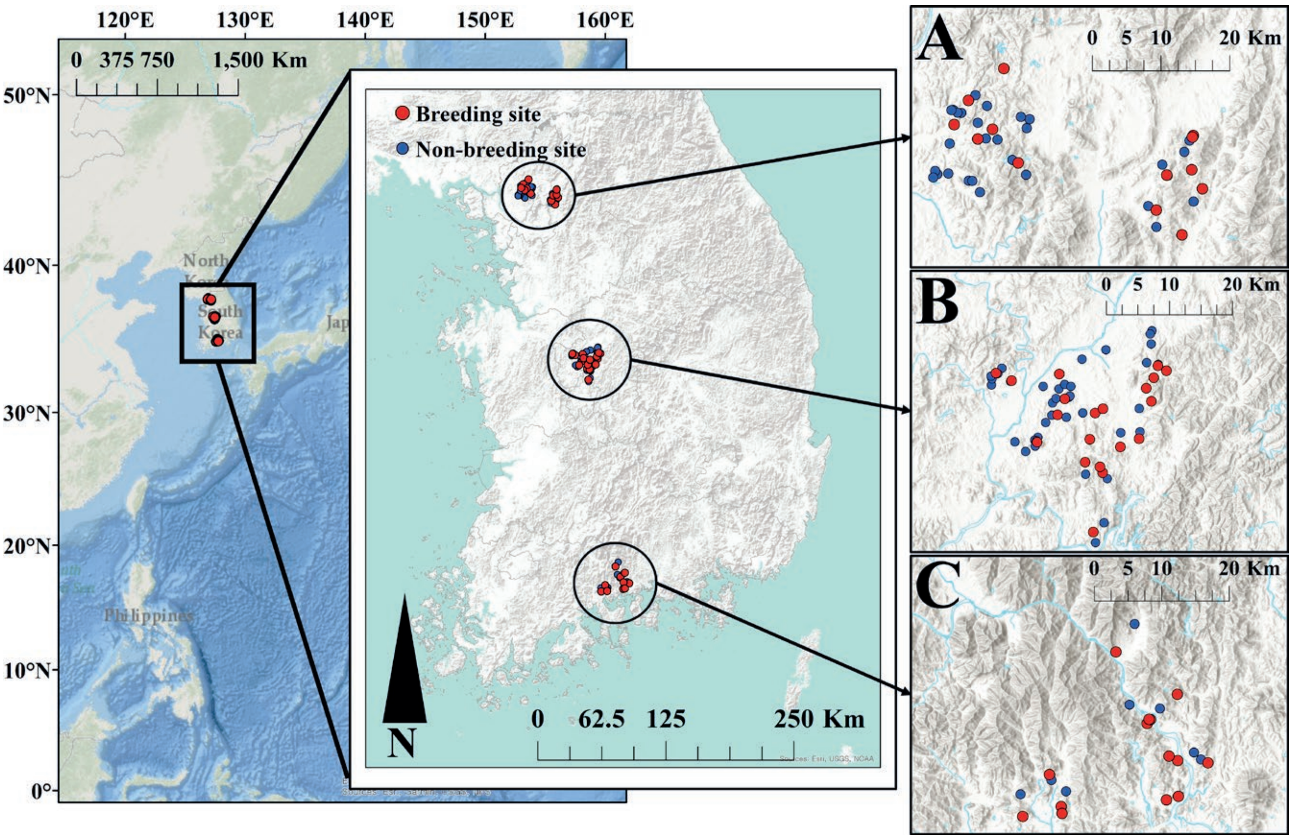

Fig. 1. Location of the study area. The study sites were selected from northern, central, and southern regions, all located along the 127° longitude. Red dots indicate breeding sites, while blue dots indicate non-breeding sites. A: Northern (Paju), B: Central (Cheongju), C: Southern (Gwangyang).

ables consisted of 7 variables collected in the field: water temperature (Wt), pH (P), dissolved oxygen (Do), the average depth of the water 1 m from the shoreline (Dp), slope (Sl), elevation (El), and the average vegetation cover of the shoreline (Vc). The measurement method involved selecting four points in the east, west, south, and north of each reservoir based on true north and measuring the values at each point. First, water temperature, pH, and dissolved oxygen were measured using a water quality meter (86031 AZ EB; AZ Instrument Corp.; Taiwan) and by scooping water samples into a small box at each point. The slope and elevation at points approximately 1 m above the ground were recorded using a GPS receiver (GPSMAP 64s; Garmin; Switzerland). The average water depth was measured using a plastic ruler 1 m from the shoreline. The average vegetation cover was assessed by placing a 2 m × 2 m grid around the measured water depth and taking photographs. The photographs were then evaluated by two researchers, including the observer, to calculate the average cover. Values recorded at each point were averaged and utilized as variables.

The macro variables consisted of 10 variables (Table 1) and were calculated using ArcMap (ver. 10.7.1; ESRI; USA). The 10 macro variables consisted of reservoir area (Ar), distance to a forest (Df), distance to the water (Dw), distance to a used area (Du), percentage of barren land within a 1km buffer (Pb), percentage of agricultural land within a 1km buffer (Pa), percentage of forest within a 1km buffer (Pf), percentage of wetland and water within a 1km buffer (Pw), percentage of used area within a 1km buffer (Pu), and percentage of grass within a 1km buffer (Pg). First, the coordinates of the reservoirs were collected using Google Earth (ver. Pro; Google; USA). Then, using the Environmental Geographic Information Services, maps containing reservoirs were identified, and the land cover maps were downloaded at a scale of 1:5,000 (EGIS, 2019). Considering the field survey period, maps from 2019 that utilized the primary classification items were used. The classification consisted of 7 items, which were barren land, such as sand or gravel; agricultural lands, such as rice paddies and agricultural fields; forests, such as broadleaf and coniferous forests; used areas, including res-

Table 1. Summary of the 17 recorded variables categorized into micro variables (1 to 7) and macro variables (8 to 17). All micro variables were collected directly from the field, while the macro variables were calculated using the ArcMap.

No.	Variable (unit)	Acronym	Breeding site	Non-breeding site
	Micro			
1	Water temperature (°C)	Wt	20.9 ± 3.0 (13.6–29.9)	20.4 ± 3.8 (13.8–29.5)
2	pH	P	8.3 ± 1.1 (6.2–10.6)	8.1 ± 1.1 (5.9–10.1)
3	Dissolved Oxygen (ppm)	Do	9.4 ± 2.3 (4.1–15.6)	8.5 ± 2.8 (1.7–17.3)
4	Average water depth of 1 m from the shoreline (cm)	Dp	48.0 ± 17.6 (5–88.8)	57.0 ± 18.7 (13.25–133)
5	Slope (°)	Sl	26.2 ± 11.5 (6.0–90)	27.5 ± 13.2 (5.3–90)
6	Elevation (m)	El	101.4 ± 89.2 (5.0–383.8)	83.3 ± 60.9 (0.7–350)
7	Average vegetation cover of shoreline (%)	Vc	30.4 ± 30.5 (0–100)	28.3 ± 32.3 (0–100)
	Macro			
8	Distance to forest (m)	Df	18.8 ± 40.7 (0–228.2)	33.8 ± 51.4 (0–249.6)
9	Distance to water (m)	Dw	181.1 ± 250.9 (0–1,153.9)	192.4 ± 275.4 (0–1,339)
10	Distance to used area (m)	Du	17.5 ± 31.3 (0–147.8)	17.7 ± 39.1 (0–241.3)
11	Reservoir area (m^2)	Ar	5,325.7 ± 7,219.8 (144.8–35,897.5)	11,289.6 ± 40276.2 (104.6–276,599.7)
	Percentage within a 1km buffer			
12	Barren (%)	Pb	3.6 ± 2.7 (0.3–11.9)	4.7 ± 3.6 (0.5–22.7)
13	Agricultural land (%)	Pa	19.8 ± 11.5 (1.6–55)	22.4 ± 13.9 (1.0–58.7)
14	Forest (%)	Pf	50 ± 18.3 (6.6–84.5)	46.2 ± 20.5 (6.9–95.9)
15	Water and wetland (%)	Pw	3.9 ± 6.4 (0.1–28.7)	2.4 ± 2.7 (0.3–12.2)
16	Used area (%)	Pu	8.8 ± 7.4 (1.3–37.7)	9.5 ± 6.3 (0.3–29.6)
17	Grass (%)	Pg	13.9 ± 5.0 (3.5–27)	14.9 ± 6.0 (1.5–37.9)

idential and industrial areas; grasslands (grass with a low proportion of trees); wetlands (consistently saturated and where water accumulates during the rainy season); and water, such as rivers and banks. However, in this study, due to the low proportions of wetlands and water (lower than 1%), they were merged into one variable: water. The reservoir shapes were extracted based on the coordinates, and the reservoir areas were calculated using these shapes. Using the 'Find Nearest' function, the distances between the reservoirs and the nearest forest, water, and used areas were calculated. To determine the surrounding landscapes of the reservoirs, the 'Buffer' and 'Intersect' functions were utilized to extract land use areas from each reservoir. The buffer range was set to 1 km, considering the maximum distance between the breeding site and the microhabitats of *B. gargarizans* within their home range (Park et al., 2021; 2024; 2025). Since the reservoir sizes varied, the areas within the 1 km buffer were converted into ratios. Based on these area ratios, the percentages of surrounding barren land, agricultural land, forest, water (including wetlands and water), used areas, and grasslands were calculated.

Statistical analysis

All statistical analyses were conducted using SPSS (ver. 20.0; IBM; USA). All variables did not exhibit normality in a Kolmogorov-Smirnov test ($p < 0.05$). Since no significant correlation (Spearman correlation; $r > 0.8$, $p > 0.05$) was observed between the variables, and no variable had a correlation coefficient above 0.8, all 17 variables were included in the analysis.

Modeling was conducted using a Generalized Linear Model (binomial logistic model), with breeding site presence as the dependent variable (1 = breeding site, 0 = non-breeding site). Model selection was based on the Akaike Information Criterion (AIC), a widely used method for comparing and evaluating habitat suitability models (Burnham and Anderson, 2004; Hu, 2007). The AIC approach requires species presence-absence data along with habitat information (MacKenzie and Bailey, 2004; Durso et al., 2011), allowing for the estimation of suitable breeding habitats. Following Ra et al. (2010), an a priori model was developed by selecting variables based on their ecological relevance and insights gained from field surveys. While testing all possible variable combinations would provide a more exhaustive evaluation, this approach was impractical due to computational constraints and the risk of model instability (Johnson and Omland, 2004; Diniz-Filho et al., 2008). Instead, ecologically meaningful combinations were prioritized based on previous research and field observations. To systematically assess the influence of different variable types, the model selection was structured

Table 2. List of the 30 models. Models 1–10 consist of only micro variables, while models 11–20 consist of only macro variables. Models 21–29 consist of combinations of micro and macro variables, and Model 30 contains all 17 variables.

Model No.	Variable
Micro variable combination	
1	Do, Sl, El, Vc
2	Do, Dp, El, Vc
3	Wt, P, Dp
4	P, Dp, El, Vc
5	Wt, Do, Dp, Vc
6	Wt, Do, Sl, El, Vc
7	Wt, Sl, El, Vc
8	Dp, Sl, El, Vc
9	Wt, P, Do, Dp, El, Vc
10	Wt, P, Do, Dp, Sl, El, Vc
Macro variable combination	
11	Df, Pf, Pg
12	Dw, Ar, Pa, Pw
13	Du, Pb, Pu
14	Df, Ar, Pb, Pw, Pg
15	Df, Pb, Pa, Pg
16	Df, Dw, Du, Pf, Pu, Pg
17	Df, Dw, Ar, Pb, Pa, Pf, Pw, Pg
18	Ar, Pb, Pw, Pu
19	Du, Ar, Pb, Pa, Pu, Pg
20	Df, Dw, Du, Ar, Pb, Pa, Pf, Pw, Pu, Pg
All variable combination	
21	Do, Dp, Pb, Pa, Pg
22	Do, Dp, Pb, Pf, Pw
23	Dp, Sl, Df, Du, Ar, Pa, Pf, Pw
24	Wt, P, Sl, Pb
25	Wt, Do, Dp, Ar, Pb, Pw
26	El, Vc, Df, Pf, Pg
27	Do, Sl, El, Df, Pf
28	Dp, El, Vc, Df, Pa, Pf, Pg
29	Do, Dp, Df, Pg
Global	
30	Wt, P, Do, Dp, Sl, El, Vc, Df, Dw, Du, Ar, Pb, Pa, Pf, Pw, Pu, Pg

into three groups: (1) 10 models using only microhabitat variables, (2) 10 models using only macrohabitat variables, and (3) 9 models incorporating both. Additionally, a global model including all variables was tested, resulting in 30 model combinations (Table 2). This approach balanced model complexity and ecological interpretability while minimizing the risk of overfitting.

Considering the small sample size, the corrected Akaike Information Criterion (AICc) values were used in this study. Generally, the relative likelihood in an AIC model is proportional to the probability of minimizing the information loss for each model (Burnham and Anderson, 2004). The relative likelihood was calculated using the formula: $\exp\left({}^{(AICc_{min}-AICc_i)}/_{2}\right)$

The calculated values were then used to confirm the model's explanatory power as weights (*wi*), ensuring the reliability of the selected suitable model. The selected suitable model evaluated the impact on the breeding sites by assessing the significance of each variable.

RESULTS

The survey included 143 reservoirs: 21 in Gwangyang in 2017, 60 in Cheongju in 2018, and 62 in Paju in 2019. Among these, breeding sites were identified in 13 reservoirs in Gwangyang, 20 in Cheongju, and 23 in Paju, totaling 57 breeding sites. For the analysis, modeling was conducted using 123 sites (46 breeding sites, 77 non-breeding sites); the modeling excluded 20 sites in Paju (10 breeding sites, 10 non-breeding sites) that did not have land cover maps.

Among the breeding site models, Model 25 exhibited the highest explanatory power (*wi*) of 0.6464, and included six variables (Table 3): water temperature (Wt), dissolved oxygen (Do), the average water depth 1 m from the shoreline (Dp), reservoir area (Ar), the percentage of barren land within a 1 km buffer (Pb), and the percentage of water within a 1 km buffer (Pw). Among the variables included in this model, three were significant. The percentage of water within a 1km buffer (Pw) had a positive correlation. In comparison, the average water depth 1 m from the shoreline (Dp) and the percentage of barren land within a 1km buffer (Pb) had negative correlations (Table 4). The average water depth 1 m from the shoreline of breeding sites was 47.9 ± 17.6 cm, and 57.0 ± 18.7 cm for non-breeding sites. The percentage of barren land within a 1km buffer of breeding sites was 3.6 ± 2.7%, and 4.7 ± 3.6% for non-breeding sites. The percentage of water within a 1km buffer of breeding sites was 3.9 ± 6.4%, and 2.4 ± 2.7% for non-breeding sites.

DISCUSSION

This study explored the suitable environmental conditions using a relatively simple model for *B. gargarizans* breeding sites. Based on a GLM, the model provides valuable information on the major environmental predictors affecting the selection of the breeding sites of *B. gargarizans*. Among the variables showing significant differences, the average water depth 1 m from the shore-

Table 3. Model selection results for breeding site suitability of *Bufo gargarizans*, including model number, variables used, number of parameters (k), AICc, ΔAICc, and model weights (*wi*). The models are ranked in order of their explanatory power, with lower AICc values indicating a better model fit.

Model No.	Variable list	k	AICc	ΔAICc	*wi*
25	Wt, Do, Dp, Ar, Pb, Pw	8	152.797	0	0.6464
22	Do, Dp, Pb, Pf, Pw	7	155.671	2.87	0.1536
29	Do, Dp, Df, Pg	6	158.423	5.63	0.0387
2	Do, Dp, EL, Vc	6	158.659	5.86	0.0345
4	P, Dp, El, Vc	6	158.726	5.93	0.0334
21	Do, Dp, Pb, Pa, Pg	7	159.344	6.55	0.0245
5	Wt, Do, Dp, Vc	6	159.437	6.64	0.0234
3	Wt, P, Dp	5	159.734	6.94	0.0201
18	Ar, Pb, Pw, Pu	6	160.164	7.37	0.0163
9	Wt, P, Do, Dp, El, Vc	8	161.313	8.52	0.0091

Table 4. The significance of the variables included in Model 25. The table presents the estimated coefficients (B), standard errors (SE), Wald Chi-Square values, and significance levels (Sig) from the binomial logistic regression analysis. The significant variables include average water depth 1 m from the shoreline (Dp), the percentage of barren land within a 1 km buffer (Pb), and the percentage of water within a 1 km buffer (Pw).

Variable	B	SE	Wald Chi-Square	Sig
intercept	1	1.6407	0.418	0.518
Wt	0.179	0.0617	1.620	0.203
Do	1.144	0.0795	3.263	0.071
Dp	**-0.035**	**0.0129**	**7.210**	**0.007**
Ar	-1.496	1.4142	1.080	0.299
Pb	**-0.203**	**0.0915**	**4.921**	**0.027**
Pw	**0.118**	**0.0525**	**5.091**	**0.024**

line (Dp) was considered an important factor. Since *B. gargarizans* are known to breed at the shoreline of reservoirs (Lee et al., 2011), it is important to maintain suitable shoreline depths (Jeong, 2017) and permanent waterbodies (Evans et al., 1996). Furthermore, suitable water depths are necessary to ensure the stable occurrence of eggs and larvae (Edgerly et al., 1998; Murphy, 2003b; Rudolf and Rödel, 2005) since lower water depths have lower temperatures, which can negatively affect breeding. In this study, the average shoreline water depth at non-breeding sites was relatively deep at 57 cm, nearly twice the 30 cm reported in a previous study (Jeong, 2017). Therefore, a water depth of 30–48 cm is a suitable shoreline water depth for *B. gargarizans* breeding sites.

The results of this study indicate that a percentage of barren land within a 1km buffer (Pb) lower than 3% and a percentage of water within a 1km buffer (Pw) higher than 3% are suitable for *B. gargarizans* breeding. *B. gargarizans* typically inhabit areas with vegetation cover or grasslands (Yu and Guo, 2010; Su et al., 2020; Park et al., 2024; Park et al., 2025), and are known to use reservoirs, ponds, rice paddies and rice paddy canals as breeding sites (Lee et al., 2011). Bufonids have thicker skin than other frog families (Lee, 2003) and are more resistant to drying out. However, they still prefer habitats with high humidity to retain moisture (Su et al., 2020). Accordingly, the amount of barren land surrounding the breeding sites was relatively low. Additionally, the percentage of water within a 1km buffer (Pw) of breeding sites was relatively high compared with the non-breeding sites. In the post-breeding period, *B. gargarizans* migrate from breeding sites (reservoirs) to the mountains (Park et al., 2021; 2024). While anurans of the family Bufonidae are known for their drought tolerance (Lee, 2003), maintaining access to water sources is crucial due to the high energy and moisture expenditure required for movement and dispersal (Yu et al., 2009; Luo et al., 2014; 2015). Therefore, additional water bodies near breeding sites likely play a vital role in the survival of metamorphosed tadpoles as they disperse into the forests.

Among the six variables included in the best-suitable model, dissolved oxygen (Do) had a p-value of 0.071, indicating marginal significance. However, it appeared in seven of the top ten models and was statistically significant ($P < 0.05$) in four of them (models 29, 2, 21, and 5), suggesting potential ecological relevance. The typical dissolved oxygen range in water is 7–10 ppm. In this study, dissolved oxygen levels were 9.4 ± 2.3 ppm in breeding sites and 8.5 ± 2.8 ppm for non-breeding sites, although the mean comparison between the two site types did not reach statistical significance (T-test, $t = 1.875$, $P = 0.063$).Previous studies have shown that toads prefer sites with higher dissolved oxygen levels (Noland and Ultsch, 1981; Semeniuk et al., 2007). While dissolved oxygen was included in 7 of the top 10 models, no significant differences were found between breeding and non-breeding sites. However, it remains an important environmental factor influencing breeding site selection. Dissolved oxygen levels can be affected by factors such as water flow, water depth, water temperature, and aquatic vegetation, which could be explored further in future studies.

The results of this study provide meaningful implications for the conservation and ecological management of *B. gargarizans* breeding habitats. Key factors such as shoreline water depth, the percentage of barren land surrounding the breeding sites, and nearby water bodies suggest practical strategies for breeding site restoration. For instance, altering artificial reservoirs with overly steep or deep shorelines to include shallower zones with depths around 48 cm may enhance breeding habitat suitability. Furthermore, improving dissolved oxygen concentrations could contribute to improving breeding site quality (Semeniuk et al., 2007). The presence of nearby water bodies for post-breeding dispersal further highlights the necessity of landscape-level conservation efforts. Preserving small wetlands and canals may facilitate metamorphism juvenile movement and connect habitats (Yu et al., 2009; Luo et al., 2014).

Nevertheless, considering the overlapping breeding periods with other amphibian species (*Hynobius* spp., *Rana dybowskii*, *R. huanrenensis*, and *R. coreana*; Lee et al., 2011), potential ecological interactions and impacts should be carefully evaluated. Moreover, *B. gargarizans* in South Korea primarily utilizes artificial reservoirs for breeding, but the effects on other organisms, including amphibians, are unknown. Future research should investigate whether these anthropogenic habitats confer ecological advantages compared with their natural breeding sites, which may, in turn, influence long-term population dynamics.

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Behavioral repertoire of the Madeiran wall lizard, *Teira dugesii* (Sauria: Lacertidae) in wildlife conditions

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Abstract. The behavioral repertoire of the Madeiran wall lizard, *Teira dugesii*, was compiled from approximately 70 hours of direct field observations conducted between July 2022 and August 2024 across seven locations along the south coast of Madeira Island. A total of 73 distinct behaviors were recorded and categorized into five groups: maintenance, aggression and defense, feeding, mobility, and reproduction. Observations took place during the species' daily activity period (09:00–15:00), primarily during the summer months. Maintenance behaviors accounted for the majority of records (86.89%), followed by mobility (6.95%), aggression and defense (3.92%), feeding (1.83%), and reproduction (0.41%). These findings provide new insights into the diverse and complex behavioral repertoire of *T. dugesii* under natural conditions.

Keywords. Behavior, Madeira Island, Lacertidae, ethogram, Macaronesia.

INTRODUCTION

Diurnal lizards serve as exceptional models for studying behavioral evolution due to the broad spectrum of traits exhibited across different taxa, populations, and species (Langkilde et al., 2003; Poma-Soto et al., 2021). In behavioral research, compiling ethograms is a crucial step in systematically documenting and classifying a species' behavioral repertoire (Martin and Bateson, 2021). An ethogram is defined as a catalog of species-specific behaviors, encompassing descriptions, classifications, and habits that constitute the fundamental behavioral repertoire of a species (Martin and Bateson, 1994; Qi et al., 2011; Mather, 2025). Since behavioral patterns can vary significantly among individuals (Ruby and Niblick, 1994), constructing an ethogram is essential for identifying similarities and differences in behavior, thereby facilitating the formulation of novel research questions (Liu et al., 2009; Qi et al., 2011). Typically, ethograms provide a structured list of observed behaviors, each accompanied by a precise definition using descriptive terminology (Martin and Bateson, 2007). Ethograms offer valuable insights into a species' life history and are particularly useful in studies focusing on courtship, intrasexual competition, and foraging behavior (Coelho et al., 2018). While behavioral research in captivity can yield important data, it often presents challenges due to the artificial nature of the environment, which may lead to stress-induced alterations in behavior (Carlstead, 1996). Whenever possible, observations should be conducted in natural settings to ensure ecological validity (Mather, 2025).

Teira dugesii (Milne-Edwards, 1829) is an endemic lacertid lizard of the Madeira Archipelago and Selvagens Islands (Jesus et al., 2009). Over time, it has been intro-

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duced to the Azores Archipelago (Ulfstrand, 1961), Gran Canaria Island (Santos et al., 2015), and in two cities of mainland Portugal, such as Lisbon (Sá-Sousa, 1995), and Porto (Ferreira et al., 2023). In its native distribution range, this species thrives in diverse habitats, from sea level up to elevations of 1,861 meters. It adapts to various substrates and degrees of sun exposure, although it is less common in shaded environments (Jesus et al., 2009). Currently, four subspecies are recognized: *Teira dugesii dugesii* (Madeira Island), *Teira dugesii jogeri* (Porto Santo Island), *Teira dugesii selvagensis* (Selvagens Islands), and *Teira dugesii mauli* (Desertas Islands) (Brehm et al., 2003; Jesus et al., 2009).

Although *Teira dugesii* is a well-known component of Madeira's terrestrial fauna, behavioral studies on this species remain relatively scarce and fragmented. Nonetheless, some aspects of its behavior have been documented. These include arboreal activity (Petrovan, 2023), instances of predation on *T. dugesii* by other animals (Câmara and Jesus, 2025a), as well as predation by *T. dugesii* itself on various invertebrate and vertebrate prey (Matias et al., 2009). Reproductive behavior has also been addressed, although primarily under laboratory or captive conditions (Sleijpen, 1996; Galán, 2003).

MATERIAL AND METHODS

Selection of observation sites and sampling periods

A total of approximately 70 hours of behavioral observations and video recordings of *Teira dugesii* were conducted between July 2022 and August 2024. Observations were distributed across four sampling periods and carried out under natural field conditions, without any experimental manipulation of temperature or other environmental variables. The first sampling period, conducted between July and September 2022, comprised approximately 36 hours of observations. Sessions were held three times per week and focused on the hours of peak activity, specifically between 10:00–12:00 and 13:00–15:00. A second period took place from 1 to 25 October 2022, during which around 10 hours of data were collected. Recordings occurred three times per week, exclusively on sunny days, between 10:00 and 12:00. Additional observations were carried out over seven days in early January 2024, yielding approximately 12 hours of data collected between 11:00 and 13:00. The final sampling period occurred between 27 July and 5 August 2024, during which a further 12 hours of observations were conducted between 11:00 and 12:30.

Behavioral observations were conducted at seven distinct locations (Table 1, Fig. 1) along the southern coast of Madeira, a region chosen for its warmer and more stable climatic conditions throughout the year, which allow for increased lizard activity and more frequent observations compared to cooler, wetter areas or dense laurel forests (Laurissilva) in the north, where *Teira dugesii* is rare or difficult to detect. In selecting these sites, we aimed to maintain a minimum distance of approximately 1 km between them to minimize the likelihood of repeatedly observing the same individuals and to increase spatial representativeness. Observations were primarily focused on substrates frequently used by *T. dugesii*, including stone walls, tree trunks, and rocky surfaces. Although no detailed studies currently exist on the species' microhabitat preferences, this approach was based on direct field observations and aimed to ensure a broad and representative sampling of behavioral patterns across a variety of

Table 1. List of the seven sites in Madeira Island where behavioral observations of *Teira dugesii* were conducted between 2022 and 2024.

Location	Coordinates	Human Pressure
Rua Nova da Igreja, Funchal	32°38′55″N, 16°53′4″W	Low
Lido, Funchal	32°38′11″N, 16°56′4″W	High
Jardim Botânico, Funchal	32°39′46″N, 16°53′42″W	High
Campanário, Ribeira Brava		Medium
Miradouro D. Sebastião, Ribeira Brava	32°40′12″N, 17°3′46″W	Low/Medium
Prazeres, Calheta	32°45′15″N, 17°12′9″W	Low/Medium
Campus Penteada, Funchal	32°39′33″N, 16°55′25″W	Medium

Fig. 1. Locations of the video recording of the behaviors of *Teira dugesii* on Madeira Island.

natural, semi-natural, and anthropogenic environments. Human presence at each site was qualitatively classified into three categories: "low", "medium", and "high". Categorization was based on direct field observations during behavioral sessions, with "low" referring to areas with rare or occasional pedestrian activity, typically in quieter residential or rural zones; "medium" included locations with regular but non-disruptive human activity, such as university campuses or suburban viewpoints; "high" was assigned to sites frequently visited by tourists or the general public, such as botanical gardens and coastal recreational areas, where constant human movement was observed throughout the day.

Sampling methods

A Nikon D3500 and a Nikon P1000 were used for video recordings and photography. Both devices allow simultaneous video and photo capture, enabling the collection of still images during active recording sessions. No tripod was used, which allowed the observer to follow rapid movements more effectively in the field. Most of the video material was recorded using the Nikon P1000, which provided higher optical zoom and flexibility in frame rate and resolution settings. The Nikon D3500 was used for only a small portion of recordings, representing approximately one hour of total footage. Recordings were conducted across all seasons, with the majority of observations and video sessions taking place during the summer months. In addition to video and photographic documentation, field notes and sketches from a notebook were also incorporated into the dataset.

To document behavioral patterns, we employed two sampling methods: (1) ad libitum sampling, which allowed for the recording of spontaneous and infrequent behaviors, and (2) focal animal sampling, which provided a structured approach to observing specific individuals (Martin and Bateson, 1994). The duration of each focal session was variable, as observations continued either through recording or field notes until the focal individual left the area or was no longer visible. The combination of these methods ensured both broad behavioral coverage and detailed individual-level data.

To prevent behavioral alterations, a minimum observation distance of approximately 5 meters was maintained during all sessions. Preliminary trials revealed that closer proximity (less than 5 meters) consistently caused *Teira dugesii* individuals to either flee or adopt a state of alert immobility, thus compromising the reliability of behavioral recordings. It is important to note, however, that in areas with high human presence, such as the Madeira Botanical Garden and coastal lidos, individuals exhibited significantly reduced wariness. In these settings, lizards often continued their natural movements without displaying typical alert or escape behaviors in response to human observers, likely due to habituation. A similar distance was used by Molina-Borja (1987) in a behavioral study of *Gallotia galloti.*

Sex differentiation

Sex differentiation followed Jesus et al. (2009), who described adult males as generally larger, with a broader head and tail base. In contrast, females and young males exhibit similar external features, making them difficult to distinguish in the field. Additionally, individuals show variable color patterns (Báez, 1990). Dorsal coloration may be uniform or divided by longitudinal stripes, with background hues ranging from light brown to black. On Madeira Island, females, juveniles, and subadults typically exhibit light longitudinal stripes, whereas adult males tend to display greater variation in dorsal patterns. Although generally more uniform, females also present some degree of variation. Ventral coloration may be uniform or mottled with spots of various colors, with background tones ranging from cream, yellowish-green, blue, orange, and brown to black (Báez, 1990).

Behavioral categories

The behaviors of *Teira dugesii* were categorized into five main groups based on Molina Borja (1981, 1987). (1) Maintenance: elementary postures and movements, as well as different types of orientation behaviors related to body positioning and environmental awareness; (2) Mobility: locomotor patterns exhibited by individuals, including walking, running, climbing, and other forms of displacement; (3) Feeding: all actions related to foraging, prey capture, and food manipulation; (4) Aggression and defense: various orientations, such as direct confrontations and visual displays, as well as complex postures and movements, which may involve gular inflation, tail displays, or other intimidation tactics used in intra- or interspecific interactions; (5) Reproduction: courtship displays, mating postures, and any observed interactions between males and females related to reproduction.

Data analysis

Video footage was systematically reviewed after fieldwork, with behavioral events manually identified and categorized. The data were then summarized as absolute counts and/or percentages of total recorded events. All video footage was reviewed and classified by a single observer (J.C.), which ensured internal consistency in behavioral identification and reduced the risk of inter-observer variability. In cases of uncertainty, video segments were reviewed multiple times and cross-referenced with field notes for clarification. No automated software or external observers were involved in the identification process, allowing for a flexible, case-by-case approach to ambiguous behaviors. Field notes were used to complement the classification process, ensuring accurate interpretation of behaviors. Each behavioral event was assigned to a single category, such as foraging, basking, locomotion, or social interaction. Although the categories were generally mutually exclusive, more complex behaviors occasionally involved overlapping or sequential components. In such cases, a hierarchical rule was applied to assign the event to the most dominant or functionally relevant category. For example, if an individual was moving while visually scanning the ground, the behavior was only classified as foraging if active prey-searching was clearly observed, and not simultaneously as both locomotion and foraging. This approach ensured that no behaviors were double-counted. Once assigned, behaviors were excluded from any other potentially overlapping categories.

The values presented in the results were obtained by counting the number of occurrences of each behavior throughout the analyzed footage. In some cases, the duration of behaviors was also recorded (e.g., time spent basking).

RESULTS

Observation context

The site with the highest number of observation hours was Rua Nova da Igreja, a quiet residential area with minimal pedestrian traffic during observation periods. The main microhabitat consisted of a stone wall approximately two meters high and several meters long, characterized by high sun exposure and the presence of ornamental potted plants. In contrast, sites such as botanical gardens experienced occasional tourist presence, and observations there were mostly restricted to vertical structures such as stone walls or tree trunks. A minimum of approximately 100 distinct individuals is estimated to have been observed throughout the study. Observed behaviors are illustrated in Table 2, and their frequencies are in Table 3.

Maintenance behaviors

Maintenance behaviors represented 86.81% of all recorded observations (3414 of 3929), highlighting their central role in the daily activity budget of *Teira dugesii*. The most frequently recorded postures included *head-up* (767 observations), *body-down* (636), *head-down* (465), and *body-up* (113). These behaviors were evenly distributed throughout the observation periods, with no marked concentration at specific times of day, suggesting a balanced expression of maintenance activities over the diurnal cycle.

In addition to postural behaviors, *basking* (516 observations, in which the lizard flattens its body against the substrate), was also commonly observed. Other thermoregulatory postures included *foot-up* (221), *toes-up* (111), and *mouth-open* (19), which may facilitate heat absorption and exchange. These behaviors occurred most frequently on sunlit stone walls, tree trunks, and rocky surfaces.

Self-directed maintenance behaviors, although less frequent, were also recorded. These included *grooming* (4 observations), *scratching* (3), *body contortion* (40), *mouth scraping* (3), and *palateo* (18) (Table 3) – a *tongue flick* behavior potentially linked to chemoreception or hydration.

Overall, maintenance behaviors overwhelmingly dominated the activity budget of *T. dugesii*, contrasting with the markedly lower frequencies of mobility (6.95%), feeding (1.83%), aggression and defense (3.92%), and reproductive behaviors (0.41%).

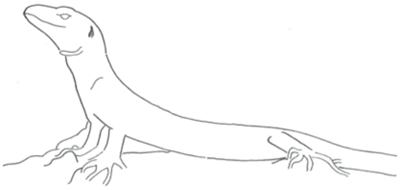
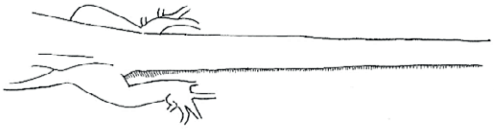
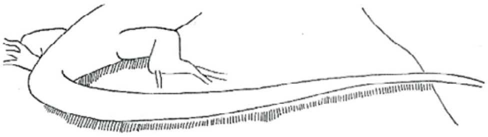
Mobility behaviors

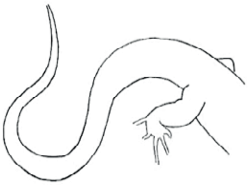
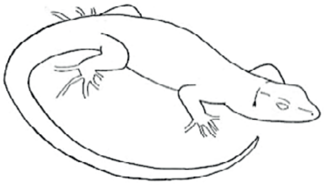
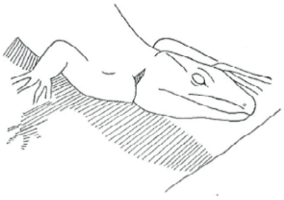
Mobility behaviors represented 6.95% of all recorded observations (273 of 3929). The most frequent behavior within this category was *walking*, with 152 records (55.68%), followed by *running* (65; 23.81%), *jumping* (20; 7.33%), and *foraging* (15; 5.49%). Less frequent actions included *arboreal locomotion* (19; 6.96%) and *move-over* (2; 0.73%) (Table 3).

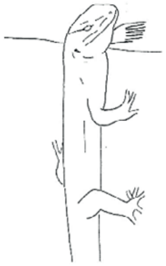
Feeding behaviors

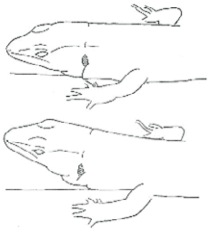
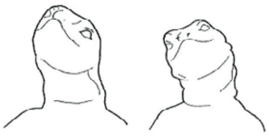
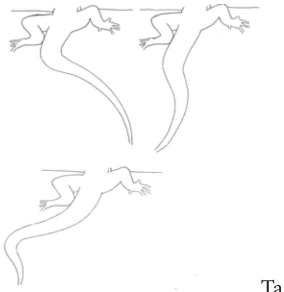
Feeding behaviors represented 1.83% of all recorded observations. The most frequently observed actions were *tongue touch* (27; 37.50%), followed by *lick* (16; 22.22%), *food-licking* (7; 9.72%), *mandible lick* (7; 9.72%), and *drinking* (7; 9.72%). Less frequent behaviors included *food-bite*, *chew*, *food-shaking*, and *defecation*, each recorded twice (2.78%) (Table 3). Feeding events involved the capture of arthropods and the consumption of *Hibiscus*

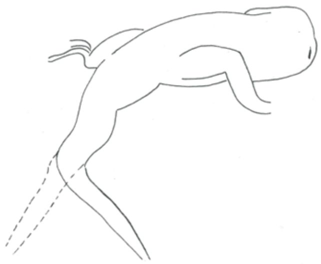
Table 2. Behavioral Description of *Teira dugesii* (Terminology is based on Carpenter Ferguson (1977) and Molina-Borja (1981)).

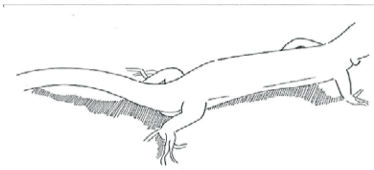
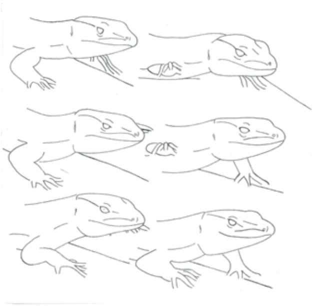
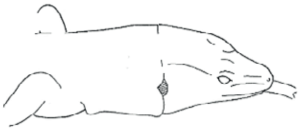
Behavior category	Behaviors	Description
Maintenance	 Head-Down	Head resting on the substrate.
	 Head-Up	Lower surface of the head detached from the substrate.
	 Body-Up	The front and back ends elevate the animal's body to different degrees.
	 Body-Down	Ventral region supported by substrates.
	 Anterior Body-Up	Posterior ends resting on the substrate while the anterior ends raise the anterior part of the animal's body in different degrees.
	 Tail-Low	Tail resting, in all its extension, on the substrate.
	 Tail-Up	Tail raised in varying degrees from the substrate.

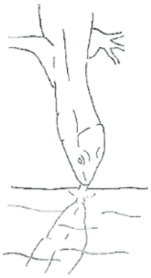
Behavior category	Behaviors	Description
	 Tail-Up-At-Base	Tail raised at the base while the distal part remains on the substrate. The tail in this position forms an arch.
	 Tail-Up-At-Tip	Tail raised at the distal part, while de base part remains on the substrate.
	 S-Tail	Tail on the ground, bent in a S shape.
	 C-Tail	Tail on the ground, bent in a C shape.
	 Foot-Up	One or more feet rise above the substrate or all four of them.
	 Toes-Up	The toes of one or more feet rise above the substrate.
	 Mouth-Open	Opening in varying degrees of the mouth.

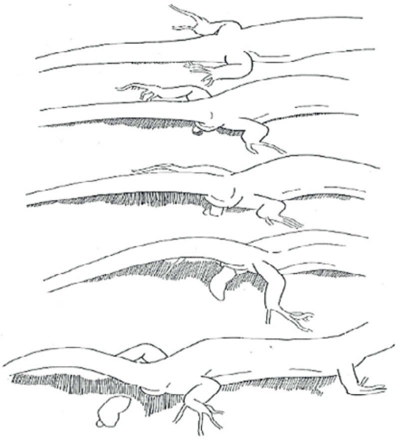
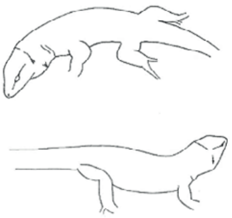
Behavior category	Behaviors	Description
	 Lie-on	A lizard rests part of its body on another animal and maintains that position.
	 Quiet/rest	Body extended against substrate; head slightly elevated.
	 Eyes closed	The eyelids close over the eyes.
	 Sleep	Eyes closed, body in all its extension resting on the substratum, head down.
	 Oblique-Orientation	Oblique position of the lizard through the support of its forelimbs on a protruding part of the substrate.
	 Extreme Oblique Orientation	The lizard clings to irregularities in the substrate, remaining almost vertical.
	 Adpress	One or more limbs are raised off the substrate and held against the lateral side of the body.
	 Bask	The body is flattened onto the substrate and oriented at right angles to a heat source.

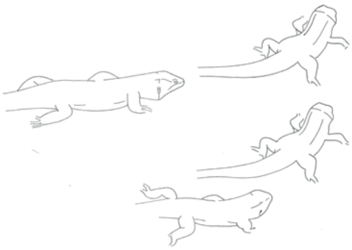
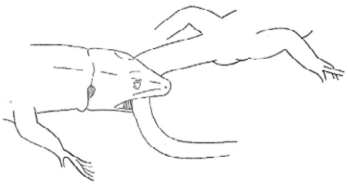
Behavior category	Behaviors	Description
	 Head-to-side	The lizard keeps its head slightly raised at an angle of 90º to its body, keeping it resting on the substrate for its entire length. The head can stand still pointing either to the left or to the right.
	 C-Body	The lizard stands still in a C-shape.
	 S-Body	The lizard stands still in an S-shape due to the irregularities of the substrate.
	 Head Up-Down	The lizard moves its head up and down along a vertical axis.
	 Head rotation	Movement in which one side of the head is oriented upwards and the other downwards.
	 Tail-Lash	Side-to-side undulation of the tail.

Behavior category	Behaviors	Description
	 Tail-Twitch	Rapid movement of the distal part of the tail.
	 Contortion of the body	Undulating movement of the body.
	 Mouth-Scrape	The lizard scratches one side of its mouth on the substrate, and slightly lifts its body on the opposite side of the scratched part.
	 Scratch	The Body is scratched by one of the hind limbs.
	 Groom	A lizard bit or licks its own body.
	 Digging	The lizard moves its head towards the substrate and starts moving its front legs from front to back, in a repetitive movement.

Behavior category	Behaviors	Description
	 Cloaca-Drag	With the body and tail - slightly Up, the lizard drags the cloaca over the substrate.
	 “Palateo”	The lizard in “head up” position strikes the substratum with one (or both) front paws in a repetitive, short and fast movement.
Mobility behaviors	Jumping	The lizard jumps from one place to another, using its rear legs to propel its body forward.
	Walking	Body slightly raised, tail down, forward movements. During the forward movement, one of the forelegs and the contralateral rear leg are moved simultaneously, followed by the corresponding symmetrical legs. As a result, the trunk of the animal shows an undulating movement (contortion of the body motion).
	Run	Fast forward movement, with body and tail raised also with contortion of the body motion.
	Forage	Foraging behaviour was distinguished from general locomotion based on consistent behavioural cues. Lizards identified as foraging paused frequently, often in vegetated or debris-rich areas, and exhibited repeated tongue-flicking, as well as marked lateral or vertical head movements indicative of active environmental scanning. In contrast, individuals engaged in simple locomotion typically moved in a more continuous manner, with little or no tongue-flicking and without pronounced head movement. These criteria allowed us to differentiate purposeful foraging from mere traversal of the habitat.
	Move-over	One lizard moves over the top of another.
	Arboreal	The arboreal movement refers to the displacement of animals through trees.
Feeding behavior	 Tongue Touch	The tongue briefly touches the substrate, water, or other animal.
	 Food-Licking	Repeated projections of a lizard’s tongue towards food.

Behavior category	Behaviors	Description
	 Lick	Repeated projections of a lizard's tongue.
	 Mandible lick	Displacement of the animal's tongue over its mandible.
	 Food-Bite	The lizard opens its mouth and grabs food with its mandible.
	 Drinking	Repetitive projection and retraction movements of the tongue over or in a liquid.
	 Chew	The lizard repeatedly moves the mandible up and down, mandibulation.
	 Food-shaking	A food item is shaken by side-to-side motion of the head.

Behavior category	Behaviors	Description
	 Defecation	The lizard assumes the Tail-Up-At-Base posture, progressively arching the tail until expelling the faecal material and producing the Cloaca-Drag movement. After a few steps, the lizard lowers its tail.
Aggression and defence behaviors	 Threat position	The lizard assumes the Body-Up posture, bends its head downwards, arches its back, and produces the Gular movement.
	 Gular movement	Expansion of the throat region.
	 Lateral Threat Orientation	Lateral orientation of one lizard relative to another during aggressive encounters.
	 Perpendicular Threat Orientation	Perpendicular orientation of one lizard relative to another during aggressive encounters.
	 Circling	Circular movement of two lizards in lateral threat orientation, with heads facing opposite sides.

Behavior category	Behaviors	Description
	 Lunge	Rapid jumping movement by one lizard toward another.
	 Stalk	A slow approach to a stimulus.
	 Head-Touch	Two lizards touch snouts facing each other.
	 Bite	The lizard opens its mouth and grabs another animal with its mandible.
	 NIP	One lizard grasp another with its jaws and then releases immediately (less than one second later).
	 Tongue Flick	Rapid movement of projection and retraction of the tongue, however, this behavior is also used for reconnaissance.

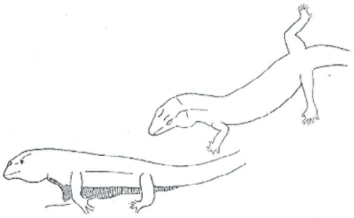
Behavior category	Behaviors	Description
	 Face-OFF	Two male lizards stare at each other.
	 Roll-up	The predated lizard wraps its entire body, including its tail, around the predatory lizard.
	 Retreat	Move rapidly towards the opposite direction of the opponent.
Reproductive behaviors	Pre copulation	The male observes the female and slowly approaches her. Once relatively close, he pauses for a few seconds before suddenly lunging toward her.
	Copulatory bite	The male bites the female's lower trunk region from the side.
	Female copulatory bite	The female bites the male's head or trunk region during copulation.
	Copulation	Following the bite, the male curves his body into a C-shape, aligning his cloaca with that of the female, and wraps his tail around her. He then inserts one of his hemipenes into the female's cloaca.
	Release bite	The male bites the female's cloacal region during copulation.
	Terminating copulation	Copulation ends with the separation of the male's genitalia from the female's cloaca, as the male straightens his body.
	Post copulatory	After withdrawal, the male raises his head and neck, keeping the anterior part of his body elevated. He rests his abdomen on the ground, lifts his cloacal region and the base of the tail off the ground, while the tip of the tail remains in contact with the substrate. The hemipenes are still externally visible.

Table 3. Frequencies of behavioral categories recorded during field observations of *Teira dugesii*.

Behavioral Category	Behavior	Counts	Percentage (%)
Maintenance	Head-Down	465	13.62
	Head-Up	767	22.47
	Body-Up	78	2.28
	Body-Down	636	18.63
	Anterior Body-Up	95	2.78
	Tail-Low	178	5.21
	Tail-Up	6	0.18
	Tail-Up-At-Base	3	0.09
	Tail-Up-At-Tip	2	0.06
	S-Tail	6	0.18
	C-Tail	33	0.97
	Foot-Up	221	6.47
	Toes-Up	111	3.25
	Mouth-Open	19	0.56
	Lie-on	6	0.18
	Quiet/rest	26	0.76
	Eyes closed	24	0.70
	Sleep	2	0.06
	Oblique-Orientation	15	0.44
	Extreme Oblique Orientation	22	0.64
	Adpress	76	2.23
	Bask	446	13.06
	Head-to-side	33	0.97
	C-Body	37	1.08
	S-Body	3	0.09
	Head Up-Down	3	0.09
	Head rotation	3	0.09
	Tail-Lash	19	0.56
	Tail-Twitch	5	0.15
	Contortion of the body	40	1.17
	Mouth-Scrape	3	0.09
	Scratch	3	0.09
	Groom	4	0.12
	Digging	1	0.03
	Cloaca-Drag	5	0.15
	“Palateo”	18	0.53
Total		3414	100
Mobility	Jumping	20	7.33
	Walking	152	55.68
	Run	65	23.81
	Forage	15	5.49
	Move-over	2	0.73
	Arboreal	19	6.96
Total		273	100
Feeding	Tongue Touch	27	37.50
	Food-Licking	7	9.72
	Lick	16	22.22
	Mandible lick	7	9.72
	Food-Bite	2	2.78
	Drinking	7	9.72
	Chew	2	2.78
	Food-shaking	2	2.78
	Defecation	2	2.78
Total		72	100
Aggression/ Defence	Alert	34	22.08
	Threat position	5	3.25
	Lateral Threat Orientation	6	3.90
	Perpendicular Threat Orientation	3	1.95
	Gular movement	5	3.25
	Circling	5	3.25
	Lunge	3	1.95
	Bite	3	1.95
	Stalk	17	11.04
	Head-Touch	3	1.95
	NIP	6	3.90
	Tongue Flick	47	30.52
	Face-OFF	1	0.65
	Roll-up	2	1.30
	Retreat	14	9.09
Total		154	100
Reproductive	Pre copulation	7	43.75
	Copulatory bite	3	18.75
	Female copulatory bite	2	12.50
	Copulation	1	6.25
	Release bite	1	6.25
	Terminating copulation	1	6.25
	Post copulatory	1	6.25
Total		16	100

rosa-sinensis flower petals, as previously documented (Câmara and Jesus, 2025b). Additionally, opportunistic feeding on anthropogenic food sources, such as fruit and biscuit remains, was observed. *Drinking* behavior was recorded at small artificial ponds and from water droplets accumulated on leaves following irrigation events.

Aggressive and defensive behaviors

Aggressive and defensive behaviors represented 3.92% of all recorded observations, occurring primarily during the summer season, coinciding with periods of heightened social interaction and territoriality. A total of 154 events were assigned to this category, with alert posture (34 observations; 22.08%) being the most frequently recorded behavior. This response was typically triggered by the sudden appearance of tourists or the proximity of other conspecifics, particularly adult males and females.

Although six complete fights were documented (four between adults and juveniles and two between adult males), at least nine additional aggressive interactions were interrupted before escalating. Commonly observed behaviors included *threat position* (5 observations; 3.25%), *gular movement* (5; 3.25%), *stalk* (17; 11.04%), *lateral threat orientation* (6; 3.90%), *NIP* (6; 3.90%), and *retreat* (14; 9.09%) (Table 3).

Gular movements were exclusively observed during threat position displays and were used by both juveniles and adult males. In male-male encounters, biting (3 observations; 1.95%) occasionally led to individuals falling to the ground, often resulting in the involuntary release of the opponent and subsequent escape.

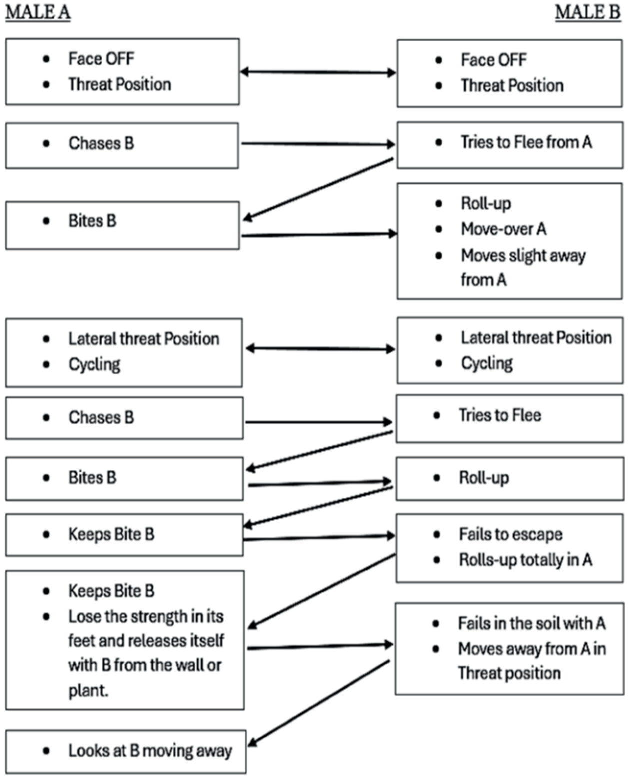


Fig. 2. Diagram of an agonistic sequence between two males.

The aggressive repertoire observed in *Teira dugesii* comprised threat displays, orientation adjustments (lateral and perpendicular threat orientation), body or head movements (circling, lunge, head-touch, face-off), and escape behaviors such as retreat and roll-up. Although diverse, these behaviors were relatively infrequent in the total activity budget. A full male-male confrontation sequence is in Fig. 2 and 3, illustrating the escalation from initial threat to resolution.

Reproductive behaviors

Reproductive behaviors were the least frequently observed, comprising only 0.41% of all recorded behaviors. A total of 16 reproductive events were documented, including one complete mating sequence.

Pre-copulatory behaviors were the most common within this category, typically involving the male slowly approaching the female before performing a sudden lunge (*Pre-Copulation*, 43.75%). During copulation, males exhibited *Copulatory Bite* (18.75%), a typical behavior in lacertid lizards used to maintain grip on the female, while Female *Copulatory Bite* (12.5%) was occasionally observed as a response. Only one instance of Copulation itself was recorded (6.25%). Additional behaviors included *Release Bite*, *Terminating Copulation*, and Post-Copulatory actions, each observed once (6.25% each) (Table 3).

DISCUSSION

Maintenance behaviors

Maintenance behaviors were by far the most frequently observed, comprising nearly 87% of all recorded behaviors. This pattern aligns with previous studies on lacertid lizards, such as *Gallotia galloti* (Molina-Borja, 1981), where maintenance behaviors also predominated. Their high frequency likely reflects their essential role in survival, requiring relatively low energetic investment when compared to more demanding behaviors such as aggression, reproduction, or locomotion (Martin and Bateson, 2021).

As ectotherms, lizards such as *Teira dugesii* do not produce sufficient internal heat through cellular metabolism to regulate their body temperature (Huey, 1982). As a result, they rely heavily on behavioral strategies to gain or dissipate heat, making thermoregulation a central aspect of their daily activity (Huey, 1982). This explains the high frequency of thermoregulatory behavior observed during our study. To maintain their physiological processes with-

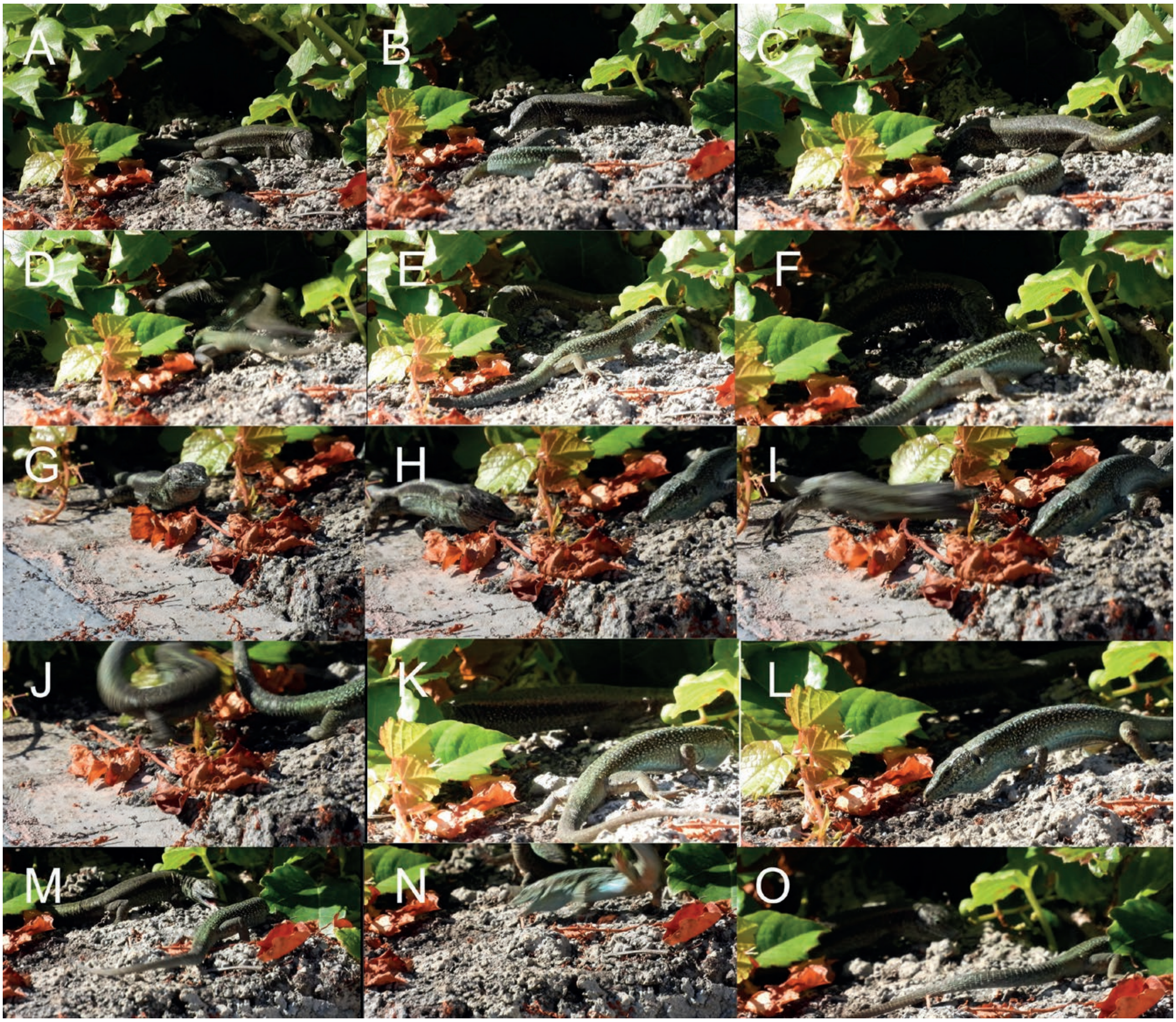


Fig. 3. One agonistic sequence between two males of *Teira dugesii*. A) One male approaches the other stealthily from behind; B) Both individuals display *The Face-Off, Circling* and *Lateral Threat Position*; C) One lizard adopts a *Perpendicular Threat Orientation*; D) The perpendicular male attempts to bite the opponent; E) *The Face-Off* and *Lateral Threat Position* continue, with gular movements; F) Continuation of *The Face-Off* and *Lateral Threat Position*; G) *Circling* with renewed gular movement; H) Head-to-head confrontation between the two lizards; I) One male attempts to bite the other; J) The biting male is countered by a tail strike from the opponent; K) Resumption of *The Face-Off* and *Lateral Threat Position* with gular movement; L) Direction change, maintaining *The Face-Off* and gular display; M) *The Face-Off* with mouth opening by one male, accompanied by tail movements; N) Intense physical combat between both individuals; O) One male retreats, leaving the other as the apparent winner.

in optimal thermal ranges, individuals must adopt postures that either facilitate heat gain or prevent overheating, depending on environmental conditions.

In our study, thermoregulatory postures were classified into two functional categories, following the framework proposed by Greenberg (1977): basking and heat-relief (thermal comfort). Postures associated with basking, such as *Body-Down*, *Head-Down*, and *Tail-Low*; or *Body-Down* combined with *Head-Up* and *Tail-Low*, were frequently observed and serve to maximize solar exposure and heat absorption (Greenberg, 1977; Molina-Borja, 1981). The second category, postures associated with heat stress relief, included *Toes-Up*, *Foot-Up*, and occasionally Tail-Up-at-Tip. Greenberg (1977), in his study of *Sceloporus cyanogenys,* observed similar behaviors at substrate temperatures between 45–50 °C. Carpenter (1961) suggested these postures minimize contact between highly vascularized regions (e.g., digits) and hot surfaces, potentially preventing overheating. In *T. dugesii*, these postures were often recorded during the warmer hours of the day and were sometimes accompanied by *Mouth-Open*, which likely aids evaporative cooling. However, *Mouth-Open* appears to be multifunctional. These findings align with previous reports for other lacertid lizards, such as *Gallotia galloti* (Molina-Borja, 1981), and underscore the central role of postural and thermoregulatory behaviors in the behavioral ecology of insular reptiles. Brattstrom (1971) distinguished four main contexts in which lizards open their mouth: drinking/eating, yawning, thermoregulation, and aggression. In *T. dugesii*, this behavior was also observed outside thermal contexts, suggesting similar functional diversity. Self-maintenance behaviors such as *Groom*, *Scratch*, *Contortion of the body*, and *Palateo* were less frequent but noteworthy.

In addition to postural and maintenance behaviors, we also recorded elementary locomotor movements, particularly in juveniles. Among these, tail movements provided intriguing insights. A lateral tail-waving motion was consistently observed prior to short displacements. Juveniles moved only a few centimeters at a time and frequently displayed this tail movement before proceeding. While the function remains uncertain, it may serve as a risk-assessment behavior or a subtle anti-predator strategy.

In sum, *Teira dugesii* appears to adopt a predominantly energy-conserving behavioral strategy, heavily focused on maintenance activities. This is consistent with the behavior of other insular lacertids and reflects ecological adaptations to relatively stable environments with predictable thermal conditions.

Mobility and feeding behaviors

Mobility behaviors represented 6.95% of all recorded actions and played a secondary role in the species' activity budget. These patterns reflect a strategy of cautious movement, likely aimed at minimizing energy expenditure while allowing efficient exploration of the environment.

Walking was predominantly observed on open ground, stone walls, and flat rocky surfaces, suggesting a calm exploratory mode used during thermoregulation or when foraging. In contrast, running and jumping were more frequent in response to disturbances or during social interactions, such as fights or courtship chases. Arboreal movements, often leading to nectar sources in plants like *Hibiscus rosa-sinensis*, underline the behavioral plasticity of *Teira dugesii*, which may take advantage of vertical structures and vegetation, particularly in anthropogenic habitats.

Jumping, though less frequent, was particularly notable in lizards navigating between branches or elevated surfaces, indicating a capacity for precise locomotion in three-dimensional space. This flexibility may be especially advantageous in the Madeiran landscape, where vegetation and human structures offer complex microhabitats.

The relative rarity of high-speed or intense locomotor activity supports the interpretation that *T. dugesii* follows an energy-conserving behavioral strategy, with bursts of mobility mostly linked to specific ecological triggers. These observations are consistent with those in other island lacertids, such as *Gallotia galloti*, where walking dominates under normal activity conditions and running is usually associated with escape or confrontation (Molina-Borja, 1981). Overall, mobility behaviors in *Teira dugesii* appear to balance caution, energy efficiency, and ecological opportunity, contributing to its adaptability across diverse habitats.

Many studies have examined the predation of vertebrates and invertebrates by *Teira dugesii* (Sadek, 1981). In this study, we observed its feeding behavior, water intake, and defecation. Regarding feeding, we recorded the lizard hunting twice and also licking nectar from flowers. During hunting, *Teira dugesii* moved rapidly toward its prey, minimizing the chance of escape. Once captured, the prey was held in the lizard's mouth while it performed *food-shaking* movements. This was followed by *chewing* before the lizard fled, likely due to the approach of other *Teira dugesii* individuals, as seen in our recordings.

The second feeding behavior recorded was nectar licking. This behavior has been documented in previous studies (Câmara and Jesus, 2025c) not only in *Teira dugesii* but also in other lizard species that rely on nectar as part of their diet (Romero-Egea et al., 2023). The lizard engaged in *arboreal movement* to reach the flower, and upon locating the nectar source, it began licking the nectar.

Regarding water intake, the Madeiran wall lizard uses a *tongue-touch* movement to drink. An interesting behavior was observed at a pond in the Madeira Botanical Garden, where the lizards approached the water one at a time to drink.

For defecation, *Teira dugesii* exhibits a behavior that may play a role in communication among individuals. After defecation, the lizards drag their cloaca and pelvic area over the substrate, potentially leaving an odor produced by the femoral pores.

Aggressive and defensive behaviors

The Madeiran wall lizard is known for being highly tolerant to humans (Sarmento, 1948). This is because many island-dwelling lizards have evolved in environments with few predators, leading to longer reaction times (Cooper Jr. et al., 2014). While escape is a common response to threats among lizards, species inhabiting islands often exhibit reduced evasive behavior due to the decreased predation pressure over generations (Goode and Pasachnik, 2018). However, when approached, the lizard often adopts an alert posture in response to stimuli such as noise or sudden movements. In these situations, head movements, including head rotation, were also observed. According to Molina-Borja (1981), alert postures in lizards may serve the function of gathering information from their immediate surroundings. Similarly, Carpenter (1967) described an alert posture in the context of challenge and fight situations.

Throughout the observations and recordings, we documented agonistic behaviors between male-male and male-juvenile interactions. This type of behavior was exclusively recorded during the summer, when temperatures were at their highest. However, as the majority of observations and recordings were conducted in summer, there was a higher likelihood of capturing such interactions. One possible explanation for the reduced frequency of aggressive interactions in *Teira dugesii* is its high population density. Increased population density often leads to greater competition for resources, which in turn shapes social behavior (MacGregor et al., 2017). In some lacertid species, such as *Podarcis hispanica*, higher densities have been associated with a greater reliance on non-aggressive dominance displays, minimizing energy expenditure and reducing the risk of injury (López and Martín, 2001).

Male-juvenile fights were recorded on four occasions. Two distinct types of interactions were observed. In the first case, the juvenile performs the gular movement to appear bigger. However, upon the sudden approach of the adult male, the juvenile move-away, avoiding the confrontation. In the second type of interaction, recorded three times, the juvenile was caught by surprise by the adult male. The male bit the juvenile on the dorsal region, prompting the juvenile to struggle by biting back and contorting its body in an attempt to escape. On one occasion, after managing to free itself from the initial bite, the juvenile was immediately grabbed again by the tail, with the adult male holding its weight solely through the strength of its bite. In response, the juvenile curled its body into a *C-shape* to aid in escaping. In both cases, the juveniles ultimately managed to break free. At times, juveniles are not as fortunate, as they may be killed or have their tails forcibly detached. Instances of adult *Teira dugesii* preying on juveniles have already been documented (Gloor and Benito, 2023).

Regarding male-male fights, we were able to record an entire combat sequence lasting seven minutes. Other fight initiations consisted only of gular display movements, followed by a chase until the losing male moved away. Another factor contributing to the quick resolution of fights was their occurrence on vertical surfaces. The *face-off* and *threat position* may serve as a way for males to display and communicate their dominance or fighting ability by utilizing various physical traits such as body length (Alberts et al., 1992; Rodda, 1992), head shape variation, dewlap color, and throat size. Previous studies with other species have indicated that color variation among males can signal fighting ability as well as aggressiveness (Molnár et al., 2016). The *face-off* can be of great importance, as the withdrawal of one of the males can prevent direct confrontation, allowing the lizards to conserve energy and avoid injuries (Briffa and Sneddon, 2006). This behavior may serve as a distraction mechanism, diverting the opponent's attention to the tail rather than more vulnerable body areas. The escalation of these male-male conflicts in *Teira dugesii* was linked to competition for a high-visibility perch – a prime location frequently occupied by a small number of lizards. In the study by Peters et al. (2016) on *Phrynocephalus vlangalii*, the intensity of the signal relative to other movements, as well as the observation that males signal more rapidly in the presence of another male, support the hypothesis that tail-lashing represents heightened aggression by the resident and that its use depends on the characteristics of the defended resource.

Both complete and incomplete agonistic sequences occurred in the same locations, which may suggest several ecological and behavioral patterns. One possibility is that these sites represent key resources for *Teira dugesii*, such as thermoregulation spots, feeding areas, or strategic vantage points for territory surveillance. The recurrence of aggressive interactions in these areas may indicate territorial behavior, where individuals, particularly males, compete for dominance over high-value locations (Cooper and Vitt, 1993; Stamps, 1994). Another potential explanation is the presence of a social hierarchy, where dominant individuals claim and defend prime areas while challenging intruders (Olsson and Madsen, 1995).

In *Teira dugesii*, several lizards were observed *Tongue Flicking* their surroundings, and *Tongue Touch* the substrate. These behaviors related to chemical senses are important in different contexts: *foraging* (prey discrimination), predator avoidance and social relationships. This last component includes discrimination between familiar and unfamiliar conspecific and self-recognition (Cooper, 1994, Cooper, 1996, Aragón et al., 2001).

Reproductive behaviors

The mating behavior of lizards has been studied in various species, including *Eremias argus*, *Podarcis liffordi*, *Takydromus septentrionalis*, *Lacerta kulzeri*, and *Lacerta agilis* (Galán, 1997; In den Bosch and Zandee, 2001; Du and Yao, 2007). In the case of *Teira dugesii*, Richter (1986), Galán and Vicente (2003) and Sleijpen (1996) described several reproductive traits, including clutch size in captivity. However, they did not provide detailed accounts of mating behavior, except for Sleijpen (1996), who noted that males bite the female's flank during copulation. According to Richter (1986), Madeiran wall lizards typically mate in early April, about 3–4 weeks after emerging from hibernation. This species also exhibits a relatively long reproductive season compared to other lacertids, which usually complete egg-laying by July. Galán (2003) observed that females lay eggs from May to August, at intervals of 5 to 8 weeks. Our observations, however, suggest that mating is not confined to these months. Small juveniles were observed throughout the year, including in January, suggesting that hatching may occur continuously rather than within a narrow seasonal window. Nevertheless, two distinct peaks of reproductive activity were detected during the main breeding season, between May and August.

We observed a complete mating event only once, which lasted approximately two minutes, the same duration reported by Sleijpen (1996) in captivity. On another occasion, copulation was avoided as the female managed to escape. Although these behaviors appeared rare, their true frequency is likely underestimated. In several instances, individuals were observed engaging in courtship or copulation-like behavior behind rocks or flowerpots, partially obstructing the observer's view. This suggests that some reproductive interactions may have gone undocumented due to limited visibility in dense or structurally complex microhabitats.

Males attempted to initiate copulation multiple times, but the female always managed to escape. Occasionally, the male succeeded in biting her, yet she still fled. Males usually tried to catch the female off guard, taking advantage of moments when she was not alert. Female rejection of mating attempts can be influenced by several factors. One possible explanation is the costly nature of copulation, where a female may gain indirect genetic benefits by rejecting all but the most persistent males (Chapman et al., 2003). Additionally, mate rejection may occur when a female is no longer in her receptive ovulation period or immediately after copulating with another male. Empirical evidence suggests that females may actively resist mating attempts, particularly after having already copulated with a previous mate. For example, in *Enyalius perditus,* Lima and Sousa (2006) documented a case in which, after courtship, a female rejected a second male following prior copulation. In response, the male attempted to force copulation by biting the female's nape to restrain her. Despite three persistent attempts, the female exhibited strong resistance by fleeing and counterattacking, biting the male's mouth. In some cases, copulation efforts can lead to significant harm or even mortality. A notable example is reported in *Ctenophorus maculosus*, where excessive force during mating resulted in severe injury, as the male's teeth punctured the female's spine (Olsson, 1995).

The copulation behavior was the rarest of all, making it extremely difficult to observe. In the study of Galan (1997) with *Podarcis bocagei* copulation behaviors were also very rare. In the pre copulation behaviors, *Teira dugesii,* the male observes the female and slowly approaches her. This may allow the male to confirm whether the individual is indeed female, using both visual and chemical cues, as observed by Carpenter and Ferguson (1977). Once he is relatively close, he pauses for a few seconds before suddenly rushing toward her.

Regarding the reproductive behavior of *Teira dugesii*, it was observed that the male bites the female's lower body to restrain her, a behavior also seen in the lacertid *Eremias argus*, before initiating copulation. At the beginning of copulation, females may bite the male in response. After the initial bite, the male grips the female just below her front limbs and then curves his body into a C-shape, aligning his cloaca with hers while wrapping his tail around her. He then inserts his hemipenes into the female's cloaca. During copulation, the male raises his head and neck, keeping his front end upright while resting his abdomen on the ground. After copulation the male lifts his cloaca and the front part of his tail off the ground, while the back end of the tail remains in contact with the substrate. The hemipenis remains visible outside the cloaca. In some lacertid lizards, females exhibit mate choice based on male morphological traits or behavioral displays (Andersson, 1994). Although *T. dugesii* males initiate copulation by directly approaching and restraining the female, it remains unclear whether female choice plays a role in mating success. Further studies examining female responses to different male traits could clarify the influence of sexual selection in this species.

This study represents the most detailed behavioral documentation of *Teira dugesii* to date, offering a broad spectrum of behavioral data that can serve as a baseline for future ecological and ethological studies. Understanding natural behavioral patterns is essential for assessing the impact of habitat modifications, climate change, and human interactions on this endemic species.

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Hierarchical response to kairomones of predator based on experience acquired through learning

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Abstract. This study investigated how prior experience modulates the anti-predator behavioral responses of *Duttaphrynus melanostictus* tadpoles to kairomones from the predatory tadpole *Hoplobatrachus tigerinus*. I compared the responses of four distinct categories of tadpoles; 1) predator-naïve (laboratory-born), 2) indirect predator-experienced (short-term exposure to caged predator cues), 3) direct predator-experienced (short-term direct encounters), and 4) wild-caught (long-term natural experience). A stimulus solution (kairomones) from the predator was used to simulate predation risk. The results showed that tadpoles of *D. melanostictus* from all experience groups exhibit antipredator behavioral responses, i.e., overall reduced swimming and less time spent swimming, but with a higher burst speed in response to water-borne kairomonal cues of predators. Crucially, the intensity of these antipredator behavioral responses was strongly dependent on experience, following a clear hierarchical gradient: Wild-caught > direct-predator experienced > indirect-predator experienced > predator-naïve > control. The significant, albeit low-level, response of predator-naïve tadpoles indicates that predator recognition is innate. However, the enhanced antipredator behavior of wild-caught tadpoles compared to predator-naïve or direct or indirect predator-experienced tadpoles suggests that learning and cumulative experience are involved. A combination of both innate and learned behaviors could allow tadpoles of *D. melanostictus* to calibrate their defensive investment necessary for survival.

Keywords. Anurans, behavioral response, prey-predator interactions, reduced activity, swimming speed, tadpoles.

INTRODUCTION

Predator-prey interactions play a crucial role in shaping the life-history strategies of animals. In both aquatic and terrestrial ecosystems, predation provides selection pressure that forces prey to maximize their fitness by recognizing and avoiding predators. Predators can impact the behavior, morphology, and life history of prey (Lima and Dill, 1990; Laforsch and Tollrian, 2004; Ferrari et al., 2010; Smith et al., 2010; Gazzola et al., 2024; Saidapur, 2025). For predator recognition, prey animals may use a wide range of cues such as visual, acoustic, electric, tactile, disturbance, chemical, or a combination thereof (Amo et al., 2004; McCormick and Manassa, 2007; Ferrari et al., 2010; Mogali et al., 2011, 2012; Batabyal et al., 2014; Landeira-Dabarca et al., 2019; Saidapur, 2025). In aquatic predator-prey systems, chemical cues are much more efficient in complex, murky ecosystems and are usually detected faster, earlier, and over larger distances than visual cues (Chivers et al., 1996; Mathis and Vincent, 2000; Ferrari et al., 2010).

Anuran larvae are an excellent model system for studying predator-prey interactions because tadpoles are highly vulnerable to aquatic predators (Heyer et al., 1975). Previous studies suggest that most species of anuran tadpoles assess predation risk using chemosensory mechanisms before responding with defense behaviors (Ferrari et al., 2010; Mogali et al., 2012; Saidapur, 2025).

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Individuals respond to alarm cues released by injured prey, kairomones of predators, and dietary cues that alter behavior to escape predation (Schoeppner and Relyea, 2005, 2009; Scherer and Smee, 2016; Saidapur, 2025). Previous studies have also revealed that anuran larvae exhibit a variety of antipredator responses to chemical cues that can be generally grouped into strategies to avoid predators and/ or strategies to escape from predation (Schmidt and Amezquita, 2001; Hossie and Murray, 2010). These responses include overall decreased activity (Saidapur, 2025), increased hiding and aggregation (Spieler and Linsenmair, 1999; Hossie and Murray, 2010), and defensive behaviors (increased swimming, Mogali et al., 2021).

In aquatic environments, predators may release various types of chemical cues, with kairomones (the odors of predator) being the most common. There is evidence that kairomones trigger antipredator behavior in a wide range of prey animals (Kats and Dill, 1998; Schoeppner and Relyea, 2005; Ferrari et al., 2010). Kairomones are generally considered to be chemical signatures of predators. However, some studies involving starved predators have shown that kairomones may not elicit antipredator responses in some prey taxa (Crowl and Covich, 1990; Stirling, 1995). In contrast, other research focused on larval anuran has demonstrated that kairomones can induce strong antipredator behavioral responses (Petranka and Hayes, 1998; Van Buskirk and Arioli, 2002; Schoeppner and Relyea, 2005, 2009; Gyssels and Stoks, 2006; Mogali et al., 2011).

The Asian common toad, *Duttaphrynus melanostictus* (Schneider, 1799), is widely distributed throughout India. In Southern India, during the early monsoon season, *D. melanostictus* generally breeds in temporary water bodies alongside other coexisting anuran species (Saidapur, 2001; Gramapurohit and Radder, 2012). The ephemeral ponds that host herbivorous tadpoles of *D. melanostictus* are also habit for a variety of predators. These include invertebrates (e.g., dragonfly and damselfly larvae, beetles, crabs, water boatmen) and both omnivorous tadpoles, *Euphlyctis cyanophlyctis* and carnivorous tadpoles, *Hoplobatrachus tigerinus* (Mogali et al., 2023a, b, c). During my regular field visits, I observed that the herbivorous tadpoles of *D. melanostictus* were primarily preyed upon by *H. tigerinus*. In studies focused on predator-prey interaction among tadpoles, most researchers have used aquatic insects, fish, or salamanders as predators, often overlooking other aquatic predators, especially anuran tadpole predators (Chivers and Mirza, 2001; Mathis, 2003). Very few studies have investigated the impact of carnivorous tadpole predators on the behavioral responses of herbivorous tadpoles (Saidapur, 2025). Most researchers have studied the behavioral responses of prey tadpoles by using either only laboratory reared (predator-naïve) or laboratory reared tadpoles with short-term direct or indirect experience with predators (predator-experienced) or tadpoles with long-term experience with predators in natural water bodies (wild-caught) (Semilitsch and Reyer, 1992; Laurila et al., 1997; Mogali et al., 2012, 2023c). In this study, I investigated the behavioral responses of different categories of *D. melanostictus* tadpoles: predator-naïve (PN), indirect predator-experienced (IPE), direct predator-experienced (DPE), and wild-caught (WC) tadpoles. I exposed them to stimulus solutions (kairomones) from a predator, *H. tigerinus*, as such studies are relatively rare. I hypothesized that all four categories of prey tadpoles would primarily exhibit antipredator behavioral responses to the kairomones of *H. tigerinus*. Additionally, I expected to find variation in the antipredator behavioral responses among the tadpole categories. Specifically, I expected that wild-caught tadpoles would display the strongest antipredator responses compared to both predator-experienced (direct and indirect) and predator-naïve tadpoles.

MATERIAL AND METHODS

Three egg clutches of *Duttaphrynus melanostictus* were collected from a temporary pond on the Karnatak University Campus, Dharwad, Karnataka State, India (15º27'N, 75º05'E, 750 m a.s.l.), during the early monsoon. Each clutch was placed in a separate 1 L plastic container filled with aged tap water and immediately transported to the laboratory. Here, the clutches were transferred separately in plastic tubs (32 cm in diameter and 14 cm in depth) containing 5 L of aged (dechlorinated) tap water. The eggs from all three clutches hatched synchronously at stage 19 (Gosner, 1960) the following day. Tadpoles from all three clutches, totaling 300 (100 tadpoles per clutch) were mixed and reared together in a glass aquarium (90 × 30 × 15 cm) containing 20 L of aged tap. In this way, two such stocks were maintained, resulting in a total of 600 tadpoles. Five days prior to the experimental trials, predatory tadpoles of *H. tigerinus* (Gosner stages 32–33; mean total length 37.80 ± 1.26 mm, $N = 50$) and prey tadpoles of *D. melanostictus* (Gosner stages 32–33; mean total length 27.32 ± 1.95 mm, $N = 50$) were collected from the same pond where the *D. melanostictus* eggs were collected. *H. tigerinus* tadpoles were reared in plastic tubs (19 cm in diameter and 7 cm in depth) in 0.5 L of aged tap water to avoid cannibalism. The *D. melanostictus* tadpoles were fed boiled spinach, while *H. tigerinus* tadpoles were fed tadpoles of *D. melanostictus*. For experimental categories classification, tadpoles of *D. melanostictus* reared in the laboratory from the egg stage were designated as predator-naïve (PN); those reared in the laboratory and exposed for a short-term to caged predators were designated as indirect predator-experienced (IPE); tadpoles reared in the laboratory and exposed directly to predators were designated as direct predator-experienced (DPE). Tadpoles of *D. melanostictus* collected from natural water bodies were designated as wild-caught (WC). The behavioral responses of all four categories of test *D. melanostictus* tadpoles were studied by exposing them to a "stimulus solution", which consisted of kairomones of the predatory tadpoles, *H. tigerinus*.

Preparation of kairomones

Tadpoles of *H. tigerinus* were placed individually in separate plastic tubs ($N = 20$ tubs; 19 cm in diameter and 7 cm in depth) containing 200 mL of aged tap water without food for 96 h. This procedure resulted in a solution with only kairomones. After 96 h of starvation period, the predators were removed from the tubs, and the stimulus solution was filtered to remove any small quantities of fecal matter. The filtered solution, rich in kairomones was used immediately for the experimental trials.

Test subjects

(1) Predator-naïve tadpoles (PN): *Duttaphrynus melanostictus* tadpoles (Gosner stages 32–33; mean total length 27.49 ± 1.80 mm; $N = 25$) were placed in plastic tubs (32 cm in diameter and 14 cm in depth) with 3 L of aged tap water. At the center of the rearing tub, a small empty plastic tub (19 cm in diameter × 7 cm in height) wrapped with cheese cloth was placed. These tadpoles were raised in the absence of predators from the time of hatching. Two duplicate tubs were maintained.

(2) Indirect predator-experienced tadpoles (IPE): *D. melanostictus* tadpoles (Gosner stages32–33; mean total length 27.49 ± 1.80 mm; $N = 25$) were also placed in similar plastic tubs containing 3 L of aged tap water. At the center, a small plastic tub (19 cm in diameter × 7 cm in height) with perforations (1.2 mm^2 holes), wrapped with cheese cloth, and housing a single starved tadpole of *H. tigerinus* (Gosner stages 32–33) was introduced for 8 h. Although the test tadpoles outside the cage had no direct contact with the predators, they were exposed to chemical cues (kairomones) released by the starved predator inside the cage. Two duplicate tubs were maintained.

(3) Direct predator-experienced tadpoles (DPE): the rearing setup for this group was identical to the previous setups, except that a single starved tadpole of *H. tigerinus* (Gosner stages 32–33) was directly introduced into the rearing tubs from 0900 to 1700 h. On average, the predator consumed 5 ± 0.3 and injured 4 ± 0.5 tadpoles during the 8–h period. After this time, the predator and the injured tadpoles were removed. The surviving uninjured that had experienced a direct predator were then used for trials on the subsequent day. Two duplicate tubs were maintained.

(4) Wild-caught tadpoles (WC): a total of 25 wild-caught tadpoles of *D. melanostictus* (Gosner stages 32–33; mean total length 27.32 ± 1.95 mm, $N = 25$) were placed in plastic tubs (32 cm in diameter and 14 cm in depth) containing 3 L of aged tap water with an empty cage at the center. In their natural temporary water bodies, *D. melanostictus* tadpoles have lived more than 15 days with various aquatic predators, potentially including *H. tigerinus* tadpoles. Two duplicate tubs were maintained.

Behavioral responses:

The behavioral responses to predator kairomones of the four tested categories of tadpoles were recorded by placing single test subjects in a rectangular glass test tank (28 × 15 × 15 cm) containing 600 mL of aged tap water. A handycam (Sony, DCR-SR300/E, Japan) was fixed above the test tank to record the entire area. The handycam was connected to a computer running the Ethovision Video Tracking System (Noldus Information Technology, The Netherlands) to track movements of the tadpole before and after the addition of the stimulus solution (predator kairomones). The Ethovision system recorded the maximum swimming speed ($V_{\max}$), distance traversed by the tadpole, number of swimming spurts and time spent swimming during the entire trial. For each trial, a new test tadpole was introduced into the tank and left undisturbed for 5 min (acclimation). A burette was placed ~1 cm above the water level and 50 mL of aged tap water (chemical blank) was added at the rate of ~1 mL/s to simulate the disturbance created by the subsequent chemical cue. The burette was then gently removed. The movement of the tadpole was recorded for 5 min using Ethovision to determine its baseline activity in the absence of any cues. Following this period, 50 mL of stimulus solution containing predator kairomones was added as described above. Movement of the tadpole was then recorded for another 5 min to determine the activity pattern after exposure to the kairomonal cues.

A total of 25 trials were carried out for each tadpole category (100 trials in total). A new test tadpole was used for each trial. The test tank was cleaned and replenished with aged tap water between each trials.

Statistical analysis

After checking for normality, the data on the behavioral responses of tadpoles of *D. melanostictus* before and after the addition of the stimulus solution were compared separately by using the paired-samples t–test. The data were analyzed using a General Linear Model for testing the overall effects of tadpole tested category, treatment type (exposure to chemical blank water or stimulus solution), and their interactions. The response variables were the different swimming activities (i.e., maximum swimming speed, frequency of swimming spurts, time spent swimming, and total distance travelled). In addition, the data on the behavioral responses between different tested categories of tadpoles of *D. melanostictus* to the stimulus solution of a predator were analyzed using a one-way ANOVA followed by a Tukeys' HSD *post-hoc* test. All these tests were performed using SPSS ver. 22.0.

RESULTS

Behavioral responses of D. melanostictus tadpoles to kairomones of H. tigerinus tadpoles

Upon exposure to the predator kairomones, the four tadpoles categories of *D. melanostictus* showed a significant increase in $V_{\max}$ and a significant decline in the number of swimming spurts, time spent swimming, and in the total distance moved when compared to their baseline activities in chemical blank water (Table 1).

Intensity of defense behaviors in D. melanostictus tadpoles to kairomones of H. tigerinus tadpoles

General Linear Model showed that both the tadpole's experience level (category) and the presence of the cue (treatment), as well as their interaction, had a significant influence on the swimming activities of *D. melanostictus* tadpoles (Table 2). Tadpoles with more experience with predator cues showed significantly stronger defensive reactions. Results of ANOVA also showed a significant difference in the $V_{\max}$ ($F_{4,195} = 1206.0$, $P < 0.05$), number of swimming spurts ($F_{4,195} = 792.89$, $P < 0.05$), time spent swimming ($F_{4,195} = 803.69$, $P < 0.05$), and total distance moved ($F_{4,195} = 432.50$, $P < 0.05$) among prey tadpoles in the different tadpole category groups (Figure 1). The intensity of the observed defense behaviors varied significantly with the prey's level of prior experience with the predator cue (Figure 1). Wild-caught (WC) tadpoles exhibited the strongest response, displaying significantly higher $V_{\max}$ ($P < 0.05$), significantly reduced time swimming ($P < 0.05$), number of swimming spurts ($P < 0.05$), and moved for a shorter distance ($P < 0.05$) compared to all other groups (Figure 1). The DpE tadpoles also exhibited significantly higher $V_{\max}$ ($P < 0.05$) and spent significantly less time swimming ($P < 0.05$) with a reduced number of swimming spurts ($P < 0.05$) and moved only a short distance ($P < 0.05$) compared to the IpE, predator-naïve, and control groups (Figure 1). The IpE tadpoles also showed significantly higher $V_{\max}$ ($P < 0.05$) and reduced swimming metrics ($P < 0.05$) compared to the predator-naïve and control groups (Figure 1). The predator-naïve tadpoles displayed the lowest-level defense, but still showed significantly higher $V_{\max}$ ($P < 0.05$), spent less time in swimming ($P < 0.05$) with a reduced number of swimming spurts ($P < 0.05$), and moved only a short distance ($P < 0.05$) compared to the control group (Figure 1). The hierarchy of intensity of defense behaviors was as follows: wild-caught tadpoles > direct predator-experienced tadpoles > indirect predator-experienced tadpoles > predator-naïve tadpoles > control group.

DISCUSSION

In aquatic environments, the survival of prey like anuran tadpoles is contingent upon effective anti-predator defenses (Schmidt and Amezquita, 2001; Relyea, 2007; Gazzalo et al., 2024; Saidapur, 2025). In such systems, various types of chemical cues (alarm cues of damaged conspecifics, dietary metabolites of predators, disturbance cues, and kairomones of predators) trigger the behavioral responses of prey (Schoeppner and Relyea, 2005, 2009; Saidapur, 2025).

The results of the present study showed that all four categories of prey tadpoles of *D. melanostictus* regardless of origin, identify the kairomones of the syntopic predator, *H. tigerinus* as a significant threat and decreased their activity levels during the trial period. When tadpoles of *D. melanostictus* moved in the stimulus solution (kairomones), their spurt speed ($V_{\max}$) was higher than in the stimulus blank solution, indicating their effort to escape from the perceived predator kairomones. This strong, specific response aligns with previous findings on tadpoles of *D. melanostictus* (Mogali et al., 2011) and *Polypedates maculatus* (Mogali et al., 2023a). The intensity of this reaction is likely linked to the co-evolutionary history and the hunting strategy of the predator. *H. tigerinus* tadpoles are active, visually-orienting predators that pose a constant threat (Mogali et al., 2023 a, b). In contrast, tadpoles of *Indosylvirana temporalis* (Mogali et al., 2012) and *D. melanostictus* (Mogali et al., 2020) did not alter their behavior in response to kairomones

Table 1. Behavioral responses of tadpole categories; predator-naïve (PN), indirect predator-experienced (IPE), direct predator-experienced (DPE), and wild-caught (WC) tadpoles of *Duttaphrynus melanostictus* to chemical blank solutions (aged tap water) or stimulus solutions (kairomones) of a predator, *Hoplobatrachus tigerinus*. Data are presented as mean ± SE and analyzed by paired samples t-test (*N* = 25 trials were conducted for each tadpole category).

Treatment/ Tadpole Category	Maximumc swimming speed (V_{max}; cm/s)	Frequency of swimming spurts	Time spent swimming (s)	Distance travelled (cm)
Predator-naïve tadpoles (PN)				
Chemical blank solution	11.79 ± 0.08	65.92 ± 1.40	63.08 ± 1.30	398.68 ± 8.72
Stimulus solution	18.49 ± 0.20	31.00 ± 0.55	29.37 ± 0.54	256.39 ± 3.89
t values	t_{24} = -30.68,	t_{24} = 20.81,	t_{24} = 21.06,	t_{24} = 14.27,
p values	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Indirect predator-experienced tadpoles (IpE)				
Chemical blank solution	11.83 ± 0.08	67.20 ± 1.39	65.31 ± 1.31	403.76 ± 10.16
Stimulus solution	19.68 ± 0.32	26.60 ± 0.81	25.16 ± 0.83	229.02 ± 6.33
t values	t_{24} = -22.21,	t_{24} = 26.89,	t_{24} = 28.05,	t_{24} = 14.18,
p values	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Direct predator-experienced tadpoles (DpE)				
Chemical blank solution	11.75 ± 0.07	65.56 ± 1.47	63.53 ± 1.46	398.98 ± 8.52
Stimulus solution	21.15 ± 0.22	22.28 ± 0.70	20.99 ± 0.67	195.91 ± 3.80
t values	t_{24} = -38.46,	t_{24} = 27.97,	t_{24} = 27.71,	t_{24} = 27.72,
p values	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$
Wild-caught tadpoles (WC)				
Chemical blank solution	11.82 ± 0.07	67.00 ± 1.36	65.45 ± 1.37	394.81 ± 6.14
Stimulus solution	24.53 ± 0.30	14.44 ± 0.52	13.42 ± 0.47	148.11 ± 3.77
t values	t_{24} = -46.24,	t_{24} = 39.80,	t_{24} = 39.66,	t_{24} = 37.10,
p values	$P < 0.05$	$P < 0.05$	$P < 0.05$	$P < 0.05$

Table 2. Results of General Linear Model for overall effects of tadpole category: predator-naïve (PN), indirect predator-experienced (IPE), direct predator-experienced (DPE), wild-caught (WC); treatment type: chemical blank solution, stimulus solution and their interactions. The response variables are various swimming activities (maximum swimming speed, frequency of swimming spurts, time spent swimming and total distance travelled) of tadpoles of *Duttaphrynus melanostictus*. Asterisks indicate significant differences.

Source	Wilks' Lambda	F	P
Tadpole category	0.342	20.825	< 0.001*
Treatment type	0.027	1676.0	< 0.001*
Tadpole category × treatment type	0.359	19.694	< 0.001*

of an insect predator, the larvae of a dragonfly, *Pantala flavescens*. Larvae of dragonflies are sit-and-wait predators that move slowly and usually wait for prey to come near before attacking (Miller et al., 2014) and may be perceived by the prey as less dangerous and pose a lower predation threat. Sit-and-wait predators, such as dragonfly larvae, exert different selective pressures and may suppress their chemical cues to attract prey (Miller et al., 2015). *D. melanostictus* exhibited strong antipredator behavioral responses to tadpoles of *H. tigerinus* because they are active predators that visually locate prey, including syntopic anuran tadpoles (Mogali et al., 2023 a, b; Saidapur, 2025). Thus tadpoles of *H. tigerinus* pose a serious predation threat to tadpoles of *D. melanostictus*. The long ecological co-existence of tadpoles of *D. melanostictus* with sympatric carnivorous tadpoles such as *H. tigerinus* may have led to the evolution of antipredator defense strategies in response to kairomones of these predators (Mogali et al., 2011, 2023 a, b).

The most significant finding of this study is the clear experience-dependent gradient in the intensity of this defensive behavior. The strong reaction of wild-caught tadpole is consistent with their long-term, cumulative experience in a high-risk natural environment (Mogali et al., 2023 c). Crucially, the significant (though low-level) defensive response of the predator-naïve tadpoles demonstrates that the recognition of *H. tigerinus* kairomones is fundamentally innate. This provides a vital baseline defense for tadpoles encountering this predator for the first time (Mogali et al., 2011). However, the results show that

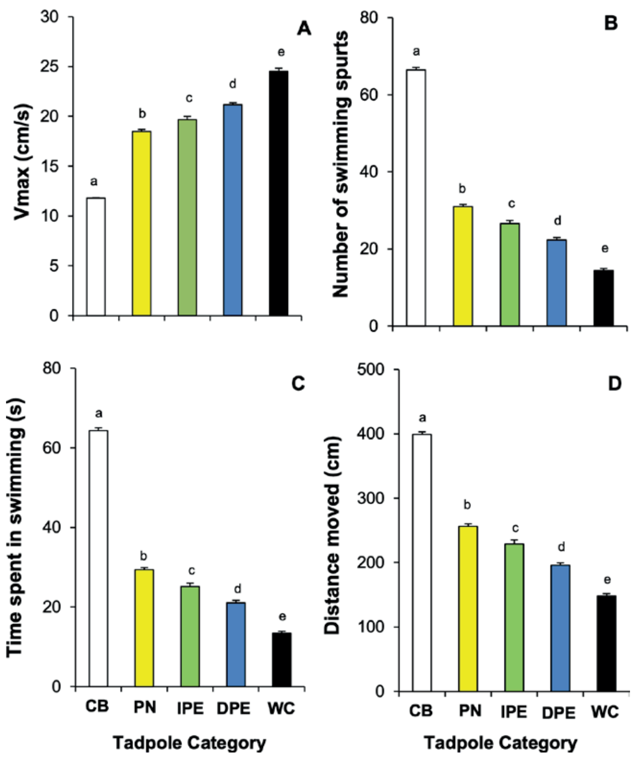

Fig. 1. Maximum swimming speed (*V*max) (A), number of swimming spurts (B), time spent swimming (C), and distance moved (D) by different categories of tadpoles of *Duttaphrynus melanostictus* exposed to chemical blank solution and to stimulus solution (kairomones) of a predator, *Hoplobatrachus tigerinus*. Data are presented as mean ± SE and analyzed by one-way ANOVA followed by Tukeys' HSD *post-hoc* test. Dissimilar letters above the bars indicate significant differences between the category groups. Twenty-five trials were conducted for each tadpole category group. CB = Chemical Blank solution, PN = Predator-Naïve tadpoles; IPE = Indirect Predator-Experienced tadpoles; DPE = Direct Predator-Experienced tadpoles; WC = Wild-Caught tadpoles.

this innate response is strongly amplified by learning and experience. The IPE group confirms that exposure to the predator's scent is sufficient to enhance the response, while the DPE group's stronger reaction suggests that direct, multi-sensory contact with the predator provides an even more powerful reinforcement (Mogali et al., 2012). The hierarchy of antipredator behavioral responses observed in tadpoles of *D. melanostictus*, wild-caught > direct predator-experienced > indirect predator-experienced > predator-naïve, illustrates this plasticity. This graded response, has also seen in *P. maculatus* (Mogali et al., 2023 c).

In summary, this study shows that all prey categories, i.e., predator-naïve, direct predator-experienced, indirect predator-experienced and wild-caught tadpoles of *D. melanostictus*, exhibit a highly plastic anti-predator strategy in response to predator kairomones of *H. tigerinus*. This behavioral defense is innately recognized, but its intensity is significantly modulated by experience. The observed hierarchy, from a low-level innate response to a maximal response in wild-caught individuals, highlights a mechanism for fine-tuning antipredator behavior based on experience acquired through learning.

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First report of overwintering tadpoles in the endemic Italian agile frog *Rana latastei*

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Abstract. Climate change is affecting organism seasonality patterns, and this can drive phenological shifts in key traits, including breeding activity. Here, we report the first record of overwintering tadpoles in the Italian agile frog (*Rana latastei*), a threatened endemic species of Northern Italy. This species usually breeds between late January and April, with tadpoles reaching metamorphosis by early summer. In February 2025, alongside the first freshly-laid egg-clutches of the usual breeding season, we observed several large-sized tadpoles at a reproductive site of *R. latastei* within Como province. A few days after hatching, six newly hatched tadpoles and six large-sized individuals at a later developmental stage were captured, photographed, and measured. Concurrently, a recently metamorphosed froglet was found at the same site. Morphological analyses revealed that individuals showed typical traits of *R. latastei*. Moreover, while newly hatched tadpoles were at Gosner's development stages 25-26, large-sized individuals were visibly bigger and at a late developmental stage, which was incompatible with the classical late-winter breeding timing of this species. Our study provides the first evidence of overwintering tadpoles in *R. latastei*, suggesting that potential shifts in development timing (prolonged larval time) or altered breeding habits (autumnal reproduction) may be occurring. Although the mechanisms behind this unusual observation remain unclear, such a phenological shift may have been favoured by variation in climatic regime.

Keywords. Amphibians, anurans, breeding, climate change, global warming, froglet, phenology, reproduction.

Global warming is causing unprecedented quick phenological shifts in animal populations (Horton et al., 2020). These shifts can involve breeding activities, including both advances (Parmesan, 2007; Ficetola and Maiorano, 2016) and delays (Dalpasso et al., 2023) in the onset of breeding season after the winter. Additionally, due to the general increase in winter temperatures, the end of the activity season in autumn is expected to be postponed (Lang et al., 2025). This may influence voltinism, potentially favouring an increase in reproductive events within a year in some populations. However, this may also produce survival risks for early stages, when suitable conditions for development are not matched (Benard, 2015; Bison et al., 2021). For instance, in temperate regions several amphibian species have been shown to extend or shift their breeding season by days or weeks in the last decades (Todd et al., 2011), with some cases of amphibian and reptile species reproducing outside their typical breeding season reported (Graña and Martínez-Freiría, 2020; Rodriguez-Muñoz et al., 2020).

In Europe, most anurans breed in late winter-spring, with juveniles metamorphosing in late spring-summer. This timing allows them taking advantage of spring rain that fills temporary wetlands and exploiting the

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warm season for larval growth and development. However, breeding patterns can be different in some species in Southern Europe. For instance, in Sicily, the green toad (*Bufo boulengeri* Laurenti, 1768) can also breed in autumn and winter, with tadpoles from autumnal mating overwintering and metamorphosing in early spring (Sicilia et al., 2006). In this case, autumnal breeding has been interpreted as an adaptation to arid environments, where waterbodies filled by autumn rain are more likely to retain water long enough to enable toadlets attaining metamorphosis (Sicilia et al., 2006). Less evidence for autumnal breeding and tadpole overwintering is available for amphibians living in Northern Italy. Among them, the Italian agile frog, *Rana latastei* Boulenger, 1879, is endemic to the lowlands of Northern Italy and adjacent areas and breeds from late January to mid-April (Ambrogio and Mezzadri, 2018; Ficetola et al., 2025). In principle, changes in temperature and/or precipitation patterns related to climate change might affect breeding phenology of this species, but information on autumn breeding and/or overwintering tadpoles is so far lacking.

Here, we report the first observation of overwintering tadpoles in the Italian agile frog. The reproductive site is a small artificial permanent pond (roughly 2 × 1 m; 0.3 m depth) located in a small wooded area in the foothills of the Como district (Inverigo, Lombardy, Italy). The site is located approximately 350 m a.s.l. and the area hosts numerous water bodies supporting a diverse amphibian community, including three urodele and six anuran species. This area is part of a long-term amphibian monitoring program covering the past two decades (Ficetola et al., 2009; Falaschi et al., 2021), which revealed the presence of a stable meta-population for *R. latastei* across interconnected breeding sites (Manenti et al., 2020). The permanent pond where overwintering tadpoles were found has been consistently surveyed multiple times during spring since 2010 and is a stable breeding site of *R. latastei*, where no other amphibian, except the fire salamander (*Salamandra salamandra* Linnaeus, 1758), has been reported reproducing. On 27 January 2025, ~5 days after the first mid-winter rainfalls that usually trigger amphibian breeding in the area, the site was monitored along with other ones in the surroundings. This survey revealed the presence of a single freshly laid egg-clutch of *R. latastei* (the first clutch of the breeding season found in the area). The site was then monitored on January 30 (one new clutch), February 6 (no new clutches), February 11 (one new clutch), and on February 26 (four new clutches). The first egg hatch was observed on February 11. On February 6, the presence of three large-sized tadpoles showing *R. latastei* traits was observed. On February 26, we collected six large-sized tadpoles and six small-sized tadpoles (Gosner's stage >25) for measuring (Fig. 1). Concurrently, a newly metamorphosed froglet was found (Fig. 2). Individuals were captured by gently netting the pool, then briefly kept in a small plastic tank, and photographed on graph paper using a 100 mm macro lens to minimize distortion. Pictures were taken by a single operator using a standardized photographic setting. After the photoshoot, the individuals were immediately released (permits listed in acknowledgements). Froglet and tadpoles were measured from scaled pictures using ImageJ software (Schneider et al., 2012) by a single operator following standard procedures (Relyea, 2001; Melotto et al., 2020, 2021). Tadpole developmental stages were determined based on Gosner's tables (Gosner, 1960). Although never observed breeding in the study site, another *Rana* Linnaeus, 1758 species with similar ecology, the agile frog (*Rana dalmatina* Fitzinger in Bonaparte, 1838), is present in the nearby woods, and tadpoles of this frog sometimes show phenotypes difficult to tease apart from *R. latastei* (Barbieri et al., 2000). Hereafter, along with morphometric and stage comparison between tadpole cohorts, key phenotypic traits differing between the two frog species are highlighted (Lanza et al., 2009; Ambrogio and Mezzadri, 2014).

We obtained tadpole's measurements from both dorsal and side pictures (Table 1A, Fig. 1A-D) and included total length (Totl), body length (BL), body height (BH), body width (BW), tail length (TL), tail height (TH), tail muscle height (TMH), and tail muscle width (TMW). Moreover, we used dorsal pictures to calculate minimum eye distance (ED) and minimum nostril distance (ND). ND was available for large-sized tadpoles only, as nostril position was not clearly identifiable in newly hatched tadpoles. All tadpole measurements, except for ND, were included in a principal component analysis to ascertain the existence of distinct size classes among tadpoles from different cohorts and if they matched the eye-based and Gosner's stage identification. Moreover, we calculated eye-distance nostril-distance ratio (ENDr) from ED and ND measurements for the perspective overwintering tadpoles (large-sized individuals). This ratio is one of the key traits that differ between tadpoles of *R. dalmatina* and *R. latastei*, being around 2 for the first one and rarely >1.5 for *R. latastei* (Lanza et al., 2009). Finally, we used ventral pictures of the froglet to take some morphological measurements (Table 1B): total length (TL), body width (BW), jaw width (JW), and few left hindlimb lengths, including proximal hindlimb (LPHL), distal hindlimb (LDHL), tarsus (LTL), and foot (LFL). Additionally, froglet ED and ND were obtained from dorsal picture (Table 1B).

Overall, all tadpoles showed characters matching the typical features of *R. latastei* [absent, poor, or incomplete

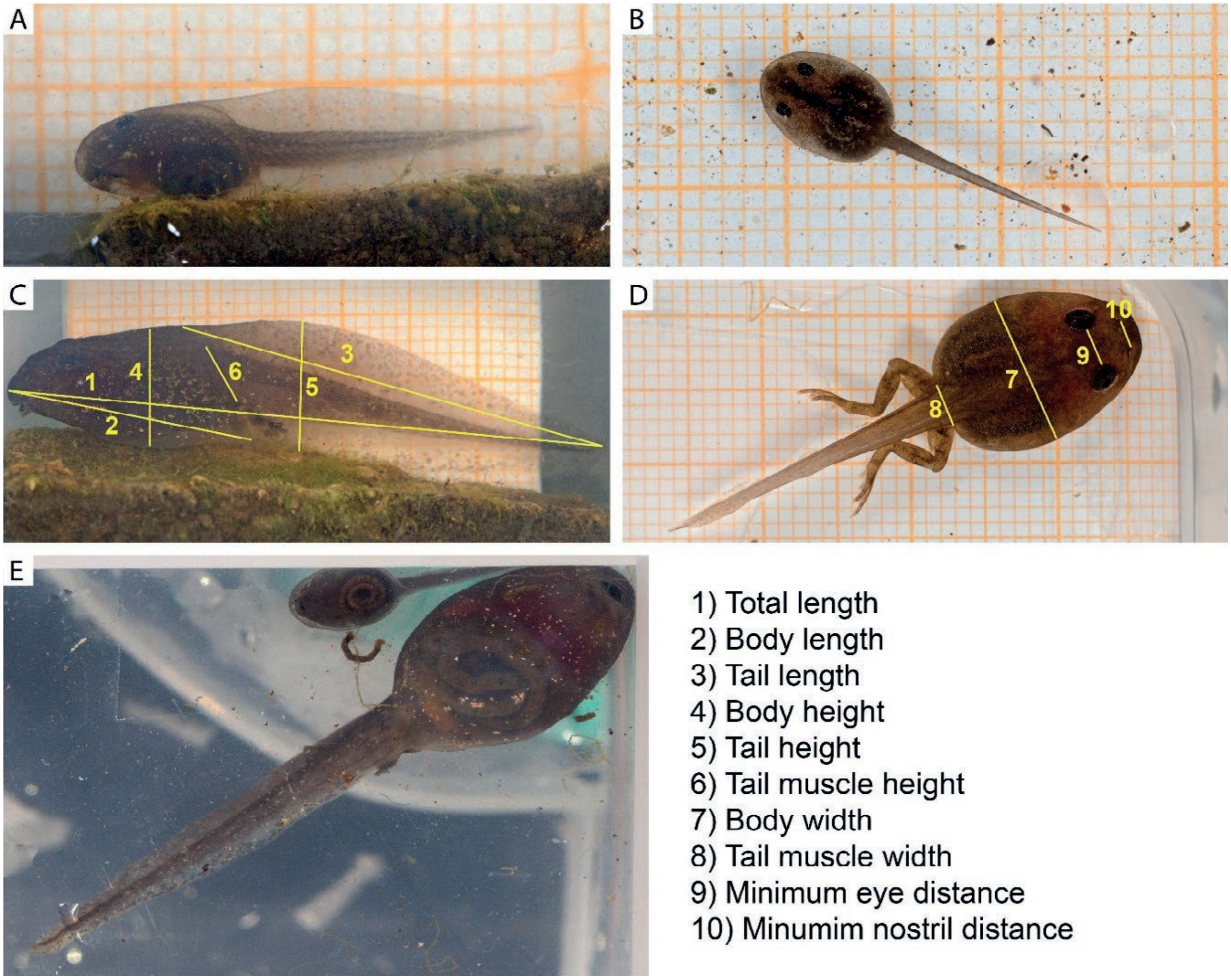

Fig. 1. Tadpoles of *Rana latastei*. A-B) Lateral (A) and dorsal (B) view of newly hatched individuals (small-sized); C-D) lateral (C) and dorsal (D) view of overwintering individuals (large-sized); E) ventral view showing size difference between a newly hatched and an overwintering individual. For C e D, examples of measures performed are provided.

ventral colouration and visible guts (Fig. 1E); EDNr of large-sized tadpoles ranged from 1.15 to 1.43 (average: 1.29) (Lanza et al., 2009; Ambrogio and Mezzadri, 2014)]. All the small-sized individuals were between Gosner's stage 25 and 26, showing functional oral canal but no trace of hindlimb formation. Instead, large-sized tadpoles were at the Gosner's stage 40 (four individuals; hindlimb with clearly differentiated toes and tubercles, cloacal tail piece visible), or between 37 and 39 (evident toes with no tubercles). The principal component analysis revealed that the first axis explained 99% of variation, identifying three distinct size classes corresponding to the three Gosner's stages identified, clearly distinguishing as different classes large-sized individuals and newly hatched ones (Fig. 3), also evident from picture comparison (Fig. 1E).

The newly metamorphosed individual measured 1.87 cm (Table 1B) and presented the typical traits of *R. latastei* (Lanza et al., 2009): the light stripe running above upper lip suddenly stops under the eye (while it is generally prolonged in *R. dalmatina*), and nostril distance was shorter than eye distance (Fig. 2A-B, Table 1B); moreover, chest and throat looked diffusely spotted and a "T" shaped stripe is noticeable between the throat and the forelimb junction (Fig. 2C), while ventral colouration is generally homogeneously pale and unspotted in *R. dalmatina*.

Taken together, our observations confirm that all individuals belong to *R. latastei*. At the same time, the size and late stage of the large tadpoles along with the presence of a froglet and the comparison with newly

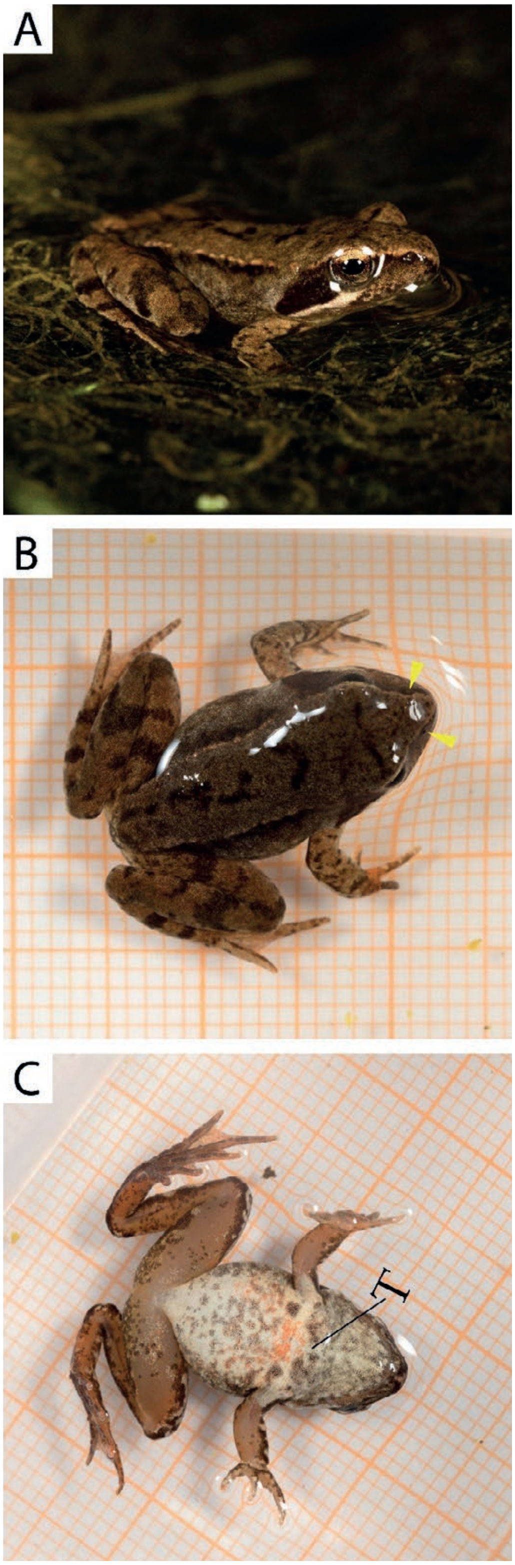

Fig. 2. Newly metamorphosed *Rana latastei* individual. A) Lateral view, white stripe stopping under the eye visible; B) dorsal view, nostrils are highlighted by yellow arrow-heads; C) ventral view, T shape between throat and forelimb junction highlighted.

hatched tadpoles, suggest that large-sized individuals originated from reproductive events occurred in the previous year and underwent overwintering at larval stage. In absence of a direct observation of autumnal reproductive events, only speculations can be made concerning the breeding period. Overwintering tadpoles are not rare in anurans (McDiarmid and Altig, 1999) and can be occasionally observed in other Northern-Italian species [e.g., green frogs, *Pelophylax* synkl. *esculentus* (Linnaeus, 1758)] and in some *Rana* species in other areas (Walsh et al., 2008; Lanza et al., 2009). However, this is generally associated with anurans facing shorter growing seasons, such as late-breeding species, or species and populations from high latitude or elevation (McDiarmid and Altig, 1999), as overwintering can extend the growth period and allow individuals attaining larger size at metamorphosis (Walsh et al., 2008; Iwai, 2024). This is not the case for *R. latastei*, which is an early breeder, whose tadpoles typically reach metamorphosis in June-July (Lanza et al., 2009).

We suggest these tadpoles originated from one or more breeding events that occurred in late summer or autumn 2024. Tadpoles exposed to low temperatures typically incur in metabolic depression, which slows down growth and development (McDiarmid and Altig, 1999; Enriquez-Urzelai et al., 2022). However, autumn and winter temperatures of 2024-2025 have been among the mildest in recent decades and 2024 has been the warmest year since consistent temperature monitoring began (https://climate.copernicus.eu/). A similar increase in temperature was recorded at the study site, where precipitation showed large variation with peaks of autumn rainfall in the last two years (see Supplementary material: Table S1; Fig. S1). This could have allowed larval development during the winter months, similarly to what happens in anurans breeding in warmer regions (Sicilia et al., 2006). Additionally, previous records of *R. latastei* males calling in autumn have been reported for nearby areas (Grossenbacher et al., 2000). While the presence of calling males does not guarantee that breeding activities are occurring, milder autumn and winter temperatures may favour egg deposition and allow tadpole development during this time.

An alternative hypothesis might be that large-sized tadpoles originated from a typical late-winter deposition event in spring 2024. The monitored breeding site is partially shaded by canopy cover, and 20 egg-clutches were laid by February 2024 (the last observed deposition). We cannot exclude that tadpole density and cold water temperatures might have induced delayed development in some individuals at the breeding site. However, tadpole density was comparable to other nearby sites monitored

Table 1. Morphological assessment of *Rana latastei* tadpoles and froglet. A) measures and approximated Gosner's development stage of tadpoles of different cohorts are reported together with individual identification code and size class; B) measures of the newly metamorphosed froglet found. Measurements abbreviations hereafter: body height (BH), tail height (TH), tail muscle height (TMH), body length (BL), tail length (TL), total length (TotL), body width (BW), tail muscle width (TMW), eye distance (ED), nostril distance (ND), jaw width (JW), left distal hindlimb length (LDHL), left proximal hindlimb length (LPHL), left tarsus length (LTL), left foot length (LFL), eye-nostril distance ratio (ENDr).

A) ID	size class	Gosner	BH	TH	TMH	BL	TL	TotL	BW	TMW	ED	ND
GIR1	large	37-39	0.884	1.042	0.510	1.858	3.149	4.525	1.089	0.349	0.341	0.244
GIR2	large	40	0.912	0.810	0.405	1.563	2.320	3.489	1.049	0.301	0.267	0.232
GIR3	large	40	0.811	0.858	0.403	1.539	2.450	3.626	1.027	0.289	0.263	0.220
GIR4	large	40	0.847	0.826	0.375	1.550	2.347	3.467	0.917	0.363	0.274	0.192
GIR5	large	40	0.857	0.983	0.423	1.563	2.461	3.698	1.076	0.312	0.323	0.247
GIR6	large	37-39	0.992	1.069	0.606	1.850	3.483	4.841	1.136	0.379	0.332	0.259
GIR7	small	25-26	0.374	0.423	0.178	0.633	1.285	1.772	0.395	0.128	0.119	-
GIR8	small	25-26	0.370	0.403	0.172	0.624	1.194	1.685	0.404	0.091	0.138	-
GIR9	small	25-26	0.347	0.387	0.168	0.662	1.206	1.740	0.455	0.095	0.157	-
GIR10	small	25-26	0.362	0.404	0.167	0.683	1.258	1.696	0.431	0.091	0.146	-
GIR11	small	25-26	0.297	0.344	0.166	0.582	1.201	1.585	0.367	0.091	0.128	-
GIR12	small	25-26	0.361	0.404	0.171	0.700	1.299	1.781	0.404	0.082	0.126	-

B) ID	TL	BW	JW	LDHL	LPHL	LTL	LFL	ED	ND	ENDr
froglet	1.867	0.888	0.819	0.968	0.952	0.480	0.899	0.437	0.195	2.241

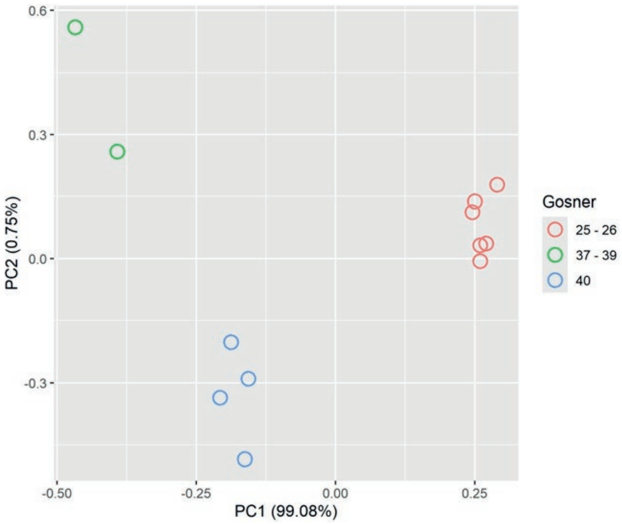

Fig. 3. Principal component analysis performed using tadpole measures in Table 1A, excluding nostril distance (ND), which were not measured for Gosner's 25 – 26 tadpoles. Colours represent Gosner's developmental stages, as indicated in Table 1A.

where *R. latastei* metamorphosed as usual in early summer. Additionally, June–September temperatures of 2024 (mean air temperature ± SD: 29.7 ± 5.1 °C) were markedly higher than those experienced over the typical growing season (March–June temperatures: 21.4 ± 6.1 °C), making it extremely unlikely that overwintering individuals originated from a late-winter 2024 deposition that experienced developmental delays due to low temperatures.

Whatever the period of deposition, the present report reveals an unusual case of shift in breeding phenology for *R. latastei*, which calls for further investigations assessing the frequency of these events and unravelling drivers and potential implications of such a shift. Indeed, variation in reproductive timing can result in crucial consequences for individual life-history and survival (Bison et al., 2021; Enriquez-Urzelai et al., 2022). For instance, amphibians developing during winter may face mass mortality risks due to freezing or desynchronization with trophic resources and increase competition (Li et al. 2013); moreover, in explosive breeders, such as wood frogs, these shifts can also trigger cascading effects on amphibian populations and freshwater community dynamics, with complex and rarely predictable outcomes (Todd et al., 2011). This first record of overwintering tadpoles in the Italian agile frog may represent an anomalous or isolated event, but correlation between increasing temperatures, altered precipitation patterns, and shifts in amphibian breeding period have been observed in multiple species as a response to global warming (Todd et al., 2011; Ficetola and Maiorano, 2016). Under the ongoing climate change scenarios, amphibian phenology is expected to be considerably impacted worldwide, and variations in their reproductive activity should deserve particular attention. Further research should aim at unravelling physiological mechanisms regulating shifts in breeding phenology and their implications on amphibian life-history traits and fitness. In light of the present case, autumnal monitoring of the study site and surrounding areas will be planned during the incoming years to ascertain the occurrence of *R. latastei* depositions outside the typical reproductive season. This study case also stresses the importance of constant monitoring of amphibian activity and phenology in a climate change scenario, calling for increased surveillance to implement appropriate conservation strategies.

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SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at: https://oaj.fupress.net/index.php/ah/article/view/17665/14575.

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Trends and main characteristics of impacted herpetological journals

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Abstract. Herpetological journals publish papers dealing with the study and conservation of amphibians and non-avian reptiles. Journals in this field are listed under the "Zoology" subject category of the Journal Citation Reports (JCR) and are assigned a two-year and a five-year impact factor (IF2 and IF5, respectively). These are among the most widely used indicators for bibliometric and academic evaluation. In this study, I present an overview of some general and bibliometric characteristics of the 18 herpetological journals listed in the 2025 JCR database. Specifically, I analysed the temporal trends of the IF2 over the 2013-2024 period. The analysis showed that most of these journals (78%) maintained a relatively constant IF2 during the study period, while four (22%) exhibited an increasing trend. I also tested if a relationship between the IF5 and the number of issues, the number of citable items, or the percentage of gold open access items per year was present. However, no significant relation was observed at least for these 2024 bibliometric indexes. Finally, journals published by scientific societies showed a significantly higher IF5 ($P < 0.03$), in comparison to other journals. Therefore, choosing these journals when submitting herpetological-focused manuscripts should be more considered.

Keywords. Amphibians, herpetology, Journal Citation Report, impact factor, reptiles, zoological journals.

The word herpetology is derived from the ancient Greek verb *ἕρπειν* (pronunciation hérpein) that means "to creep" or "to crawl" (Schlesinger, 1911). Historically, the zoological discipline of Herpetology pools and studies two terrestrial clades of cold-blooded Tetrapoda: amphibians and (non-avian) reptiles (Shine, 2014). These two animal taxa generally share a relatively small body size, low rates of energy consumption, and are often sampled in the field by overlapping or similar methods (e.g., Dodd, 2010, 2016). Therefore, the exclusion of birds (avian reptiles) makes of Herpetology an artificial category that, however, it is still considered useful from both an ecological and a practical point of view (Shine, 2014). Jointly, amphibians and reptiles are called herptiles or herps, while the whole amphibian and reptile community living within an ecosystem or a geographic defined area is named herpetofauna. For these reasons, academic societies, conferences and scientific journals dedicated to amphibians and reptiles are called "herpetological".

When speaking about scientific journals several electronic databases and different bibliometric indexes describe and rank the scientific interest or status of academic journals for librarians, readers and authors (e.g., Pajic, 2015; Lazarides et al., 2023). The most influential indicators are usually those calculated upon citation rates based on international bibliographic databases. Currently, among the many proposed bibliometric indicators, the most popular is probably the two-year journal Impact Factor (IF2), published since 1975 for journals listed in the Journal Citation Reports (JCR), now edited by Clarivate (Garfield, 2006). This indicator is obtained yearly for each journal as the total number of citations enumerated in the considered year divided by the total number of citable items published in that journal during the previous two years (Hubbard and McVeigh, 2011).

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In this note, I retrieved several characteristics of impacted herpetological journals listed in the JCR database. I also analysed the IF2 level of variability and its recent temporal trend during the 12-year period 2013-2024. In addition, I evaluated if the journals' IF5 was correlated to specific features, such as the number of issues published per year and the total number of citable items. I also evaluated if bibliometric differences were characterising journal published or edited by scientific societies and those owned by private companies. The scope of this note was not to evaluate editorial policies, accountability or transparency as these aspects were already examined by Marshall and Strine (2021), or to endorse a specific ranking for herpetological journals, but to give a general overview about impacted herpetological journals to better understand their status and temporal trends.

I retrieved all the herpetological journals listed in the 2025 JCR subject section Zoology. From this database, I collected for each journal the first year in which the journal was listed in the JCR, the number of issues per year, the total number of citable items, the five-year impact factor (IF5), the percentage of gold open access (GOA) items published per year (i.e., published articles that are freely and permanently available online without any cost for readers), and the IF2 scores over the period 2013-2024. Moreover, I retrieved the journal publisher (scientific society or private company) and its nationality. From the journals' official websites, I also obtained information about the journal aims and scope and the submission procedure (i.e., through an online portal or by emailing directly the editors). The presence of temporal trends in the 12-year IF2 was evaluated by means of the Mann-Kendall nonparametric test (Zhang, 2021). I analysed if the number of issues per year, the number of citable items per year and the percentage of golden open access items (% GOA) were correlated to the journal five-year impact factor (IF5) values by means of Spearmans' rank correlation coefficient. A generalised linear model with binomial distribution (i.e. trend present or absent) and logit link function was used to assess if the year of first listing on JCR site was related to the presence of a positive IF2 trend. Differences between journal published or owned by scientific societies or private companies were assessed by Mann-Whitney non-parametric test (M-W). Statistical analyses were performed using PAST software 5.02 (Hammer et al., 2001) and statistical significance was set at $\alpha = 0.05$.

In the 2024 JCR database 18 journals dedicated to the study of amphibians and/or reptiles were listed in the subject category "Zoology", which contains overall 183 journals, (Table 1; Supplementary Material Table S1). Among these 18 journals, 13 included in their titles a word derived from *ἕρπειν* such as*:* herpetology, herpetozoa, herpetological and *herpetologica* (in Latin). Two journals were named after specific genera of amphibians, two explicitly referenced amphibians and reptiles, while one was exclusively dedicated to chelonians and therefore, dedicated only to turtles, terrapins, and tortoises. Seven journals were published in the United States, two each in Brazil and England, and one each in Austria, Germany, Italy, Japan, the Netherlands, the People's Republic of China, and Russia (Table 1).

The JCR database listed for the first time three herpetological journals in 1997, and the most recent addition occurred in 2021, following a title change (Supplementary Material Table S1). The aims and scope of 16 out of the 18 journals were globally inclusive and accepted manuscripts dedicated to all groups of herptiles from all regions of the world. In contrast, one journal focused exclusively on herptiles from the African region, while another was dedicated exclusively to chelonians (Supplementary Material Table S1). Submission procedures were diverse: 14 journals are using an online portal, while the remaining four are requiring submissions by direct emailing the appropriate editors (Table 1). The number of issues published per year ranged from 1 to 4, with half of the journals producing four annual issues.

There were no significant correlations between the IF5, and the number of issues per year, the number of citable items per year and the % GOA items (Spearman's rank correlation: $\rho = 0.325$, $P = 0.188$, $\rho = 0.061$, $P = 0.811$ and $\rho = -0.181$, $P = 0.472$, respectively).

Four journals showed a significant positive trend of their IF2 while the IF2 of 13 journals remained constant over the study period (Table 1). Although the sample size was relatively small, there was some evidence that a significantly increasing IF2 trend was related to the year in which the journal was first listed on JCR. In fact, all increasing trends were observed only for journals listed after 2010 (Table 1; Supplementary Material figure S1). Society-owned journals possessed a slight but significant higher IF5 in comparison to private-owned (1,3 versus 0.9, respectively; M-W: $U = 15$, $z = 2.112$, $P = 0.029$), but there were no differences concerning the number of issues per year (M-W: $U = 28$, $z = 0.982$, $P = 0.328$).

From this analysis it emerges that herpetological journals listed in the 2024 JCR are showing highly heterogeneous and variable scenarios. For example, there are journals that do not use an online platform for the submission of manuscripts, and the GOA proportion of articles showed a huge variation, ranging from 0 to 100% (Table 1).

Recently the real value of the journal impact factor as a "quality" indicator has been questioned, especially when zoological taxonomy journals are concerned (e.g., Pinto et al., 2021). This is the reason why I focused on general fea-

Table 1. Main bibliometric features of Herpetological Journal listed in the Journal Citation Report (ICR) 2024, obtained in June 2025. % GOA = golden access articles; IF5 = five-years impact factor; IF2 trend calculated by means of Mann-Whitney rank statistical test; * indicates a significant trend < 0.05.

Journal	Scientific Society	Nationality of Publisher	First JCR	IF5	Citable Items	% GOA	Issues per year	IF2 trend P
Acta Herpetologica	Societas Herpetologica Italica	Italy	2010	0.9	50	100	2	0.137
African Journal of Herpetology	Herpetological Association of Africa	England	2007	0.9	44	27	2	0.266
Amphibia-Reptilia	Societas Europaea Herpetologica	Netherlands	2000	1.6	128	24	4	0.299
Amphibian and Reptile Conservation	-	USA	2016	1.0	36	0	3	0.038*
Asian Herpetological Research	-	PR China	2011	1.2	76	7	4	0.019*
Chelonian Conservation and Biology	-	USA	2007	1.0	86	1	2	0.090
Current Herpetology	-	Japan	2017	0.7	60	15	2	0.054
Herpetologica	Herpetological League	USA	1997	1.8	86	0	4	0.158
Herpetological Conservation and Biology	-	USA	2010	0.9	156	0	3	0.721
Herpetological Journal	British Herpetological Society	England	1997	1.1	64	16	4	0.725
Herpetological Monographs	Herpetological League	USA	2001	2.1	10	0	1	1.000
Herpetozoa	Österreichische Gesellschaft für Herpetologie	Austria	2010	1.1	113	98	4	0.830
Ichthyology and Herpetology (from 2021)	American Society of Ichthyologists and Herpetologists	USA	2021	1.7	144	15	4	NA
Journal of Herpetology	Society for the Study of Amphibians and Reptiles	USA	1997	0.9	136	1	4	0.828
Phyllomedusa	-	Brazil	2015	0.9	63	98	2	0.008*
Russian Journal of Herpetology	-	Russia	2015	0.8	113	0	4	0.002*
Salamandra	Deutsche Gesellschaft für Herpetologie und Terrarienkunde	Germany	2012	1.6	64	1	4	0.351
South American Journal of Herpetology	Brazilian Society of Herpetology	Brazil	2015	1.1	66	0	3	0.431

tures and temporal trends, rather than on absolute values and relative ranking within the JCR Zoology subject list.

One of the main findings of this study concerns the analysis of the IF2 temporal trend of herpetological journals listed in the JCR. In fact, most of these journals showed constant impact factor values over the 12-year period, while only four journals possessed significantly increasing values. In fact, these journals were among the most recently listed on the JCR portal, suggesting that their starting IF2 values were relatively low and that they are still increasing and have not reached the asymptotic values for their impact factor indexes. Finally, the absence of correlation between the number of issues, the total number of citable items and the percentage of gold open access items per year with the journal IF5 seems to suggest that, at least in the subcategory of Herpetology, journals are cited more on the specific merit and quality of papers rather than on a mere quantitative basis. The strategy of publishing more issues or more papers per year to increase visibility and bibliometric indexes does not seem successful, at least for the herpetological journals analysed. Finally, the outcome that society-published herpetological journals were showing a higher IF5 in comparison to private-owned journals appears surprising. Usually, society-published journals provide rigorous peer review by experts, and possess ethical, and transparent standards which enhance the credibility of their papers. For this reason, these society journals should be more considered when selecting for a publication venue for herpetological-focused manuscripts, in addition to other economic, academic and ethical considerations (e.g., Chytrý et al., 2023; Dolan et al., 2024).

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SUPPLEMENTARY MATERIAL

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Trophic plasticity and novel predator-prey interaction between two introduced snakes in a Mediterranean island

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Abstract. We report the first documented case of interspecific ophiophagy by the naturalised false smooth snake (*Macroprotodon mauritanicus*), preying on the introduced horseshoe whip snake (*Hemorrhois hippocrepis*) in Mallorca (Balearic Islands, Spain). The event occurred in a suburban garden and was photographically recorded. While *M. mauritanicus* is known to consume reptiles, interspecific ophiophagy has not previously been documented. This observation represents a novel trophic interaction between two introduced colubrids. It was likely facilitated by the increasing local abundance of *H. hippocrepis* and by the ecological constraints of insular systems. Together with a previous report of avian predation on *H. hippocrepis* in Mallorca, it suggests that both native or naturalised predators may be incorporating this species into their diets. This finding adds new information on the trophic ecology of *M. mauritanicus* and highlights the dynamic nature of predator-prey interactions in island ecosystems, potentially involving juvenile individuals due to size-related constraints.

Keywords. Interspecific ophiophagy, invasion dynamics, insular ecology, biotic resistance, Colubridae.

Introduced snakes are widely recognised as drivers of ecological disruption on islands, where native faunas often lack evolutionary defences against novel predators. Well-documented cases involving invasive colubrids include *Boiga irregularis* (Bechstein, 1802) in Guam (Rodda and Savidge, 2007), *Lampropeltis californiae* (Blainville, 1835) in the Canary Islands (Piquet and López-Darias, 2021), and *Hemorrhois hippocrepis* (Linnaeus, 1758) in Ibiza (Hinckley et al., 2016; Montes et al., 2022). In each case, the arrival of an invasive colubrid had measurable impacts on native species and predator-prey dynamics, illustrating the ability of introduced snakes to establish novel trophic interactions and restructure island ecosystems. Despite this, little is known about interactions between non-native colubrids, particularly when both species are introduced.

The island of Mallorca, part of the Balearic archipelago, has experienced multiple reptile introductions, including the colubrid *Macroprotodon mauritanicus* (Guichenot, 1850), currently considered naturalised (Pinya and Carretero, 2011). This species was probably introduced to Mallorca and Menorca by the Romans in the 2nd century BCE (Pleguezuelos et al., 1994), as supported by its current distribution, genetic affinity with Northwest African populations (Carranza et al., 2004), and historical land-use patterns. A more recent arrival, *H. hippocrepis*, is considered an introduced species in Mallorca under Spanish Law 42/2007. First recorded in 2006 and linked to the ornamental olive tree trade (Álvarez et al., 2010), its presence is now well established on the island (Pinya and Carretero, 2011). Beyond the main island, this species has reached several small islets inhabited by endemic and protected species such as *Podarcis lilfordi* (Günther, 1874) (Ayllón, 2015), and has been implicated in the extinction of at least one population of this endemic lizard in Mallorca (Picó et al., pers. comm.). Unpublished reports

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from the Government of the Balearic Islands also suggest impacts on other *P. lilfordi* populations in islets around Ibiza and Mallorca. Management efforts, mainly through snake-trapping campaigns and legislative measures, have so far been ineffective in containing this species (Febrer-Serra, 2023). Meanwhile, ecological interactions among introduced reptiles remain poorly documented and largely overlooked in conservation planning.

On the Iberian Peninsula, predation on *H. hippocrepis* has been reported primarily from birds of prey (Feriche, 2017), although occasional cases involving reptiles have also been recorded, including *Timon lepidus* (Daudin, 1802) (Pleguezuelos, 1998) and *Malpolon monspessulanus* (Hermann, 1804) (Díaz-Paniagua, 1976). In the Balearic Islands, studies indicate that *Macroprotodon* spp. (Guichenot, 1850) feeds mainly on small mammals (*Mus* spp. Linnaeus, 1758) and reptiles (*Podarcis siculus* (Rafinesque-Schmaltz, 1810) and *Tarentola mauritanica* (Linnaeus, 1758), with occasional records of avian and invertebrate prey (Mayol, 1985; Barbadillo, 1987; Pleguezuelos et al., 1994). Therefore, *M. mauritanicus* may be considered a euryphagous species, and ophiophagy is not ecologically unexpected. Consistent with this, intraspecific ophiophagy has been previously reported both in Mallorca (Capellà et al., 2011) and Lampedusa, Italy (Faraone, 2020). However, interspecific ophiophagy by *M. mauritanicus* has never been documented to date.

On 27 May 2025, at approximately 11:30 h, an adult *M. mauritanicus* was observed preying on a juvenile *H. hippocrepis* in the garden of a private residence, near a greenhouse in a suburban area of Sa Casa Blanca in Mallorca (39.5835° N, 2.7474° E; WGS84, 23.12 m a.s.l.). The garden features ornamental vegetation and a maintained lawn adjacent to the greenhouse. The two snakes were discovered on the lawn, beside a stone wall bordering the structure, with *M. mauritanicus* tightly coiled around *H. hippocrepis*, in an apparent act of constriction. The juvenile *H. hippocrepi*s appeared lifeless, and the predator was initiating ingestion head-first. The snake showed no defensive reaction to the observer's presence and continued swallowing its prey without interruption. After completing ingestion, it retreated into the stone wall. The observation was made by Arnau, X. and documented photographically (Fig. 1).

The surrounding area of the observation includes a small area of Mediterranean shrubland and cultivated fields. Less than 250 meters away lies a water reservoir with a storage capacity of 11,000 m^3 and an average depth of 3 meters. This reservoir, the largest freshwater body in southwestern Mallorca, is listed in the Balearic Inventory of Wetlands as Bassa de rec de Son Artigues (code MAZHA08; Govern de les Illes Balears, 2023).

Fig. 1. Predation of a juvenile *H. hippocrepis* by an adult *M. mauritanicus* in Sa Casa Blanca (Mallorca, Spain). Photo: X. Arnau.

This case represents the first documented instance of interspecific ophiophagy by *M. mauritanicus*. It involved the incorporation of a recently established introduced prey into the diet of a naturalised predator, suggesting a functional trophic response to a novel resource within the insular ecosystem. Such interactions may arise from increased encounter rates and ecological overlap, particularly in simplified island communities. This observation therefore expands the known trophic plasticity of *M. mauritanicus* and highlights the dynamic nature of predator–prey relationships involving introduced reptile species.

Although both species coexist in parts of North Africa and the Iberian Peninsula, no predation events have been reported from those regions. This may reflect low encounter rates, possibly related to lower population densities or habitat segregation. By contrast, in Mallorca, the expansion of *H. hippocrepis* has likely increased its local density, enhancing the probability of trophic interactions with resident predators. Notably, the present case involved a juvenile individual, consistent with the pronounced body size disparity between the two species: while adult *H. hippocrepis* can exceed 1.5 meters in length (Feriche, 2017), *Macroprotodon* spp. rarely surpasses 60 cm (Pleguezuelos and Vasconcelos, 2015). Thus, such predation events are probably restricted to the juvenile segment of the *H. hippocrepis* population rather than indicating a generalised dietary shift. This finding highlights how insular contexts, characterised by confined space, simplified food webs, and elevated encounter rates, may facilitate interactions that are rare or absent in continental ecosystems.

This pattern aligns with broader ecological principles observed in insular environments, where limited space, reduced species diversity, and the absence of specialist predators or competitors often promote ecological release and novel interactions (Carlquist, 1974). In such simplified systems, generalist species may expand their dietary niches or adjust behaviours in response to increased encounter rates with available prey (Savidge, 1987; Fritts and Rodda, 1998; Henderson and Powell, 2009). The observation of interspecific ophiophagy in *M. mauritanicus* may therefore reflect a context-driven shift in foraging strategy, shaped by the ecological constraints and opportunities of Mediterranean islands such as Mallorca.

Although based on a single event, this record suggests that *M. mauritanicus* could exert some predation pressure on local populations of *H. hippocrepis* in Mallorca. As a naturalised and generalist predator, it may be exploiting the growing abundance of this alien species. In island ecosystems, it is not uncommon for introduced predators to assume new functional roles, especially when food resources are limited and novel prey becomes available (Fritts and Rodda, 1998). While the ecological impact of this interaction remains uncertain, the incorporation of juvenile *H. hippocrepis* into the diet of resident predators could influence invasion dynamics if such events occur more frequently. Additional records will be necessary to determine whether this represents a sporadic occurrence or a recurring ecological interaction.

A previous observation of *H. hippocrepis* predation by a grey heron (*Ardea cinerea* Linnaeus, 1758) in Mallorca (Solà et al., 2015) supports the idea that native or naturalised predators may be beginning to incorporate this introduced colubrid into their diets. Together, these cases suggest the emergence of novel trophic pathways involving *H. hippocrepis*, with potential implications for its long-term establishment and spread on the island.

Beyond documenting a single event, this finding contributes to the broader discussion on predator flexibility, the formation of new food-web links, and the dynamics of biological invasions in islands. Continued monitoring and reporting of such interactions will be essential to clarify how introduced species reshape ecological communities and to inform effective conservation and management strategies in insular ecosystems. This case also underscores important conservation implications. The emergence of new trophic interactions among introduced predators and prey can influence invasion trajectories and the stability of insular food webs. Understanding these dynamics is essential for anticipating cascading effects on native fauna and for developing effective management responses in Mediterranean islands.

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Contributing to the breeding phenology of a temperate nocturnal gecko

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Abstract. This short descriptive note updates and summarises knowledge on the breeding phenology of the nocturnal Gekkota *Euleptes europaea*. A forty-year dataset covering the entire range of the species is provided. Between 1982 and 2024, a total of 39 populations were studied in 85 sampling sessions. Reproduction is seasonal, with gravid females appearing from early spring until mid-June when oviposition begins. Hatching occurs from mid-August to early October.

Keywords. Ectotherm, *Euleptes europaea*, gravid, hatchling, Sphaerodactylidae, squamate.

Understanding the natural history of species, particularly the timing of their life cycles, is critical in a warming world (Parmesan et al., 1999; Ibáñez et al., 2010; Forrest, 2016). Because temperate ecosystems are highly seasonal and reproduction is costly, animals are expected to invest in reproduction when both abiotic and biotic conditions are optimal (Brown and Shine, 2006). In squamates, developmental success is strongly related to temperature and, especially in oviparous species, to moisture. In temperate latitudes, temperature and relative hygrometry vary seasonally. As stated by Van Dyke (2015), the reproductive success of squamates is maximised when reproduction is initiated at the right time of the year. Seasonal variations in temperature and precipitation provide direct cues to the environmental conditions favourable to reproduction, while photoperiod provides an indirect metric of season and should be correlated, at least in part, with seasonal changes in temperature and humidity. Accordingly, squamates appear to use all three as seasonal cues to reproduce (Van Dyke, 2015). The thermal constraints are particularly critical for strictly nocturnal ectotherms living in temperate regions, i.e., in thermal deficit for most of their annual activity period (Saint-Girons and Saint-Girons, 1956; Chukwuka, Monks, and Cree, 2023). Among the Gekkota, all kinds of reproductive strategies have been observed, even in sympatry, ranging from the continuous to the highly cyclical (Vitt, 1986; Righi, Nascimento, and Galdino, 2012)

The aim of this note is to assess whether the reproduction of a nocturnal gecko, *Euleptes europaea*, is seasonal, as it would be expected since females of most temperate reptile species reproduce during the warmest part of the year (Pianka and Vitt, 2003). However, given the efficient thermoregulatory abilities of this gecko (Delaugerre, 1984) and its dessication-resistant hard-shelled eggs (Mourgue, 1910), one might expect reproductive activity to be less dependent on temperature and humidity, and spread over weeks or months, but still seasonal rather than continuous (Brown and Shine, 2006).

E. europaea is a strictly nocturnal, small-sized, Mediterranean gecko mainly living on islands. Along with suitable climatic conditions, the availability and the quality of retreat sites and egg laying sites (narrow rock cracks) are likely the key factor governing its presence and its abundance. It is no longer considered a pure rock specialist, as recent studies have shown it to be arboreal as well (Salvi et al., 2023; Deso et al., 2024). For comprehensive information on the natural history and the dis-

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tribution of the study species, see Salvidio, Lanza, and Delaugerre (2010).

The current state of knowledge on the phenology of this species is based on Knoepffler (1973) studies carried out on the island of Port-Cros (Provence) in the 60s and on Delaugerre (1981) studies on Port-Cros in 1978 and 1979. On Port-Cros, mating takes place in March. Gravid females are observed from April to the end of July. Egg-laying begins in mid-May and continues until the end of June or July (two successive clutches). Hatching occurs 65-80 days after oviposition from late July to early October, a female usually lays a pair of eggs, hatchlings measure about 17 mm in Snout-Vent-Lenght (SVL).

In this study, we use a 40-year dataset based on the survey of populations across most of the species range, from Tunisia in the south to Provence in the north-west (from 37.3° to 43° north latitude), including the islands of Sardinia and Corsica (Table 1). The populations of Tuscany and Liguria were not part of the survey. Between 1982 and 2024, a total of 39 populations were studied in 85 sampling sessions (repeated sessions of some populations). Most of the populations studied are Mediterranean landbridge islets, except two from the continental island of Corsica and one from the 'large' island of Port-Cros. All of these localities are below 200 m above sea level, and 75% of them are below 50 m. All of the islets are uninhabited and often included in protected areas where access is prohibited. Although they are not subject to direct anthropogenic disturbance, they are affected by the effects of globalisation, such as climate change, rising sea level and colonisation by alien species. Observations were carried out from early April to mid-October. Sampling sessions lasted from 1 to 6 nights per population. Active geckos were searched for using battery-powered lamps, starting one or two hours after dusk and continuing until dawn if necessary. Geckos were carefully caught by hand and temporarily stored in bags. Animals were gently held between thumb and forefinger under the anterior members until their posture was in extension, and SVL was measured to the nearest mm using a digital caliper.

Females were checked for gravidity by visual examination of the belly, the eggs -even smaller than 2 mm-being visible through the thin skin (Figure 1). After examination, geckos were later released in the area of the original sighting. In order to avoid double-counting, when more than one night of prospection was planned, the animals were marked with non-toxic acrylic ink before being released. Gravidity was examined in 36 populations (68 sessions); censuses without females and those without examination of females for eggs were discarded [N = 2450 geckos, of which 729 adult females, of which 262 with visible eggs (Table S1)]. Limitation: It is possible that, just after the first egg-laying, very small eggs in formation may have gone unnoticed by some females.

The presence of newborn juveniles (Figure S1) with a SVL < 22 mm, i.e., aged less than 4-6 weeks (Delaugerre, 1981), was recorded together with the number of juveniles and subadults [N = 2276 geckos; 318 subadults, 487 juveniles of which 127 < 22 mm SVL] Populations without juvenile observations were discarded (Table S2). Limitation: at hatching, the newborns measure ≈ 17 mm SVL (weight ≈ 0.25 g). They grow rapidly in the first month (+ 4.5 mm) (Delaugerre, 1981; 2003). For this reason, we set the threshold at > 22 mm SVL, below which the young were considered to be only a few weeks old. Even if stature is highly variable from one microinsular population to another (Delaugerre and Cheylan, 1992; Delaugerre et al., 2019; Delaugerre and Corti, 2020), with tendencies towards gigantism and - rarely - dwarfism, we believe that these variations have little effect on these early stages of growth, as hatching size appears to be invariable between populations (M. Delaugerre pers. obs.). This statement might not be true for the dwarf population of Gargalu island.

So, to sum up; the two landmarks of reproduction in this study are: 1) observing the timing of egg-bearing females; the frequency of non-pregnant females marking the egg-laying period, and 2) measuring hatchlings in natural populations; the presence of newborns marking the hatching period.

Gravid females were observed from early April to early October. 258 females out of 439 (59%) were gravid between 2 April and 25 July, while 4 females out of 290 (1.4%) were gravid between 1 August and 14 October (Table S1, Figure 2)

All young age classes (sub-adults, juveniles and hatchlings under 22 mm SVL) were observed from early April to mid-October. From early April to the end of July, the juvenile class represented 18.7% of the population and hatchlings represented 9.9% of the juveniles, but only 3% if the Gargalu population is excluded from the analysis (N geckos 978, N juveniles 183, N hatchlings 18, N = 5 without Gargalu). From early August to mid-October, juveniles account for 23.7% of the population and hatchlings for 35.5% of the juveniles (N geckos 1298, N juveniles 307, N hatchlings 109); see Table S2 and Figure 3.

As this species is able to live above 1000-1500 m above sea level (Salvidio, Lanza, and Delaugerre, 2010), our results only apply to the phenology of the coastal populations.

At sea level, the European leaf-toed gecko doesn't actually go into brumation, it just slows down its activity during the coldest periods. Most adults are inactive

Table 1. Populations of *Euleptes europaea* studied. All localities are Mediterranean islets except 3 (*italics*)

Region	Population	Geographic coordinates	Date	Day/Month
Corsica	Gargalu	42°22’12”N 8°32’22”E	2-3/04/1985	3/4
Corsica	Toro G	41°30’33”N 9°22’53”E	14/04/2005	14/4
Provence	*Port-Cros*	43°00’18”N 6°23’49”E	23-26/04/2001	23/4
Corsica	Locca	41°53’09’N 08°36’03’E	30/04/2010	30/4
Corsica	Cornuta	41°37’55’N 09°22’07’E	26/05/2017	26/5
Provence	Gabinière	42°59’18”N 6°23’43”E	25/05/2016	25/5
Tunisia	Gallo	37°33’28”N 8°57’21”E	3-6/05/2008	3/5
Corsica	Gargalu	42°22’12”N 8°32’22”E	18-19/05/2022	18/5
Corsica	Lavezzu	41°20’25’N 09°15’15’E	6-9/05/2024	6/5
Corsica	*Falaises de Bonifacio*	41°22’37”N 9°10’34”E	22/05/2020	22/5
Corsica	Mezzumare	41°52’38’N 08°35’34’E	28/05/2024	28/5
Corsica	Nord Pinarellu	41°40’22”N 9°23’40”E	25/05/2017	25/5
Sardegna	Piana Tavolara	40°53’18”N 9°39’04”E	03/05/2022	6/5
Sardegna	Porco	41°10’27”N 9°27’39”E	29/05/2012	3/5
Corsica	Porro	41°53’20’N 08°36’22’E	29/05/2014	29/5
Sardegna	Spargiotto	41°15’00”N 9°19’25”E	08/05/2014	29/5
Provence	St Féréol	43°30’22”N 7°03’28”E	20/05/2016	8/5
Provence	Tradelière	43°30’56”N 7°04’23”E	21/05/2016	20/5
Corsica	*Scandula*	42°22’06”N 8°34’19”E	06/05/1982	21/5
Corsica	Lavezzu	41°20’25’N 09°15’15’E	2-4/06/2021	2/6
Corsica	Lavezzu	41°20’25’N 09°15’15’E	2-4/06/2010	2/6
Sardegna	Piana Tavolara	40°53’18”N 9°39’04”E	04/06/2024	4/6
Sardegna	Reulino Tavolara	40°52’39”N 9°40’17”E	04/06/2024	4/6
Corsica	Sperdutu G	41°22’12’N 09°18’18’E	15/06/2011	15/6
Corsica	A Botte	41°56’32”N 8°35’22”E	21-28/06/2011	28/6
Corsica	Brocciu	42°38’46”N 8°55’56”E	29/06/2012	29/6
Corsica	Cala d’Alga	41°52’56’N 08°35’55’E	26/06/2017	26/6
Corsica	Porro	41°53’20’N 08°36’22’E	20/06/2011	20/6
Corsica	Gargalu	42°22’12”N 8°32’22”E	28-29/06/2022	28/6
Corsica	Giraglia	43°01’30’N 09°24’24’E	21/06/2020	21/6
Corsica	Lavezzu	41°20’25’N 09°15’15’E	14-17/06/2011	14/6
Corsica	Lavezzu	41°20’25’N 09°15’15’E	14-15/06/2012	14/6
Sardegna	Piana Tavolara	40°53’18”N 9°39’04”E	14/06/2022	14/6
Provence	Rascas	43°00’52”N 6°23’21”E	17-18/06/2008	17/6
Sardegna	Reulino Tavolara	40°52’39”N 9°40’17”E	13/06/2022	13/6
Corsica	Sperdutu g	41°22’12’N 09°18’18’E	15/06/2011	15/6
Sardegna	Toro	38°51’40”N 8°24’34”E	28/06/2015	28/6
Corsica	Giraglia	43°01’30’N 09°24’24’E	07/07/2014	7/7
Corsica	Vacca	41°33’23’N 09°23’14’E	09/07/2012	9/7
Sardegna	Reulino Tavolara	40°52’39”N 9°40’17”E	10/07/2024	10/7
Sardegna	Piana Tavolara	40°53’18”N 9°39’04”E	12/07/2024	12/7
Corsica	A Botte	41°56’32”N 8°35’22”E	15/07/2023	15/7
Corsica	Gargalu	42°22’12”N 8°32’22”E	16;28/07/1990	16/7
Corsica	Garganellu	42°21’55’N 08°32’23’E	17/07/1986	17/7
Corsica	Lavezzu	41°20’25’N 09°15’15’E	29/06-4/07/2020	29/6
Sardegna	Molarotto	40°52’27”N 9°46’42”E	11/07/2024	11/7
Corsica	Palazzinu	42°22’47”N 8°33’01”E	19-20/07/1985	19/7
Corsica	Porragia G	41°23’30’N 09°15’47’E	25/07/2024	25/7
Corsica	Porri	42°23’10’N 08°34’55’E	23-24/07/1983	23/7

(Continued)

Table 1. (Continued).

Region	Population	Geographic coordinates	Date	Day/Month
Corsica	Porri	42°23’10’N 08°34’55’E	21/07/1986	21/7
Corsica	Toro G	41°30’33”N 9°22’53”E	24/07/2014	24/7
Corsica	Giraglia	43°01’30’N 09°24’24’E	02/08/2022	2/8
Corsica	Giraglia	43°01’30’N 09°24’24’E	07/08/2023	7/8
Corsica	A Botte	41°56’32”N 8°35’22”E	11/08/2022	11/8
Corsica	2nd rock Toro P	41°30’30”N 9°22’47”E	05/08/1986	5/8
Tunisia	Fauchelle	37°29’39”N 8°52’43”E	30/08/2022	30/8
Corsica	Gargalu	42°22’12”N 8°32’22”E	10-11/08/2023	10/8
Corsica	Garganellu	42°21’55’N 08°32’23’E	12/08/2023	12/8
Corsica	Giraglia	43°01’30’N 09°24’24’E	5;9/08/2012	5/8
Corsica	Lavezzu	41°20’25’N 09°15’15’E	02/08/1986	2/8
Corsica	Mezzumare	41°52’38’N 08°35’34’E	16/08/2012	16/8
Corsica	Palazzu	42°22’49”N 8°32’47”E	22/08/1986	22/8
Corsica	Porragia G	41°23’30’N 09°15’47’E	06/08/1985	6/8
Corsica	Porragia P	41°23’35’N 09°15’52’E	01/08/1986	1/8
Corsica	Ruscana	41°41’00’N 09°24’07’E	07/08/1986	7/8
Corsica	Sperdutu G	41°22’12’N 09°18’18’E	04/08/1986	4/8
Corsica	Toro G	41°30’33”N 9°22’53”	04/08/1986	4/8
Corsica	Vacca	41°33’23’N 09°23’14’E	2-4/08/1985	2/8
Corsica	Giraglia	43°01’30’N 09°24’24’E	29/08/2015	29/8
Corsica	A Botte	41°56’32”N 8°35’22”E	3-6/09/2010	3/9
Sardegna	Carpa	41°17’15”N 9°21’45”E	06/09/2011	6/9
Corsica	Giraglia	43°01’30’N 09°24’24’E	12/09/2000	12/9
Corsica	Ruscana	41°41’00’N 09°24’07’E	10-13/09/2012	10/9
Tunisia	Gallina	37°33’07”N 8°56’56”E	19/09/2024	19/9
Corsica	Gargalu	42°22’12”N 8°32’22”E	28/09/2023	28/9
Provence	Rascas	43°00’52”N 6°23’21”E	02/09/1985	2/9
Provence	Gabinière	42°59’18”N 6°23’43”E	13/10/2003	13/10
Corsica	Gargalu	42°22’12”N 8°32’22”E	04/10/2022	4/10
Corsica	Giraglia	43°01’30’N 09°24’24’E	04/10/2014	4/10
Corsica	Giraglia	43°01’30’N 09°24’24’E	06/10/2012	6/10
Corsica	Garganellu	42°21’55’N 08°32’23’E	14/10/2024	14/10
Corsica	Sperdutu g	41°22’12’N 09°18’18’E	1-2/10/1984	1/10
Corsica	Garganellu	42°21’55’N 08°32’23’E	01/10/2022	11/10
Provence	Gabinière	42°59’18”N 6°23’43”E	13/10/2010	13/10
Corsica	Ruscana	41°41’00’N 09°24’07’E	7-11/10/2008	7/10

and frequently only juveniles are active at air and substrate temperatures below 10° or 5°C (author pers. obs.). Mating takes place at the end of the winter and is facilitated by winter aggregations, which can consist of one or more dozens of individuals, depending of the size and the quality of the retreat site (Delaugerre, 2003). Most females are gravid from April to mid-July. Gravid females are the exception in autumn. Some may retain their late clutch throughout the winter. As in the case of a female with fully developed eggs, observed during nocturnal activity at the beginning of May 2021 in an alpine location of central Corsica (elevation of 1450 m asl, air temperature 3.7°C) author pers. obs. The first hatchings take place at the beginning of August and most occur from mid-August to early October. The 100% of juveniles below 22 mm SVL observed on the Corsican island of Gargalu (Table S2) in the early days of April were most likely not hatchlings, but juveniles around 6 months old with very low growth, as Gargalu are dwarf sized (Delaugerre and Cheylan, 1992). This dataset confirms and refines what was previously published from the single population of Port-Cros. Most females are gravid in April; oviposition lasts from mid-May to the end of July and hatchlings appear from

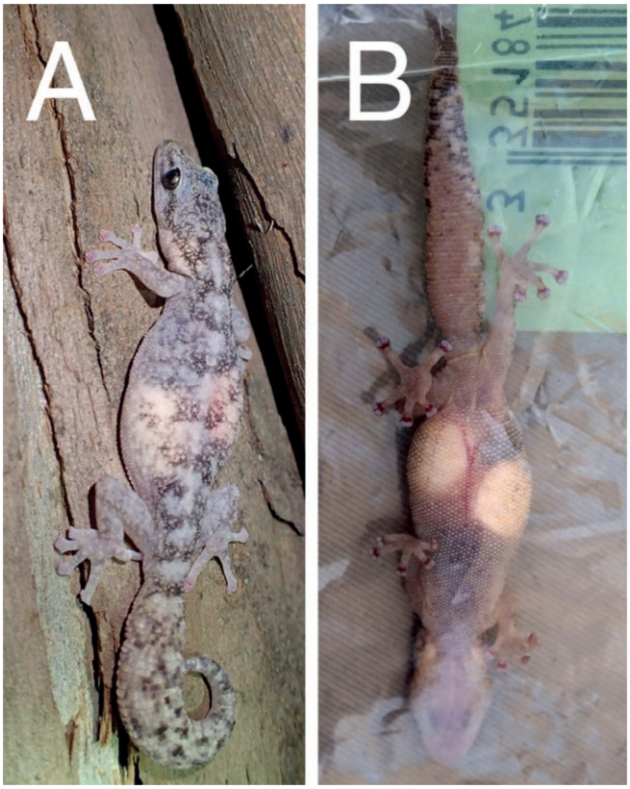

Fig. 1. Gravid *Euleptes europaea* females; the two eggs are clearly visible through the dorsal (A) and ventral (B) skin (in a plastic bag). A: ©Julien Renet, Levant island, Provence; B: ©Michel-Jean Delaugerre, Bonifacio, Corsica.

mid-August to early October. Growth appears to be constant for the first two years, then slows in the third year, when functional sexual maturity is reached in both sexes (Salvidio and Delaugerre, 2003). The timing of the reproduction is indeed seasonal, but it is spread out over several weeks. With the comprehensive data set provided here, further studies and meta-analysis will be able to investigate a change in the timing of reproduction in relation to variations in weather conditions (Marco and Pérez-Mellado, 1998) even night-time temperatures (Rutschmann et al., 2016), clinal variation or a seasonal shift induced by climate change (Parmesan et al., 1999; Henle et al., 2008; Beaumont et al., 2015; Ljungström, Wapstra, and Olsson, 2015).

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SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at: https://oaj.fupress.net/index.php/ah/article/view/17238/14577.

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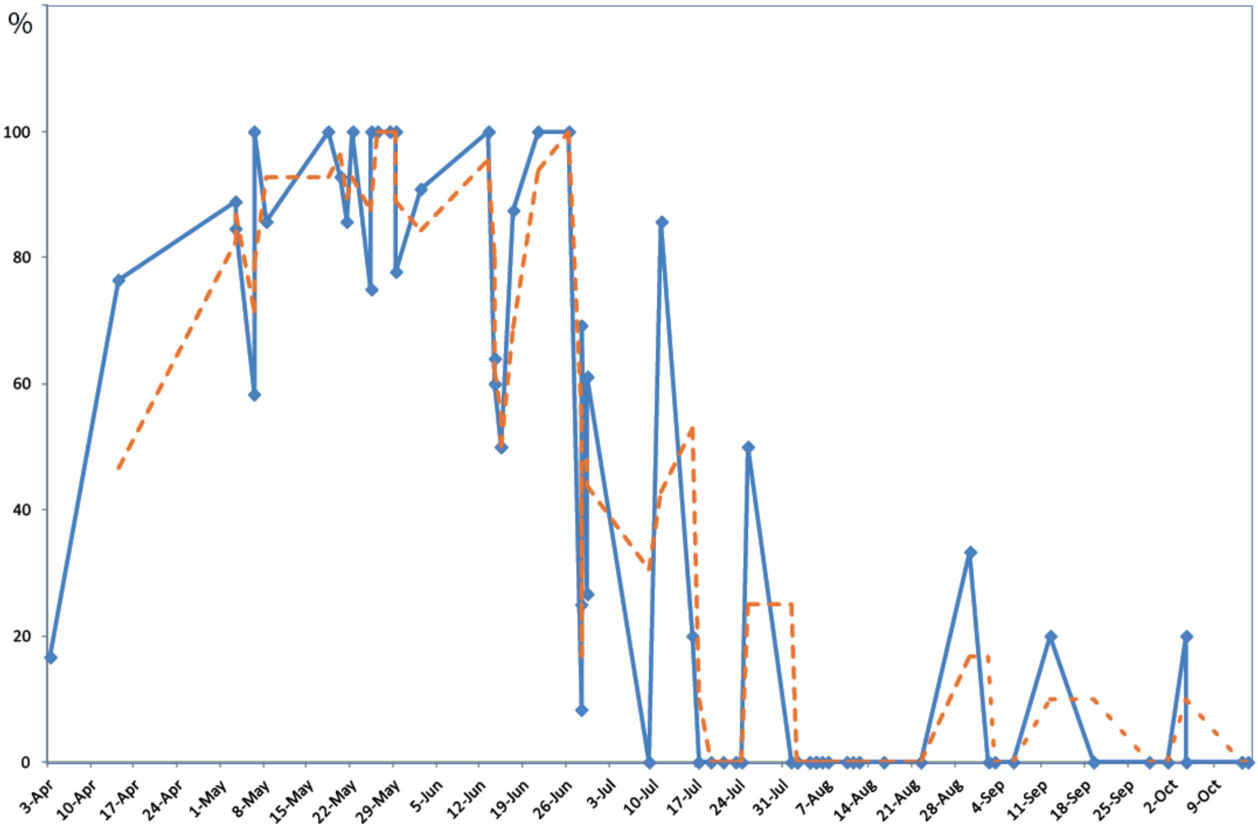

Fig. 2. Annual cycle of gravidity in female *Euleptes europaea* :68 surveys between 1982 and 2024; in 36 coastal populations in Tunisia, Sardinia, Corsica and Provence. A total of 2462 individuals, including 729 females, of which 262 were gravid. Dotted line: moving average. Data from Tab S1.

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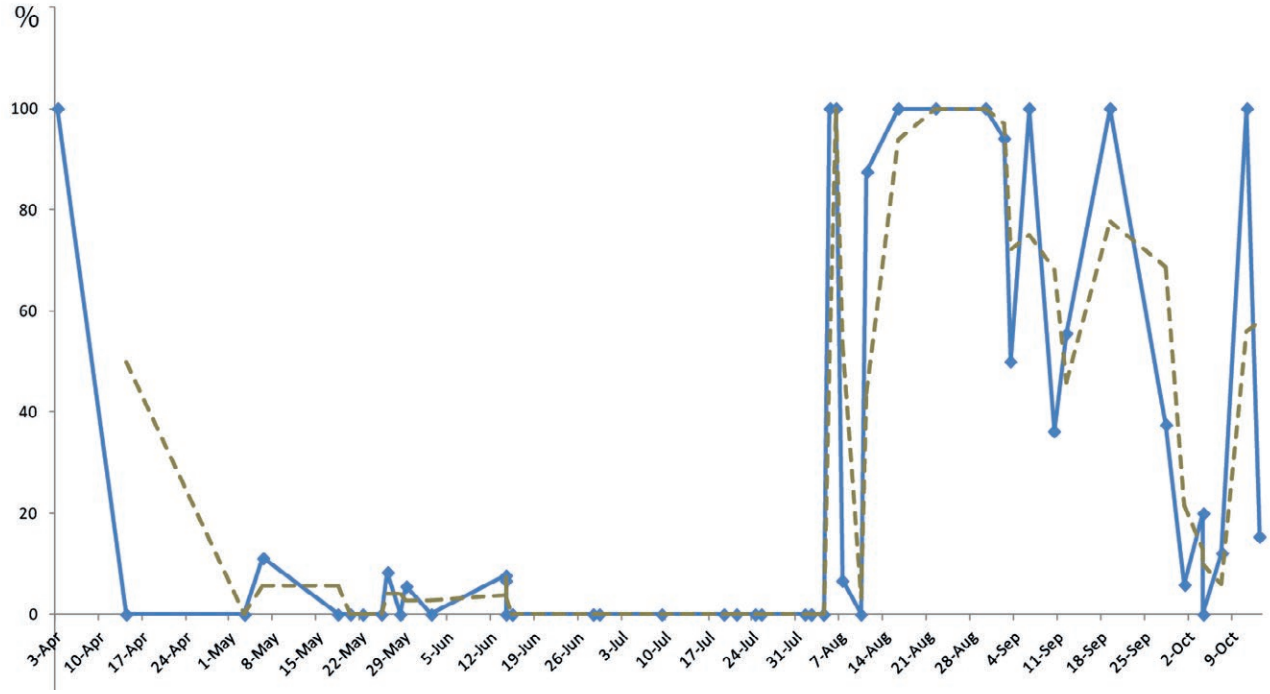

Fig. 3. Occurrence of newborn juveniles of *Euleptes europaea* along the annual cycle: 53 surveys between 1984 and 2024; in 29 coastal populations in Tunisia, Sardinia, Corsica and Provence. 2276 individuals in total, including 487 juveniles, 127 of which < 22 mm in SVL. Dotted line: moving average. Data from Tab S2.

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Shadowboxing salamanders: Defensive behavior in two Amazonian *Bolitoglossa* species (Amphibia: Plethodontidae)

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Abstract. Salamanders employ diverse anti-predator strategies, yet many tropical species remain poorly documented. We describe a previously unreported defensive display in two Amazonian plethodontids, *Bolitoglossa equatoriana* and *B. altamazonica*. During nocturnal surveys in the Apayacu Reserve, Ecuador, an individual of *B. equatoriana* perched on a leaf performed a striking "shadowboxing" display when illuminated: it repeatedly raised and waved its forelimbs while swaying from side to side for several minutes. A comparable behavior was observed in *B. altamazonica* in Peru after gentle handling for photography. Neither species exhibited skin secretions or bright warning colors during the display, although anecdotal reports suggest mild cutaneous reactions to *B. altamazonica* secretions. Shadowboxing may therefore function as a visual deterrent, complementing chemical defenses documented in other species of *Bolitoglossa*. Similar posturing is rare in salamanders, previously noted only in a few species such as *Cryptotriton nasalis*. These records expand knowledge of antipredator behavior in neotropical salamanders and underscore the importance of detailed natural-history observations for understanding behavioral diversity and survival strategies in Amazonian amphibians.

Keywords. Antipredator behavior, defensive display, Plethodontidae.

Salamanders use a lot of different ways to avoid being eaten, and these ways vary across species, individuals, and environmental contexts (Brodie, 1977). These strategies include behavioral responses, physical adaptations, and chemical defenses (Brodie, 1977; Arrivillaga and Brown, 2018). For instance, the Northern two-lined salamander, *Eurycea bislineata* (Green, 1818) responds to predator cues such as snake tongue-flicks by running, jumping, or staying still (Dowdey and Brodie, 1989). Running is the most common strategy in areas with a lot of predators (Ducey and Brodie, 1983). Savage (2002) described body-flipping as a defensive behavior shown by salamanders of the genus *Oedipina*, especially in reaction to violent disruptions or direct physical contact. To protect important body parts from predators, many salamanders take defensive positions. They commonly focus on their tails, which they can give up to escape (Myette, 2019). Some species also have bright colors on their bellies that act as a warning sign that their skin secretions are poisonous or unpleasant (Brodie et al., 1979). Although the chemical composition of the skin of most Caudata species is unknown (de Vasconcelos et al., 2021), the cyan newt, *Hypselotriton cyanurus* (Liu, Hu, and Yang, 1962) rolls onto its back to prominently display its warning colors (Brodie, 1977).

The genus Bolitoglossa, part of the family Plethodontidae, is the biggest group of salamanders. It can be

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found all over tropical America (Frost, 2024). These salamanders live in a wide range of places, from grasslands at high elevations to rainforests in lowlands, and may display a lot of genetic and phenotypic variety (Jaramillo et al., 2020). Many species, including *B. ramosi, B. rostrata*, and *B. subpalmata,* produce potent skin toxins that deter predators (Brodie et al., 1991; Medina et al., 2022). Other species in the genus secret viscous substances that may be toxic (*B. diaphora, B. conanti, B. dunni*; Arrivillaga and Brown, 2018). However, the chemical and behavioral anti-predator strategies of this genus are still not well documented.

This study documents an antipredator behavior not yet described for *Bolitoglossa equatoriana* and *B. altamazonica* in the Amazon rainforest of Ecuador and Peru, respectively. The Ecuadorian climbing salamander, *Bolitoglossa equatoriana (*Brame and Wake, 1972), is endemic to the western Amazon basin and is found in several provinces of Ecuador, including Napo, Orellana, Pastaza, Sucumbíos and Morona Santiago (Almendáriz et al., 2004). This species is common at night in the eastern forests of Ecuador (Raffaëlli, 2013). On the other hand, *B. altamazonica* (Cope, 1874) is restricted to just a few localities in Loreto, Peru (Cusi et al., 2020). The behavior of these species remains poorly understood, highlighting the need for natural history observations to be documented.

During a field expedition to the Apayacu Reserve (-1.067° S, -77.670° W, 400 m a.s.l.) in Tena Province, Ecuador, in November 2023, we found an individual of *Bolitoglossa equatoriana* during an active search for amphibians at night. The salamander was perched on a leaf, and as we approached with the light of a headlamp to observe it more closely, it began to perform a fascinating behavior, resembling shadowboxing movement (Fig. 1, Supplementary material Video 1). Shadowboxing refers to the act of striking into the air without an opponent, typically used to practice movements, refine technique, and simulate combat scenarios. The salamander individual raised both front legs and swayed back and forth while holding them up. This behavior continued for several minutes, during which the salamander intermittently rested its legs before starting the movement again. We observed this sequence on at least three occasions, totaling six minutes during the same encounter.

We observed the same behavior in *Bolitoglossa altamazonica* on 18 February 2014 in the Allpahuayo-Mishana National Reserve (-3.965° S, -73.421° W, 132 m a.s.l.). After handling the individual for laboratory photography (Figure 2), it exhibited the same defensive shadowboxing behavior. The display did not last more than a couple of minutes, and no secretions or other responses were observed after handling.

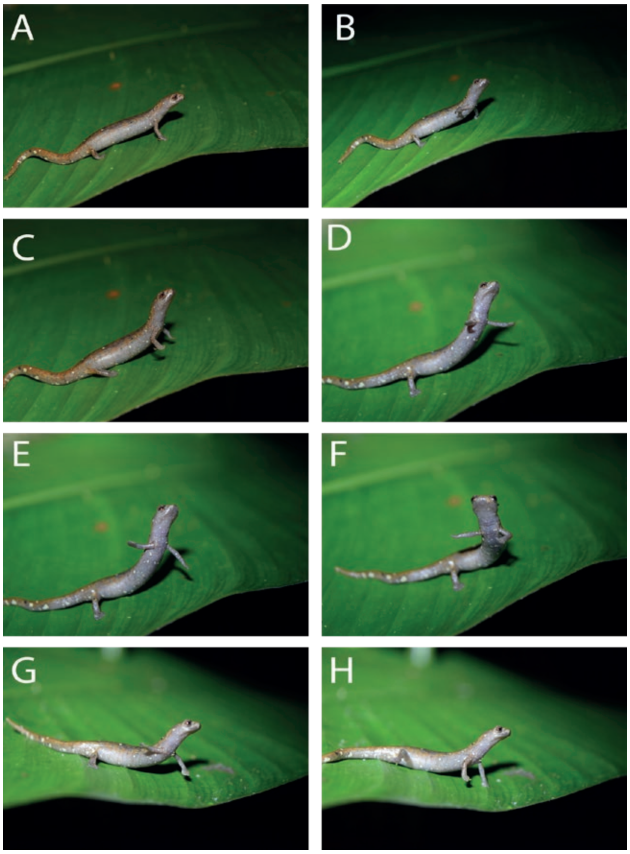

Fig. 1. Shadowboxing sequence (A-H) as a defensive posture in *Bolitoglossa equatoriana*.

The present record represents a novel behavior for these species. Similar posturing has also been observed in the Cortes salamander, *Cryptotriton nasalis* (Dunn, 1924), which adopts and maintains an elevated defensive posture when threatened (Arrivillaga and Brown, 2018). In many terrestrial salamanders, defensive postures are closely tied to the use of skin secretions. These may involve revealing bright warning colors on the underside or directing specialized skin glands toward the predator (Brodie and Gibson, 1969). Although these species lack bright colors, we have anecdotal evidence of allergic reactions to the secretions of *B. altamazonica.* After handling the reported individual, a localized sensation of heat appeared in the hand, which triggered redness. The sensation lasted from a few minutes to an hour, depending on the length of contact with the animal (*comm. pers.* Giussepe Gagliardi-Urrutia). This shadowboxing behavior suggests that these species rely on physical positioning to deter predators, emphasizing the role of different strategies in salamander survival. This finding not only enhances our understanding of the behavioral ecology of *B. equatoriana* and *B. altamazonica* but also emphasizes the importance of continued field

Fig. 2. Shadowboxing posture in *Bolitoglossa altamazonica.*

research to uncover the hidden complexities of amphibian life in the Amazon rainforest.

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SUPPLEMENTARY MATERIAL

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Bread and cookies: allotriophagy in a worm lizard (Squamata: Amphisbaenia)

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Abstract. Allotriophagy refers to the consumption of food items considered abnormal for a given species. In this note, we report the recurrent consumption of bread and cookie crumbs by a free-ranging worm lizard (*Amphisbaena* sp.) in Brazil. While there are a few isolated reports of worm lizards consuming unusual food items, this is the first documented case of such behavior in a wild specimen occurring over several months, potentially involving the same individual. Allotriophagy in wild worm lizards may be triggered by nutritional deficiencies or opportunistic feeding. Documenting these cases is important for advancing our understanding of worm lizard behavior, particularly in urban environments.

Keywords. *Amphisbaena*, diet, food, lizard, trophic ecology.

The diet of burrowing reptiles reflects their morphological adaptations and ecological flexibility (Pianka and Vitt, 2003; Vitt and Caldwell, 2014). Amphisbaenians (worm lizards), a group of snake-like squamates adapted for life underground (Gans, 1978) feeds mainly on arthropods and their larvae (López et al., 1991; Cruz Neto and Abe, 1993; Webb et al., 2000; Esteves et al., 2008; Amorim et al., 2024), although some species may be specialized in particular prey, such as gastropods (Pregill, 1984; Martín et al., 2013). Due to their elusive nature, studies on the diet of worm lizards are conducted through the dissection of preserved specimens from natural history collections, allowing for the examination of gut contents (White et al., 1992; Cruz Neto and Abe, 1993; Esteves et al., 2008). However, occasional observations of live specimens in natural and captive conditions provide insights into their feeding behavior, sometimes revealing unusual food choices (Navas et al., 2004; Moraes and Recchia, 2011; Aragão et al., 2019; Nascimento et al., 2019; Reiche et al., 2021; Bezerra et al., 2022).

Between April and September 2022, in the Central-West Region of Brazil, Alessandra Sá observed an *Amphisbaena* sp. individual in Goianésia (15.326°S, 49.120°W), state of Goiás. The animal was regularly seen in the morning near the sidewalk, between Alessandra's ice cream shop and a house behind it. The first sighting, on April 1st, 2022, was incidental – the worm lizard was found feeding on cookie crumbs scattered on the ground. On several subsequent occasions, the same individual (or likely the same) was seen emerging from an underground tunnel to feed on bread and cookies left on the sidewalk. However, it often remained hidden for 7 to 10 days before reappearing.

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It appears that, after the initial observation, the observer began intentionally offering food to the animal. It is important to note that these recordings were not part of a scientific experiment, but rather the result of casual observations made by a layperson with no formal training in biology, motivated by curiosity about the animal's behavior.

Due to the low quality of the images (Fig. 1), it was not possible to confirm the species with certainty. However, based on the animal's size and coloration, as well as the known distribution of Brazilian amphisbaenians (Colli et al., 2016; Guedes et al., 2020, 2023; Assis et al., 2022), the specimen most likely belongs to *Amphisbaena vermicularis* or *A. mertensii* (Gans, 1966; Gans and Amdur, 1966). The videos recorded are available as supplementary material.

Eating unusual food items has been observed in lizards from different regions around the world, such as captive *Gekko monarchus* eating bread (Weterings and Weterings, 2018), free ranging *Hemidactylus platyurus* eating rice, cucumber, and eggs from a trash bin (Weterings, 2017), *Teira dugesii* eating pieces of bread, cake, and other items accidentally dropped to the floor (Lunn, 1991), and *Trachylepis atlantica* eating cookie crumbs (Gasparini et al., 2007).

There is only one known report of amphisbaenians consuming unusual food items in a natural environment: sea turtle eggs (Nascimento et al., 2019). In contrast, most such observations come from urban settings, where individuals have been seen feeding on atypical materials such as an oil-soaked paper towel (Moraes and Recchia, 2011), chicken eggshells (Aragão et al., 2019), and even a cockroach significantly larger than the animal's head (Bezerra et al., 2022).

The behavior of consuming food items considered abnormal is known as allotriophagy (Bender, 2006). Allotriophagy is also often referred to as pica syndrome (Lewbart and Christian, 2007) and is better documented in humans (Walker et al., 1997) and domestic animals (Demontigny-Bédard et al., 2016; Santos Campos et al., 2024). In veterinary literature, this term is often used specifically to describe the ingestion of non-food items, distinguishing it from the opportunistic consumption of unusual but edible food items (Lewbart and Christian, 2007; Cardona et al., 2017; Popova and Mano, 2018; Santos Campos et al., 2024). However, considering a broader interpretation of the term (Bender, 2006), the recurrent consumption of unusual food items by *Amphisbaena* sp., which likely provides little to no nutritional value to the individual, can be classified as allotriophagy.

The consumption of unusual food items may be triggered by nutritional deficiencies or opportunism. In urban areas, the decrease of terrestrial arthropods (Buczkowski and Richmond, 2012; Fenoglio et al., 2020) and the presence of human food waste may push reptiles to explore alternative food sources. Additionally, the strong smells of processed foods can attract these animals, leading them to

Fig. 1. *Amphisbaena* sp. consuming cookie crumbs in April 2022 (A) and September 2022 (B) in Goianésia, state of Goiás, Brazil. Images captured from videos recorded by Alessandra Sá.

eat inappropriate materials (Lunn, 1991; Weterings and Weterings, 2018). Individuals of certain species can quickly become accustomed to consuming human-provided food on a regular basis. For instance, in Italy, a *Podarcis siculus* lizard was observed feeding on bread for five consecutive days at the same location where researchers had placed it (Valerioti and Sperone, 2024). A similar pattern was observed in the *Amphisbaena* sp. reported here. Although this observation spanned for months and occurred on non-consecutive days, the animal consistently appeared at the same location to feed, suggesting a learned habit of seeking out and consuming food items left by humans.

The consistent consumption of unusual food items can pose health risks to reptiles, potentially leading to death (Lewbart and Christian, 2007). In the case of sugar-rich foods, such as the bread and cookies consumed by the *Amphisbaena* reported in this study, there is evidence that their intake may cause changes in the immune system and gut microbiota (Ki et al., 2024), as well as other physiological and even behavioral alterations (French et al., 2022, and references therein). Regarding behavioral changes, the available evidence suggests that the presence of anthropogenic food in the environment did, in fact, has altered the foraging behavior of the *Amphisbaena*, prompting it to seek out these items on the surface with some regularity.

Documenting cases of allotriophagy in wild reptiles is essential, particularly in the Tropics, where such reports remain scarce. The limited availability of published data restricts our understanding of both the frequency of this behavior and the broader occurrence of unusual food consumption within the group. Further investigation into this subject may yield valuable insights into its ecological and physiological implications, enhancing our understanding of reptile feeding behaviors and the effects of unusual food items on trophic interactions. A particularly compelling question emerging from this framework concerns how the indirect consequences of allotriophagy influence both trophic and non-trophic ecological interactions.

SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at: https://oaj.fupress.net/index.php/ah/article/view/17538/14579.

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Cover: Adult female *Enyalioides heterolepis* on a nocturnal trunk perch from Gorgona Island, Colombia (photo by Álvaro Javier Fierro).

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