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Editorial

XI International Symposium on the Mediterranean Lacertid Lizards

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It has been a long time since an Editorial opened a new issue of *Acta Herpetologica* (it was 2012, volume 7, first issue). The occasion is to inform the readers that the journal of the *Societas Herpetologica Italica* is pleased to host, from the present issue on (and, possibly, in the coming two), some contributions presented during the last “International Symposium on the Mediterranean Lacertid Lizards”, held (on line, sigh!) in Lipari last September 2021.

The International Symposium on the Mediterranean Lacertid Lizards was conceived during the 1st World Congress of Herpetology held in Canterbury (UK) in 1989 to share scientific results, project ideas, conservation initiatives, recruit new enthusiastic students and, overall, enjoying what we are most passionate about studying Lacertid lizards in the Mediterranean and surrounding areas. The 1st Symposium took place in Mytilini, Lesvos (Greece) in 1992. The idea of informally joining scientists, students and conservationists across the Mediterranean and beyond in a stimulating ambient

was so successfully that it has become an appointment every, more or less, three years: Faro (Portugal), 1995; Čres (Croatia), 1998; Maó, Menorca (Spain), 2001; Lipari (Italy), 2004; Mytilíni, Lesvos (Greece), 2008; Palma, Mallorca (Spain), 2010; Koper, (Slovenia) 2013; Limassol (Cyprus), 2016; Tel Aviv (Israel), 2018. The last one has been hold online from the Island of Lipari (Sicily, Italy) in 2021 in an unusual form due to pandemic, but sharing the same spirit as always.

Considering that promoting the study of amphibians and reptiles and the growth of young herpetologists has always been also one of the SHI goals, AH has welcomed the request by the Organization Committee to give the opportunity to publish some of the contributions of the XI Symposium (according to the peer-review standard). You will find such contributions, along with the “normal” ones, marked as follows: “Presented at the XI International Symposium on the Mediterranean Lacertid Lizards, Lipari 27-28th September 2021”.

Enjoy the reading!

The directional testes asymmetry increases with temperature in seven plateau brown frog (*Rana kukunoris*) populations

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Abstract. Environmental stress is generally regarded as an important evolutionary force for promoting the differentiation of shape, structure and function of animal organs closely related to survival and reproduction. Geographical variation of temperature and corresponding change in intensity of male-male competition might drive inter-population differences in directional testes asymmetry (DTA). Here, we investigated inter-population variation in DTA of the brown frog (*Rana kukunoris*) at seven different altitudes on the eastern Tibetan Plateau. We found that the size of right testes increased with temperature, but not left testes. We also found that male age, body mass or body condition, and testis mass had not effect on DTA, suggesting that heavier or older *R. kukunoris* males or those with larger testes had not stronger DTA. The operational sex ratio did not affect DTA, but there was a positive correlation between DTA and temperature, suggesting that differences in the length of activity period and resources availability across locations may affect the energy budget of this frog, resulting in a gradual change in reproduction energy parallel to increasing temperature.

Keywords. Environmental factor, testes asymmetry, body condition, age, the brown frog.

INTRODUCTION

Environmental stress (e.g., resource availability, competition, or temperature) has generally been an important evolutionary force for promoting the differentiation of life-history traits (Blanckenhorn and Demont, 2004; Liao et al., 2015). For example, variation in energy acquisition under great differences in environmental conditions and environmental stress might lead to differences in the size, structure and function of animal organs and tissues among populations (Jönsson et al., 2009; Chen et al., 2011).

In most species of birds, the left testis mass is larger than the right one (e.g., Friedmann, 1927; Møller, 1994; Rising, 1996; Jamieson et al., 2007; Idriss et al., 2018; Sara et al., 2019). Møller (1994) hypothesized that

the increase in size of the right testis is only to compensate for a reduced function of the left one, and thus, the degree of directional testes asymmetry (DTA) in testis size is a measure of male body condition. Moreover, the degree of DTA is correlated with age in that the older males in a population because they could allocate more energy to reproduction than younger individuals (Birkhead et al., 1997; Graves, 2004). Thus, there is correlation between possibilities for energy acquisition and male quality, thus the energy acquisition might have a potential impact on the degree of (DTA). In recent years, although the testis asymmetry has been proved in some anuran species (Zhou et al., 2011; Liu et al., 2011, 2012; Mi et al., 2012; Yu and Guo, 2015; Wu and Liao, 2017), there only few studies have focused on exploring geographical variation in DTA (Hetttye et al., 2005).

Sperm competition may be attributed to drive the evolution of directional testes asymmetry. Males owning large testis mass indicate experiencing strong sperm competition when the male/female sex ratios was highly male-biased (Gage, 1994; Pitcher et al., 2005; Soulsbury, 2010; Zeng et al., 2014). For instance, compared to control treatments, males increased investment in testis mass, ejaculates or accessory glands when they lived at large population density or high male/female sex ratios (Gage, 1995; Tan et al., 2004; Ramm and Stockley, 2009). In this case, two larger testes may be more effective in increasing overall larger testes size because it might be very costly to produce directional asymmetry (Møller, 1994).

However, although relevant substantial data were collected, an understanding of the causes for geographical variation in DTA remains ambiguous and contentious. Hence, independent datasets, especially on different populations within species, that do help us to have a better understanding of the general geographical patterns of variation in male quality, age and DTA. In this study, we explore the occurrence of DTA in all study populations of the brown frog (*Rana kukunoris*), as well as the association between the degree of DTA and the body condition or age. This species is endemic to the eastern Tibetan plateau, inhabits open alpine marshes, and their habitats are located from 2200 to 4400 m in altitude. *R. kukunoris* deposits larger energy reserves in fat bodies and liver, but pre-hibernation energy stores decrease with increasing altitude (Chen et al., 2013). Further, degree of DTA was not related to altitude or body size across three populations of *R. kukunoris* (Chen et al., 2014). However, we expected a positive correlation between degree of DTA and temperature as a consequence of decreasing developmental stress (sensu Møller, 1994; Hettyey et al., 2005). Then, we expected degree of DTA increased with body condition or age. Finally, we expected degree of DTA decreased with increasing Operational sex ratio (OSR, the ratio of the sexually competing males to fertilisable females in a breeding aggregation at a given time) in response to high sperm competition level.

MATERIAL AND METHODS

Study site and sample collection

We collected *Rana kukunoris* individuals from seven populations (elevations ranging from 2506 to 3478 m, Fig. 1) along the eastern Tibetan Plateau, China. We randomly collected 10–53 individuals by hand in the medium spawning period from late-March to mid-April

in 2012 at each site. In this study, all individuals were sampled at the same time in their breeding cycle. Then, these frogs were identified as adult males if they displayed nuptial pads on the fore digits, others as females. All adult females examined were released to the original spawning sites. The population-specific OSR was calculated as the number of males to the number of fertilizable females in a breeding aggregation at a given time (3–5 days; Mai et al., 2017). In this case, OSR was used to estimate under the specific assumption that they could reflect the average number of males mating with each female. All captured males were brought to our field laboratory close to breeding sites. At room temperature, they were put into individual plastic opaque containers (diameter = 16.75 cm), filled 2 cm deep with fresh water. Then, the snout-vent length (SVL, to the nearest 0.1 mm) was measured with a vernier caliper (LXZ919160, Shenzhen luxianzi Technology Co., Ltd.), and body mass (to the nearest 0.01 g) was weighed with an electric balance (SL202N, Shanghai Minqiao Precision Scientific Instrument Co., Ltd.).

A plastic bucket was prepared with the TMS (Tricaine methane sulfonate, CAS: 886-86-2, Purity: > 97.0%, Sigma-Aldrich) at 2g/L in 5000 mL fresh water aerated for at least 48 h for dechlorination and oxygenation (Paduano et al., 2013). Then, every five frogs were put into this plastic bucket containing the 5000 mL anesthetic bath. The degree of sedation was assessed through testing of the limb retraction reflex in response to gentle pinching of a toe. Once anesthesia was achieved, adult males were euthanized by two-pithing and dissected (Liao et al., 2016). Both testes were removed and then weighed them to the nearest 0.1 mg with an electronic balance (CAV264C, OHAUS instrument (Shanghai) Co., Ltd.). Following the protocol of Hettyey et al. (2005) and Chen et al. (2014), directional testes asymmetry (DTA) was calculated with the following equation: $DTA = \text{right testis mass} - \text{left testis mass}$.

Age determination

We removed the longest phalange of the left hindfoot of male adults in each population and preserved in 10% aqueous solution of formaldehyde. Following the protocol of Ma et al. (2009), we produced histological sections of the frog phalanges and determined age by counting the number of lines of arrested growth (LAG) in the sections. Numerous studies have confirmed that improved method of paraffin section and Ehrlich's haematoxylin stain display seasonal growth of amphibian species (e.g., Yu and Lu, 2013; Yu et al., 2019; Yu et al., 2021).

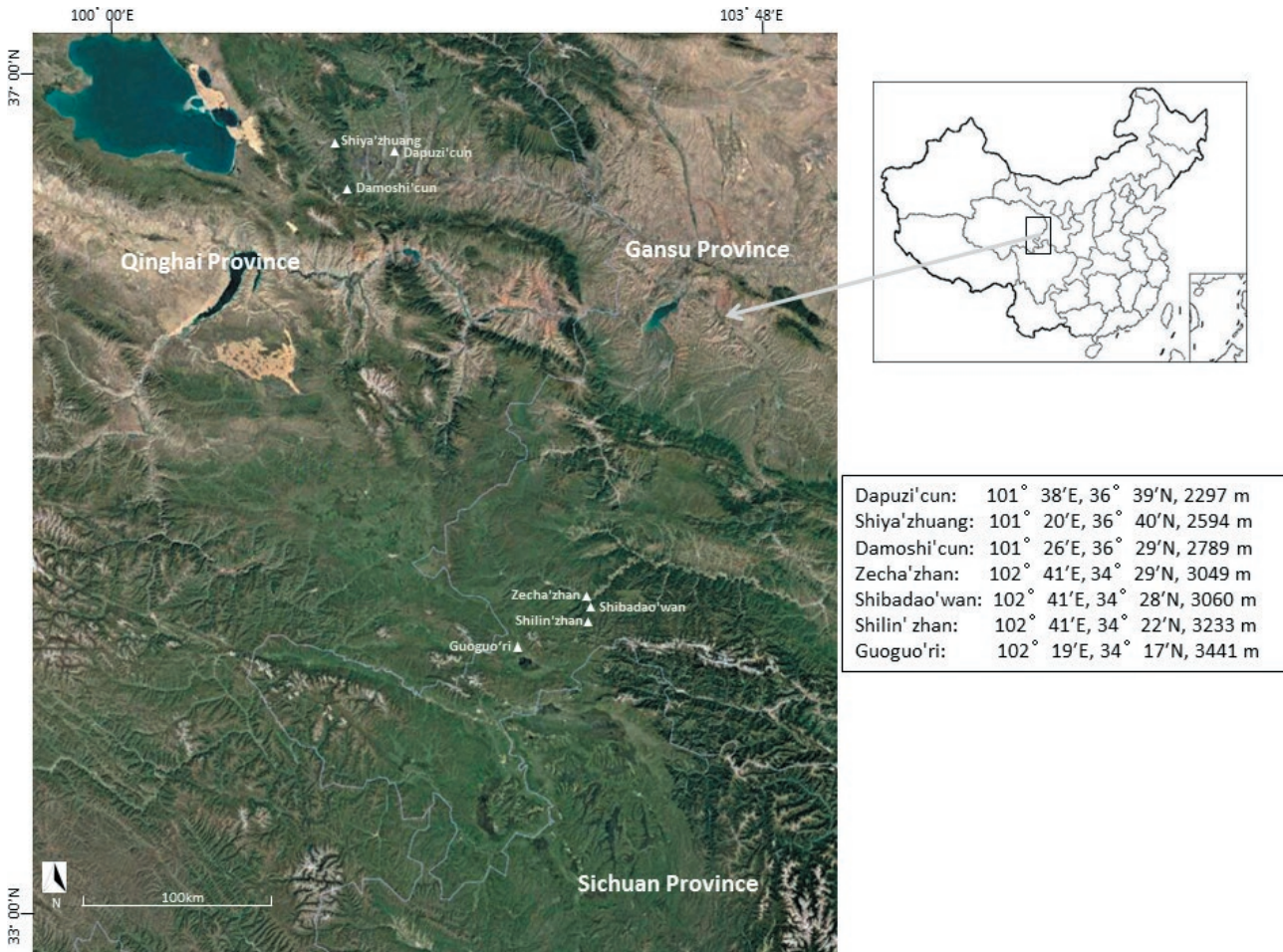


Fig. 1. Topographic map showing the location of the 7 *Rana kukunoris* study populations in the eastern Tibetan plateau.

Environmental factor collection

The annual mean temperature did not decrease significantly with elevation (Spearman's correlation: $r = -0.643$, $P = 0.119$), and latitude ($r = 0.607$, $P = 0.148$). Thus, we used annual mean temperature as environmental factor in this study. We downloaded temperature data from WorldClim (<http://www.worldclim.org>; Hijmans et al., 2005). WorldClim data were for the period of 1950–2000 at a resolution database of $0.167^\circ \times 0.167^\circ$ grid cells.

Statistical analyses

We used one-way ANOVA to test whether the male size, age, the left or right testis mass and DTA differed among populations. The body condition was measured using residuals of body mass regressed against SVL. Then, we performed a linear mixed models (LMMs) to

test variation in the left or right testis mass, where the left or right testis mass as the dependent variable, body condition, OSR, temperature and age as fixed effects, population as a random effect. To test whether degree of DTA covaried with the population-specific OSR as proxies of sperm competition levels, we also performed linear mixed models (LMMs) to test variation in degree of DTA among populations, where body condition, OSR, temperature and age as fixed effects, population as a random effect. In the subsequent analyses of reproductive traits against sperm competition levels (OSR) the temperature remained in a simplified models as a covariate, but not include body condition. Prior to analyses, we log-transformed body mass, the left or right testis mass and operational sex ratio of each population to better attain normality and enhance homogeneity of variance. SPSS 20.0 (SPSS Inc., Chicago, Illinois, USA) was used for all analyses.

Table 1. Comparisons of SVL, age, body mass, testis mass and directional testes asymmetry of *Rana kukunoris* from seven altitudes in the east Tibet Plateau, China. Values represent mean \pm SE for each measure; n = number of individuals.

| Population | Altitude (m) | Latitude (degrees) | Annual mean temperature (°C) | n | OSR | SVL (mm) | Body mass (g) | Age (years) | Right testis mass (mg) | Left testis mass (mg) | Directional testes asymmetry |
|--------------|--------------|--------------------|------------------------------|----|------|------------------|------------------|-----------------|------------------------|-----------------------|------------------------------|
| Dapuzi'cun | 2297 | 36.65 | 5.30 | 17 | 2.43 | 54.70 \pm 0.90 | 17.38 \pm 0.86 | 3.06 \pm 0.13 | 14.26 \pm 1.08 | 11.51 \pm 1.32 | 2.76 \pm 0.87 |
| Shiya'zhuang | 2594 | 36.68 | 3.50 | 39 | 2.79 | 51.27 \pm 0.58 | 18.35 \pm 0.62 | 3.13 \pm 0.08 | 14.32 \pm 0.76 | 14.05 \pm 0.76 | 0.27 \pm 0.50 |
| Damoshi'cun | 2789 | 36.49 | 0.20 | 53 | 1.23 | 50.25 \pm 0.58 | 15.39 \pm 0.69 | 2.81 \pm 0.10 | 10.92 \pm 0.72 | 10.97 \pm 0.73 | -0.05 \pm 0.54 |
| Zecha'zhan | 3049 | 34.49 | 1.50 | 19 | 1.90 | 54.57 \pm 0.59 | 18.23 \pm 0.79 | 3.32 \pm 0.13 | 12.53 \pm 1.24 | 14.26 \pm 1.21 | -1.74 \pm 0.85 |
| Shibadao'wan | 3060 | 34.47 | 1.40 | 11 | 1.67 | 51.23 \pm 0.90 | 14.74 \pm 0.72 | 3.45 \pm 0.16 | 8.79 \pm 1.09 | 9.05 \pm 0.99 | -0.26 \pm 0.52 |
| Shilin' zhan | 3233 | 34.37 | 1.00 | 24 | 2.20 | 47.40 \pm 0.70 | 12.46 \pm 0.41 | 2.96 \pm 0.14 | 8.07 \pm 0.64 | 8.42 \pm 0.54 | -0.35 \pm 0.45 |
| Guoguo'ri | 3441 | 34.29 | 0.80 | 10 | 1.48 | 51.74 \pm 0.98 | 17.56 \pm 0.94 | 3.20 \pm 0.13 | 12.07 \pm 0.99 | 11.64 \pm 0.79 | 0.43 \pm 1.01 |
| F | | | | | | 6.44 | 7.64 | 5.41 | 6.44 | 5.41 | 2.91 |
| P | | | | | | < 0.001 | < 0.001 | < 0.001 | < 0.001 | < 0.001 | 0.01 |

RESULTS

Mean values of male size, age, left or right testis mass, and degree of DTA differed significantly between populations (Table 1). The LMMs showed that the right testes was not correlated with the OSR ($t = -2.105$, $P = 0.158$), but increased with temperature ($t = 3.284$, $P = 0.042$, Table 1, Fig. 2), body condition ($t = 5.447$, $P < 0.001$) and age ($t = 3.235$, $P = 0.001$) when controlling for

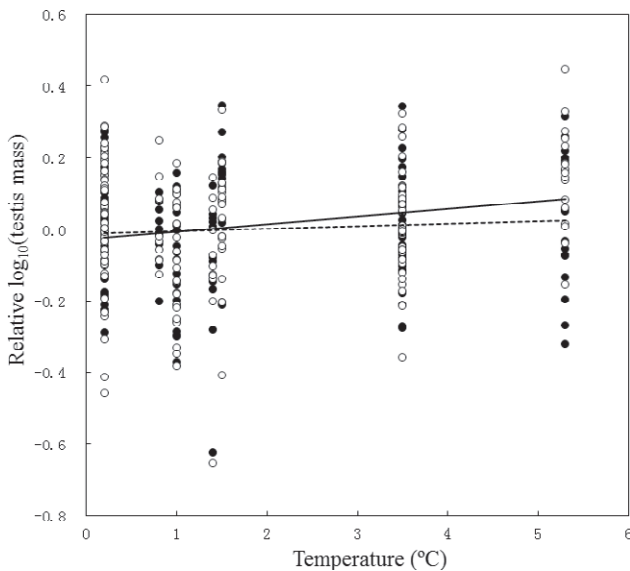


Fig. 2. Relationship between annual mean temperature and left testis mass (open circles) and right testis mass (solid circles) in 7 *Rana kukunoris* populations. Solid lines: the right testes and $P < 0.05$; dashed line: the left testes and $P > 0.05$. P values were computed using linear mixed models. Each dot represents the residual value for a given individual corrected for the effect of operational sex ratio, body condition and age.

population (random effect: $Z = 0.537$, $P = 0.591$). Similarly, the left testes was not correlated with the OSR ($t = -0.262$, $P = 0.809$) and temperature ($t = 0.643$, $P = 0.558$, Fig. 2), but increased with body condition ($t = 6.361$, $P < 0.001$) and age ($t = 5.564$, $P < 0.001$) when controlling for population (random effect: $Z = 1.077$, $P = 0.281$).

The degree of DTA was not significant correlated with age ($t = -1.540$, $P = 0.125$), body mass ($t = 0.788$, $P = 0.432$) and testis mass ($t = 0.107$, $P = 0.915$), when controlling for population (random effect: $Z = 1.057$, $P = 0.290$).

The LMMs showed that the degree of DTA was not correlated with the OSR ($t = -1.623$, $P = 0.248$), body

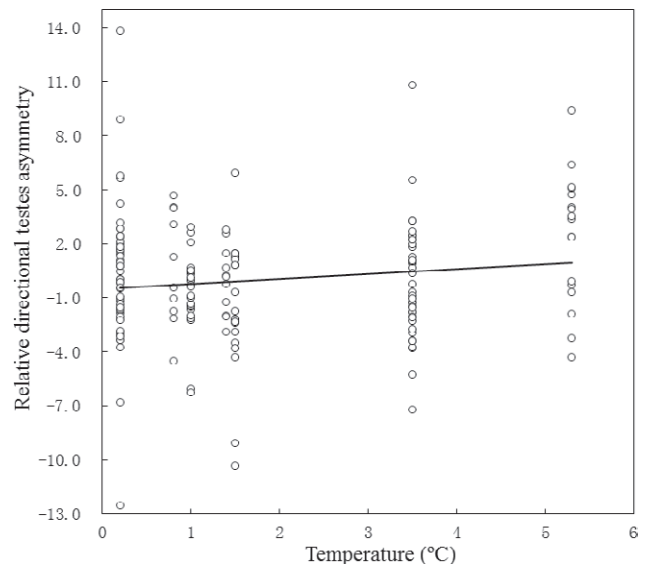


Fig. 3. Relationship between annual mean temperature and directional testes asymmetry in 7 *Rana kukunoris* populations. Each dot represents the residual value for a given individual corrected for the effect of operational sex ratio, body condition and age.

condition ($t = -0.108$, $P = 0.914$) and age ($t = -1.369$, $P = 0.173$), but increased with temperature ($t = 2.899$, $P = 0.053$; Fig. 3) when controlling for population (random effect: $Z = 0.280$, $P = 0.780$). Meanwhile, in a simplified model controlling only for population ($Z = 0.253$, $P = 0.800$), age ($t = -1.377$, $P = 0.171$) and temperature ($t = 2.952$, $P = 0.050$), DTA did not increase with the OSR ($t = -1.676$, $P = 0.244$). Furthermore, the right testis was significantly heavier than the left testis in only one of seven studied populations (5.3°C, paired test: $t = 3.165$, $df = 16$, $p = 0.006$; other population, $p = 0.055-0.928$, Table 1) where the temperature was warmest among all the studied locations.

DISCUSSION

DTA is common in male animals (Møller, 1994; Simmons and Kotiaho, 2002). Specially, this phenomenon has been proved in some anurans, including *Rana temporaria* (Hettyey et al., 2005), *Rhacophorus omeimontis* (Mi et al., 2012) and *Rana nigromaculata* (Zhou et al., 2011). The degree of DTA may be used to reveal individual quality (Møller, 1994; Simmons and Kotiaho, 2002) because some studies found that there were positive correlations between DTA and body condition (e.g., Møller, 1994; Hettyey et al., 2005). However, other studies (e.g., *Rana omeimontis*, Mi et al., 2012; *Hylarana guentheri*, Liu et al., 2011; *Rana kukunoris*, Chen et al., 2014) do not show this pattern. In this study, our results showed that DTA did not co-vary with male condition or body mass, suggesting individuals with good condition or heavier did not tend to have a larger degree of DTA. Thus, the degree of DTA as a good measure of male body condition remains ambiguous and contentious in male animals (Birkhead et al., 1997; Kimball et al., 1997; Kempnaers et al., 2002; Wu and Liao, 2017).

Environmental stresses may drive the evolution of testis asymmetry (Møller, 1994). Although directional asymmetry in testis size has been observed in some anurans within a population (Mi et al., 2012; Zhou et al., 2011; Yu and Guo, 2015), most studies so far have found the level of DTA was not correlated with latitude or altitude in spite of large differences in environmental conditions and environmental stress (Hettyey et al., 2005; Chen et al., 2014; Zhang et al., 2018). However, our results support this hypothesis as the degree of DTA covaried with temperature in the study populations of *Rana kukunoris*. Furthermore, the right testis was significantly heavier than the left testis in the populations with locations of warmest temperature. One possible explanation is that adaptive asymmetry might be difficult to evolve. For example,

the development of testes asymmetry is adaptive, but very costly because reduction or loss of an organ on one side of the body could commonly not be compensated, leading to physiological or morphological handicaps. Males living in warm regions could allocate more energy to reproduction, in that they increase resource availability in relative longer the length of the activity seasons than those living in cold regions. This suggests that low environmental stresses (e.g., warm temperature) provide the opportunity for males to increase in size of the right testis, thus compensate for a reduced function of the left one. In addition, we found no evidence for a geographical trend in genetic stress, and this result was consistent with other anurans species (Palo et al., 2003; Hettyey et al., 2005).

Previous studies have shown that DTA covaries with age (e.g., Birkhead et al., 1997; Graves, 2004; Liu et al., 2012), suggesting that males with a larger degree of DTA have a longer life span. However, the degree of DTA was not positively correlated with age in current study. This result was consistent with Liu et al. (2011) and Zhou et al. (2011), suggesting that older males did not indicate a higher degree of DTA than younger males. An explanation for this phenomenon was males have already reached complete reproductive maturity during the first breeding year (Liu et al., 2011).

Sperm competition is also attributed to drive the evolution of ejaculate quality in a wide range of taxa, in that higher levels of sperm competition tend to result in larger testes (Møller, 1991; Møller and Briskie, 1995). Assuming that it is costly to have high the level of DTA, thus two large testes might be advantageous to increase testes size. However, we found annual mean temperature did not effect on testis mass (Yu, unpublished data). Moreover, our results also showed that degree of DTA did not decrease with the OSR, suggesting that male-male competition did not lead to an increase in levels of sperm competition.

In conclusion, we did not find evidence for the suggestion that DTA is related to male condition, age and OSR, although there is a significant directional testes asymmetry in *R. kukunoris*. We found a positive correlation between the level of DTA and temperature, suggesting that differences in the length of activity period and resources availability across locations may affect the energy budget of this frog, resulting in a gradual change in reproduction energy parallel to increasing temperature.

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The Gahai-Zecha National Nature Reserve Management Bureau approved this project (approval number GHZCRMB/03-212014), and gave permission for fieldwork. Handling and processing of frogs followed approved protocols from the Animal Scientific Procedures Act 1988 by the State Department of China. All experiments involving the sacrifice of live animals were approved by the Animal Ethics Committee at Xinyang Normal University. The study was funded by Emergency Management Program of National Natural Science Foundation of China (Grant no. 31741019).

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Influence of tail injury on the development of Neotropical elegant treefrog tadpoles

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Abstract. Anuran larvae in aquatic environments are important prey items for several vertebrate and invertebrate species. Besides avoiding predation, there are some strategies that may reduce the physical damage in those tadpoles that survive the predation attempt. For example, the injured tadpole tail can regrow after a predator bite, but few studies have examined the consequences of such injury. We examined the consequences of three levels of injury to the tail and how this influenced development and feeding behavior of tadpoles of the Neotropical elegant treefrog, *Dendropsophus elegans*. We collected spawns and kept them in the laboratory until tadpoles reached Gosner's stages 28 to 35. Then, they were separated in four experimental groups: individuals with tail trimmed in 30, 50 or 70 % of its length, and a control group, with no tail removing. We counted the days until metamorphosis, calculated the Scaled Mass Index (SMI) through weight and length of newly-metamorphosed, and evaluated the feeding frequency to evaluate the influence of tail amputation on them. We found that the time until metamorphosis was positively related to the extent of the amputation, but SMI and feeding behavior were not influenced. As the time to metamorphose is related to the survivorship chances of individuals: i.e., if the aquatic environment is with high density of predators, it would be advantageous to rapidly metamorphose out of the water. However, tail injury delays the metamorphose process, which could influence the survival of the individual.

Keywords. Anuran larvae, *Dendropsophus elegans*, Atlantic rainforest, tail loss, development, feeding.

INTRODUCTION

Most anurans present aquatic larval stages and terrestrial post-metamorphic (adult) life stages, and are susceptible to predators of both environments. In this context, several defensive strategies were already reported for tadpoles in face of predators' attack. For example, tadpoles of *Pelophylax lessonae* can alter their behavior in the pres-

ence of dragonfly larvae (van Buskirk and Arioli, 2002), and tadpoles of *Dryophites crysoscelis* can change the morphology of their tails in order to increase swimming speed, which consequently promotes a higher probability of escaping in a possible attack of predators (McCollum and Leimberger, 1997). Also, Other species rely on visual aspects to avoid predation, such as tadpoles of *Scinax machadoi*, which select background colors to improve

their camouflage (Eterovick et al., 2018; Gontijo et al., 2018), *Pseudacris regilla*, which alter their tail color to avoid predator attacks (Benard, 2006), and *Boana semi-lineata*, which uses aposematic coloration to avoid predation (D’Heursel and Haddad, 1999). Hence, other species, such as *Bufo bufo*, rely on chemical defenses to avoid attacks of predators (Üveges et al., 2019).

Anurans are well known to be centralized in trophic webs (Blanco-Torres et al., 2020) since they are both prey and predators (Rebouças et al., 2013; Rebouças and Solé, 2015). In this way, they evolved several strategies to avoid predation (e.g., Lourenço-de-Moraes et al., 2016; Toledo et al., 2007). In larvae, one of the possible sublethal consequences of a predation attempt is the partial tail loss or injury (Morin, 1985; Touchon and Wojdak, 2014; Wilbur and Semlitsch, 1990), but the consequences of it to individual survival are very variable. For some species, past evidence suggest that it incurs little cost for tadpoles, since they, after escaping the predation, can regenerate the tail completely (Wilbur and Semlitsch, 1990). For example, van Buskirk et al. (2003) observed that tails may play a role as a lure, in which larger tail fins reduced predations in 16 % of the observations. Indeed, although firstly reported that enlarged tail fins enables predator escaping by enabling faster swimming (Smith and van Buskirk, 1995), posterior studies showed that tadpoles with injured tails did not lost speed in relation to those with an intact tails (van Buskirk and McCollum, 2000a). The effect on speed was significant only if large portions of the tails were removed (Hoff and Wassersug, 2000; van Buskirk and McCollum, 2000b). However, for some species tail injuries result in less swimming performance, and consequently a higher predation risk. In *Dryophytes chrysoscelis*, for example, tadpoles with no tail injury presented a survival almost twice as high as those with 75 % of tail loss (Semlitsch, 1990). Also, in *Bombina orientalis* tadpoles presented less survivorship and longer larval period (Parichy and Kaplan, 1992).

Beyond the ecological consequences, tail loss in tadpoles can also present feeding activity modification. Theoretically, if individuals need no regenerate tails after a predation attempt, they should acquire more energy through feeding to reach the maximum of tail length as less time as possible, and consequently reach the full swim performance, which is related to tail shape (van Buskirk and McCollum, 2000b). However, although modification of feeding behavior is already observed in presence of predators (e.g., Feminella and Hawkins, 1994; Pueta et al., 2016), the effects of tail loss on it, which is the most common consequence of predation attempt, still were not observed. Hence, while regenerating the tail, tadpoles are in continuous growth, which *per se* requires

a constant food intake until reach the metamorphosis stage. Thus, the tail injury, and an extra acquisition of nutrients during its regeneration, must affect the feeding-growth-time until metamorphosis balance. It is relevant because tail injuries may impact on the population survivorship coupled with the fact that this species occurs in Atlantic rainforest, one of the most diverse and vulnerable environments of the world, where pandemic diseases (Carvalho et al., 2017) climatic changes (Moura-Campos et al., 2021; Rebouças et al., 2021), habitat fragmentation (Becker et al., 2010; Dixo et al., 2009), and introduced predators (da Silva et al., 2009; de Oliveira et al., 2016; Forti et al., 2017) are threatening endemic anurans.

Therefore, this study evaluates the consequences of tadpole’s tail injuries in a Neotropical anuran species, *Dendropsophus elegans* (Anura; Hylidae), testing the following hypotheses: i) different levels of tail injury result in less healthy newly-metamorphosed; ii) different levels of tail injury increase the time to complete metamorphosis; and iii) tail injury reduces foraging activity of tadpoles.

MATERIALS AND METHODS

Tadpoles of *Dendropsophus elegans* (Fig. 1) were obtained through the maintenance of egg masses collected at Reserva Betary, Iporanga, São Paulo, Brazil. After hatching, each tadpole was kept in an individual aquarium (40 x 45 x 30 cm), to avoid pseudo-replicates and the influence of one individual in another, maintained at room temperature (25 °C), and half of the water was replaced twice a week after tadpoles reach the stage 28. We used tadpoles between Gosner’s (1960) stages 28 and 36 for the experiments. These stages were chosen because they comprehend most of growth and development of anuran larvae (Pfab et al., 2020). Environmental conditions of laboratory were constantly monitored and individuals were observed until metamorphosis. Thus, our experiment began before hatching and finished after metamorphosis. After the experiment, all individuals were released in the original sampling locality.

Tadpole development. To evaluate the influence of tail loss in the size and growth of individuals, we selected tadpoles that measured 25 mm of total length. Individuals were measured with a digital caliper (to the nearest 0.01 mm) and weighted with a digital scale (to the nearest 0.01 g). We then arranged these tadpoles into four groups, following Semlitsch (1990) and Figiel Jr and Semlitsch (1991), representing each of the treatments: i) tadpoles with 30 % of the tail clipped; ii) tadpoles with 50 % of tail clipped; iii) tadpoles with 70 % of tail clipped;

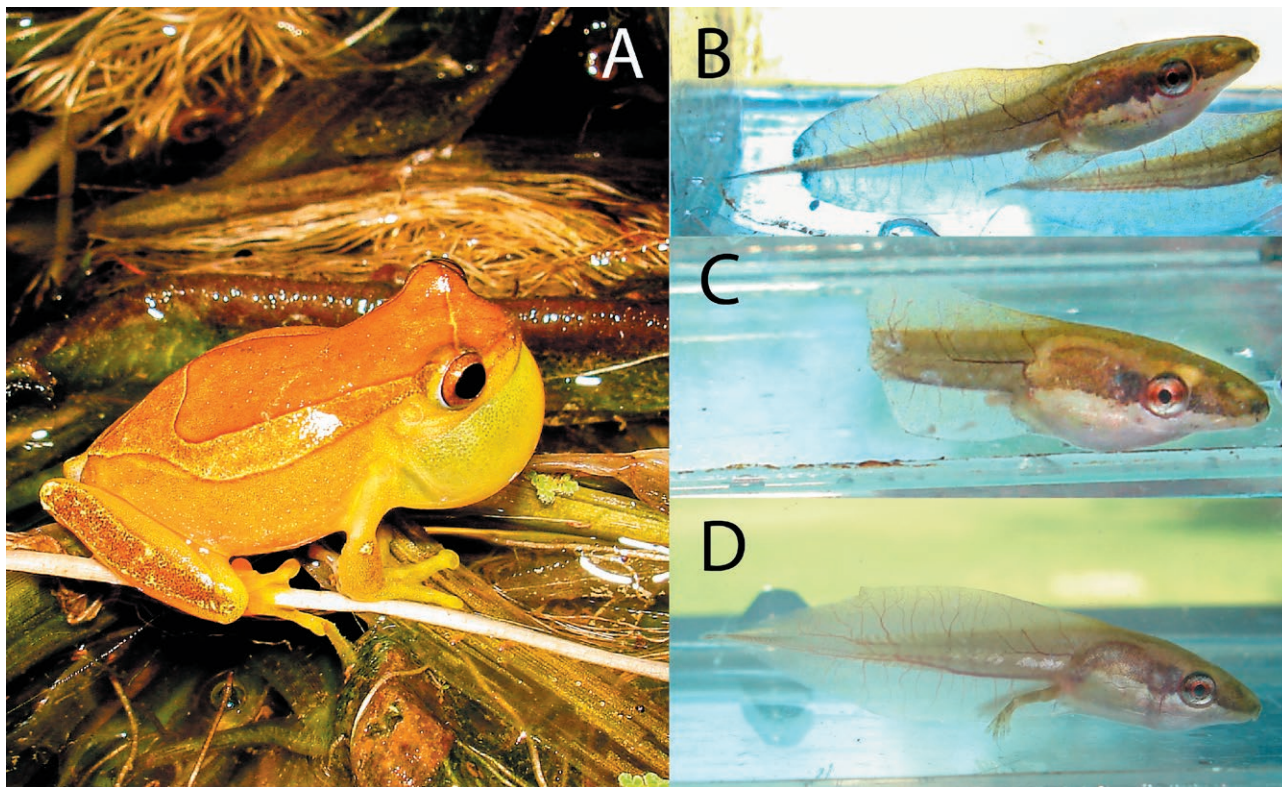


Fig. 1. An adult individual of *Dendropsophus elegans* (A), tadpoles of control (B) and 50 % of the tail clipped treatments (C), and with regenerated tail (D).

and iv) tadpoles with intact tails, which was the control group (Fig 1). Each group contained between 8 and 10 individuals (Table 1), which were isolated in each aquarium. Tail modifications were performed using a sterilized scalpel blade. Individuals in all treatments were equally fed with a standard fish food (extruded AquaLine), with 0.1 g every day. Individuals were observed until the metamorphosis was completed (complete tail absorption), and snout-vent length (SVL) of newly-metamorphosed individuals was measured with the digital caliper and body mass was weighted with the digital scale. Body mass and weight were used to calculate the Scale Mass Index (SMI), which is an index that can be used as a proxy of animals' health and fitness (Peig and Green, 2009).

Foraging. In order to evaluate the influence of partial tail loss in tadpoles foraging, we performed a second experiment also using 10 individuals measuring 25 mm in total length and between Gosner's (1960) stages 28 and 36. These individuals were separated in two treatments: i) individuals with 70 % of tail amputated; and ii) individuals with intact tails, treated as the control. Tadpoles were kept individually in glass jars measuring 6.5 cm in diameter and 6 cm height, with 120 ml of water and 0.1 g

of fish food. After two min of acclimation, tadpoles were observed for 12 min. During this time, the feeding frequency was observed in intervals of 20 s, and during each observation we evaluated if were feeding or not.

Statistical analyses. Firstly, we used an Analysis of Variance (ANOVA) and a Student's *t* test to evaluate of SMI present difference between treatments (tail amputations of 30 %, 50 % and 70 %; and tail amputation *per se*, respectively). To evaluate the influence of tail injury on SMI and on time until metamorphosis, we ran two Generalized Linear Models analyses (GLM), both using treatment (30 %, 50 % and 70 % of tail amputations and control, coded as 1, 2, 3 and 0, respectively) as predictive variable, the first one with SMI of newly metamorphosed individual as response, and the second with days until metamorphosis as response. Both analyses were performed using gaussian family and identity link. Additionally, we ran other two GLM's, with the same parameters, to evaluate if SMI or days until metamorphosis were influenced by amputation *per se* (all treatments were classified as "amputated", for treatments which the tail was clipped, coded as 1, and "intact" for the control treatment, coded as 0). Finally, in order to evaluate the

influence of tail loss in foraging we also used a GLM, but with quasipoisson family and logit link, considering “treatment” as predictive variable (control, coded as 0, or amputation, coded as 1), and the feeding frequency as response variable.

All models were checked through residuals deviance, and models with more than one predictive variable and collinearity was checked through Variance Inflation Factor (VIF) through the “vif” function of “car” package (Fox & Weisberg, 2019). We considered levels higher than 4 as an indicator of multicollinearity (Hair et al., 2010). Hence, as pos hoc tests, we used estimated marginal means to compare groups of tail-trimmed individuals with the control group through the “emmeans” package (Lenth, 2020). All analyses were carried out in R 4.1.0 (R Core Team, 2021) considering a significance level of 5 %.

RESULTS

During the experiment about tadpole development, we recorded the death of four individuals: one from the control group, one from the 50 % amputation group, and two from the 70 % amputation group. All individuals from the treatment groups presented the tail totally regenerated within 12 days after the beginning of the experiment (Table 1). We observed tail regeneration in all individuals that had their tail clipped (Fig. 2).

The average time until metamorphosis (from eggs until newly-metamorphosed) was 87.5 days for the control group (room mean temperature of 26.5 °C; Table 1). We observed no difference between treatment groups ($F = 0.91$, $P = 0.44$) or between individuals with tail amputation or not ($t = -0.06$, $P = 0.95$). Newly metamorphosed individuals presented an average SMI of 0.148 ± 0.012 , with control group presenting 0.148 ± 0.011 , 30 % group presenting 0.143 ± 0.013 , 50 % group presenting 0.153 ± 0.012 , and 70 % group presenting 0.149 ± 0.014 . During foraging experiment, individuals with injured tail were

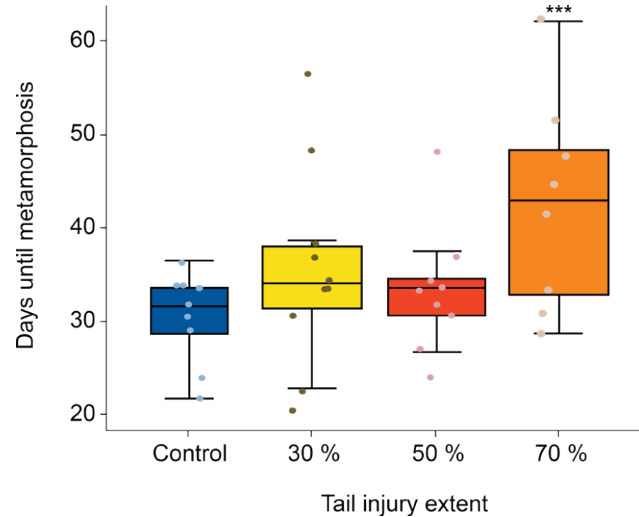


Fig. 2. Days until metamorphosis of *Dendropsophus elegans* tadpoles subjected to four treatments: control (intact tail), and 30, 50 and 70% of tail removal. The top and bottom of the boxes represent the first and last quartiles, the horizontal line within the box represents the median, the whiskers represent the tenth and 90th percentiles. Asterisks represent the category of tail amputation that showed significant reduction of time until metamorphosis.

observed feeding in an average of 36.6 ± 25.9 times, while individuals with no tail injuring were observed feeding in an average of 55.1 ± 22.9 times.

In our analysis, none of the variables presented VIF higher than 4 ($SVL = 3.02$, $weight = 3.35$, $days\ until\ metamorphosis = 1.2$). We observed no influence of treatment on SMI or in weight, but treatment presented a significant influence on time until metamorphosis. Amputation *per se* showed no influence in any of our variables. Regarding to foraging, we observed no influence of tail injury on feeding frequency. All model outputs are in Table 2 and estimated marginal means in Table 3.

Table 1. Time until metamorphosis, snout-vent length (SVL) and body mass of newly metamorphosed individuals during experimentation. Values presented as mean \pm standard deviation (minimum – maximum; number of individuals tested; standard error).

| Treatment | Time until metamorphosis (days) | SVL (mm) | Weight (g) |
|-----------|---------------------------------|---------------------------|--------------------------|
| Control | 29.8 ± 11.5 | 12.06 ± 0.44 | 0.15 ± 0.02 |
| | (18.3 – 41.3; 9; 3.83) | (11.62 – 12.49; 9; 0.14) | (0.13 – 0.17; 9; 0.007) |
| 30 % | 35 ± 10.8 | 11.86 ± 0.46 | 0.14 ± 0.02 |
| | (24.2 – 45.8; 10; 2.98) | (11.40 – 12.32; 10; 0.14) | (0.12 – 0.16; 10; 0.006) |
| 50 % | 32.8 ± 7.1 | 12.13 ± 0.24 | 0.16 ± 0.02 |
| | (25.7 – 39.9; 9; 2.36) | (11.90 – 12.37; 9; 0.08) | (0.14 – 0.18; 9; 0.007) |
| 70 % | 42.0 ± 15.1 | 12.01 ± 0.44 | 0.15 ± 0.02 |
| | (26.9 – 57.1; 8; 5.34) | (11.57 – 12.46; 8; 0.14) | (0.13 – 0.17; 10; 0.006) |

Table 2. Coefficients of Generalized Linear Model analysis, which considers the percentage of tail injury as a predictor of (1) Scaled Mass Index (SMI) and (2) days until metamorphosis; tail injury *per se* as a predictor of (3) SMI and (4) days until metamorphosis; and (5) tail injury as a predictor of feeding frequency. All models present degrees of freedom = 35 and significant values are in bold.

| | Estimate | Std Error | t value | P |
|--|----------|-----------|---------|--------------|
| (1) SMI ~ % tail injury | | | | |
| Intercept | 0.15 | 0.004 | 34.42 | <0.001 |
| 30% | -0.005 | 0.006 | 0.79 | 0.44 |
| 50% | 0.005 | 0.006 | -0.81 | 0.42 |
| 70% | 0.001 | 0.006 | 0.25 | 0.81 |
| (2) Days until metamorphosis ~ % tail injury | | | | |
| Intercept | 29.78 | 2.99 | 9.95 | <0.001 |
| 30% | 5.22 | 4.13 | 1.27 | 0.21 |
| 50% | 3 | 4.23 | 0.71 | 0.48 |
| 70% | 12.22 | 4.36 | 2.8 | 0.008 |
| (3) SMI ~ tail injury | | | | |
| Intercept | 0.15 | 0.004 | 34.05 | <0.001 |
| tail loss | 0.0003 | 0.005 | 0.05 | 0.96 |
| (4) Days until metamorphosis ~ tail injury | | | | |
| Intercept | 29.79 | 3.15 | 9.56 | <0.001 |
| tail loss | 6.56 | 3.6 | 1.82 | 0.08 |
| (5) Feeding frequency ~ tail injury | | | | |
| Intercept | 4.01 | 0.18 | 22.56 | <0.001 |
| tail loss | -0.41 | 0.28 | -1.45 | 0.17 |

Table 3. Summary contrasts of Estimated Marginal Means, used as a pos hoc test to compare groups of different levels of tail injury with the control group. Significant value is in bold.

| | Estimate | Std. Error | P |
|--|----------|------------|-------------|
| SMI ~ % tail injury | | | |
| 30% - control | -0.005 | 0.006 | 0.73 |
| 50% - control | 0.005 | 0.006 | 0.75 |
| 70% - control | 0.001 | 0.006 | 0.98 |
| Days until metamorphosis ~ % tail injury | | | |
| 30% - control | 5.2 | 4.13 | 0.44 |
| 50% - control | 3 | 4.23 | 0.79 |
| 70% - control | 12.22 | 4.36 | 0.01 |
| SMI ~ tail injury | | | |
| injured - control | 0.0003 | 0.005 | 0.96 |
| Days until metamorphosis ~ tail injury | | | |
| injured - control | 6.56 | 3.6 | 0.07 |
| Feeding frequency ~ tail injury | | | |
| injured - control | -0.41 | 0.28 | 0.15 |

DISCUSSION

We showed that although tadpoles reach metamorphosis with the same weight and size in all classes, the time spent until the end of the metamorphosis tends to increase, and it was significantly longer when 70 % of tail is removed. It means that individuals with a severe damage in tail tend to spend more time under larval stage, which can submit individuals that were already threatened by a predator under aquatic predation pressure for a longer time. Also, it delays the development of adult life stage, and consequently reproduction can be retarded. Therefore, a high predation pressure can influence other life stages of individuals, and in a larger scale, can impair the permanence of a population.

We also observed that the feeding frequency was not significantly higher in the group with tail trimmed. Some similar results were observed in other experiments involving artificial tail removing in tadpoles of *Aquarana catesbeiana*, where individuals also had a delay in growth and development (Wilbur and Semlitsch, 1990). A possible explanation for these observed results is that a predation attempt does not result in increasing of uptake but in reallocation of energy, since feeding presented no increasing, and it consequently could cause a delay in development. Additional studies are necessary to further elucidate the physiology of this possible energy reallocation and verify this hypothesis.

We did not observe influence of tail removal on the SMI of newly-metamorphosed individuals, similarly to what was reported for size in *Osteopilus septentrionalis* (Koch and Wilcoxon, 2019) and *Hoplobatrachus rugulosus* (Ding et al., 2014). However, opposing results were found for other species. For example, in *Bombina orientalis*, for which the time until metamorphosis was the same independently of the tail injury extent, newly metamorphosed individuals were smaller than those without tail injury (Parichy and Kaplan, 1992). Likewise, tadpoles with 55 % of the tail removed resulted in smaller newly-metamorphosed individuals in *Pelobates cultripes* (Zamora-Camacho et al., 2019). Besides, such effect lead to a reduction in the jumping performance of post-metamorphic individuals (Zamora-Camacho and Aragón, 2019), which could expose them to higher risk of predation on land. So, these cases highlight a trade-off: tadpoles will either stay longer in the water, exposed for a longer time to aquatic predators but with newly metamorphosed with an 'ideal' size, with less exposure to terrestrial predators (Semlitsch, 1990; Wilbur and Semlitsch, 1990), or they could leave the water smaller and with some mobility handicaps, which could limit the exposure to aquatic predators but exposing them more to terrestrial predation

in the developmental stage that they are most susceptible to predation (Toledo et al. 2007). In *D. elegans* we observed that the strategy adopted is the first one. Tadpoles threatened by a predator spend more time under larval stage, i.e., reduce the growth rhythm, but reach the same size after metamorphosis, and consequently the same SMI, than unharmed individuals.

We also did not observe change in feeding frequency as a result of tail injury. It probably implies that the tail regeneration was not provided by an extra acquisition of energy – expected by a more frequent feeding. Although these stages (stages 28 until 36) are those when generally tadpoles present the most significant growth and energy uptake (Pfab et al., 2020), we did not observe any difference when the tail was lost. Considering that for some species locomotion is more important than feeding, such as in *Pleurodema thaul* (Pueta et al., 2016) and *Pelophylax lessonae* (Steiner, 2007), and that tail fins enable fast swimming (Smith and van Buskirk, 1995), perhaps for *D. elegans* the regeneration of tail is energetically more important than time until metamorphosis. Consequently, there is not an increase in feeding to regenerate the tail, but a reallocation of the energy that otherwise would be used to growth. Thus, such observation supports the hypothesis of a probable reallocation of the energy from the regular development/metamorphosis process directed to tail regeneration. However, different results were reported for other species. For example, in *Ascaphus truei*, a simple clue of predators' presence was enough to modify the foraging in tadpoles, which reduced up to six-folds its foraging activity (Feminella and Hawkins, 1994). Also, similar results were observed for *Rana sylvatica* (Fraker, 2010) and *Rana clamitans* (Fraker, 2008, 2009). It efforts that more studies are necessary to elucidate this process of energy reallocation during larval stage until metamorphosis.

Our experiments showed consequences of predatory events in *D. elegans* tadpoles. Tail injury caused by predators can result in several consequences for the individuals, decreasing their survivorship, affecting tadpole morphology (Nunes et al., 2010), and swimming speed (Figiel Jr and Semlitsch, 1991). Besides, as tadpoles of *D. elegans* remained more time in the larval stage when the tail was injured, this fact may have several consequences, since evolutionary approach until conservation of native populations.

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The effect of weight and prey species on gut passage time in an endemic gecko *Quedenfeldtia moerens* (Chabanaud, 1916) from Morocco

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Abstract. Gut passage time (GPT), a key factor in digestive procedure, is of pivotal importance for digestion. Several parameters may affect GPT, such as temperature, length of gastrointestinal tract and body size. Here, we examine the influence of prey weight and prey species on GPT in the endemic diurnal gecko *Quedenfeldtia moerens*, from the Anti-Atlas Mountains in central Morocco. We used two prey species, house crickets (*Acheta domesticus*, AD) and mealworms (*Tenebrio molitor*, TM). Lizards were fed with the larval stage of TM and nymphs of AD. The influence of prey weight and prey species was tested at a constant temperature. We used three weight classes of each prey species to test the influence of prey weight on GPT. Our results showed that prey species affected GPT in a distinct way: mealworms induced a longer gut passage time compared to house crickets. Moreover, GPT increased with the increasing weight of prey for both prey species. Our finding demonstrates that the effect of prey species and prey weight affect digestion and thus should be better clarified in future studies.

Keywords. GPT, prey weight, *Acheta domesticus*, *Tenebrio molitor*, *Quedenfeldtia moerens*, Morocco.

INTRODUCTION

Given that digestive activity regulates energy flow to animals, effective digestion is a prerequisite for survival (Karasov and Douglas, 2013). Among the many important parameters that shape the digestive repertoire of animals, the time required for food to pass through the gastrointestinal tract from consumption to defecation, known as gut passage time (GPT) stands out (Hume, 1989; Van Damme et al., 1991). GPT shapes digestive efficiency, as increasing the time food remains in the gastrointestinal tract provides more time for effective digestion (Van Damme et al., 1991; Alexander et al., 2001).

GPT may be affected by numerous factors in lizards, among which temperature is maybe the most important. Indeed, GPT is temperature-dependent and varies from few hours to several days (Christian et al., 1984; Karasov et al., 1986), decreasing with increasing temperature (Du et al., 2000; Pafilis et al., 2016, 2007; Sanabria et al., 2020). The reptilian digestive system is characterized by high plasticity and reptiles can control the time food remains in the gut (Herrel et al., 2008; Sagonas et al., 2015), by elongating the gastrointestinal tract and thus increasing GPT (Sagonas et al., 2015; Vervust et al., 2010; Pafilis et al., 2016). Furthermore, the existence of specialized digestive microstructures, such as cecal valves, may prolong the time it takes

for food to pass through and boost GPT (Herrel et al., 2008; Sagonas et al., 2015). Furthermore, GPT may also be influenced by age (Karameta et al., 2017a) or tail autotomy aftermaths (Sagonas et al., 2021; 2017).

Here we aim to clarify whether prey characteristics have an effect on gut passage time. To this end, we assessed the impact of prey weight and prey species on the GPT of the Atlas day gecko. We expected that the increasing size of a given meal (prey weight) would consequently prolong GPT. We also predicted that different prey species would distinctly affect GPT.

MATERIALS AND METHODS

Study species

The Atlas day gecko (*Quedenfeldtia moerens*) (Chabanaud, 1916) is a small diurnal lizard, belonging to the Moroccan endemic genus *Quedenfeldtia* of the Sphaerodactylidae family. It is widely distributed in the Atlas Mountains, from 10 to 2,700 m above sea level. The study population originates from the Anti-Atlas Mountains (29°51'N, 09°01'W; 1,900 m a. s. l.). During a field survey in February 2020, we captured, by noose, 12 adult males with snout-vent length (SVL) between 40 and 48 mm (mean \pm SD = 45.43 \pm 1.85) and weight ranging from 2 to 3 grams (mean \pm SD = 2.76 \pm 0.08).

Prey species and marking technique

Captured lizards were transferred to the laboratory and housed individually in transparent plastic terraria (11 x 17 x 7 cm³), with *ad-libitum* access to water. All lizards were maintained in natural photoperiod and acclimated, for two weeks, inside a temperature-controlled room (25 \pm 1°C) (Sagonas et al., 2021; 2017). Prior to the experiment, we fed the lizards with house crickets (*Acheta domesticus*, AD) nymphs and mealworms (*Tenebrio molitor*, TM) larvae to familiarize them with the specific prey items. Both insect species originated from an in-house breeding colony.

To test the effect of prey species on GPT, we selected AD nymphs and TM larvae. In order to evaluate the effect of prey weight on GPT, we categorized three weight classes for each prey species (in total six feeding regimes, three weight classes for TM and three for AD). For TM larvae we distinguish three classes, in ascending order of weight (mean \pm SD): L1 (0.013 \pm 0.0019 g), L2 (0.033 \pm 0.0017 g) and L3 (0.064 \pm 0.0036 g). For AD nymphs, the respective weight classes were: N1 (0.032 \pm 0.0035 g), N2 (0.065 \pm 0.0047 g) and N3 (0.1 \pm 0.0057 g).

Gut passage time

Prior to the experiment trials, food was withheld from lizards for three days, until no feces were found in the terraria (Sagonas et al., 2017). Then, we marked the prey items before feeding them to lizards. Prey items are typically marked with small pieces of plastic (PVC) that serve as indigestible markers (Van Damme et al., 1991; Pafilis et al., 2007). To avoid possible injuries to gecko's digestive tracts because of their small body size, we marked prey species using soft thread tied around the abdominal-thorax junction of the insect prey (Fig. 1). We fed all 12 lizards with the marked prey. Terraria were inspected every hour for the appearance of the marker. After the detection of the marker, we withheld food for another three days. After this period, we repeated trials by feeding lizards with other prey species and weight classes. At the beginning of each trial, we noted the lizard code, the prey species and weight class. Gut passage time was determined as the time elapsed from the consumption of the marked prey to the defecation of fecal pellets with the marker (Van Damme et al., 1991). Each lizard was tested six times (with feeding regimes L1, L2, L3, N1, N2 and N3) and respective GPTs were recorded. Using this specific protocol, all lizards are tested identically, thus minimizing noise associated with individual particularities.

Statistical analysis

We constructed mixed-effects models in R (version 4.1.1; R Core Team 2021) using the package lme4 (v1.1-15; Bates et al., 2015) with prey species (two levels) and prey weight (continuous covariate) as fixed effect factors (including the interaction between them). To avoid pseudo-replication, we included the lizard's ID as a random effect factor. We used quantile-quantile plots and residual plots to check the models' assumptions, and we assessed the significance of both comparisons using the Anova function in the package car (Fox and Weisberg, 2019) with type II sums of squares and the chi-square test statistic.

RESULTS

The marker used in the present study was effective and the thread tied around the prey was easily visible in fecal pellets from both prey species (Fig. 1F). Before comparing the effect of prey species on GPT, feeding regime to lizards were classed on three weight classes each (Table 1). The variance explained by the random effect (lizard ID) was nearly equal to 0, which means that the major

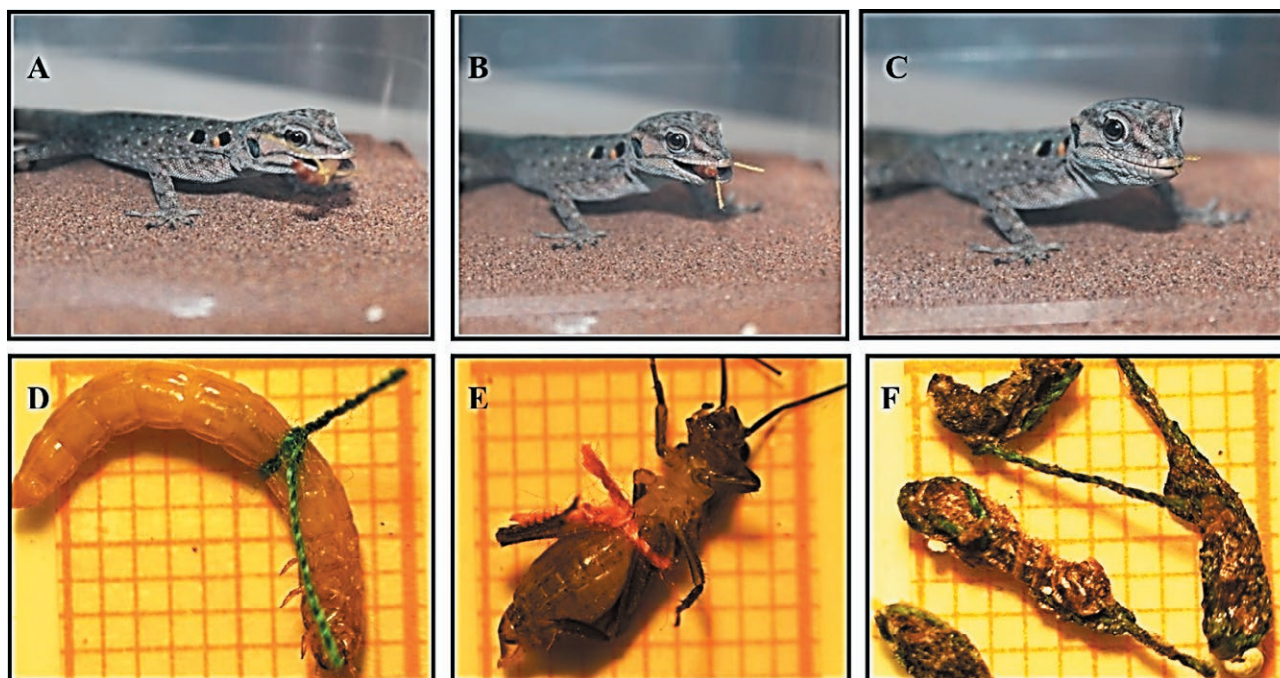


Fig. 1. (A to C): successive photos showing Atlas day gecko *Quedenfeldtia moerens* eating a marked mealworm larva. (D and E): the technique used to mark prey (here respectively mealworm larva and house cricket nymph); a thread was tied to prey as shown in photos. (F): fecal pellets where the marker (the thread tied around the prey) was clearly visible.

Table 1. Variation on gut passage time, GPT (mean \pm SE), as a function of the prey species and prey weight classes in *Quedenfeldtia moerens*. (N: nymph, L: larvae. Numbers 1 to 3, designed the weight class of each prey species).

| Prey species/class | Mean weight (g) | Weight range (min-max) | GPT (hour) |
|--------------------|-----------------|------------------------|------------------|
| Crickets/N1 | 0.03083 | 0.024 - 0.039 | 48.20 \pm 2.99 |
| Crickets/N2 | 0.0656 | 0.054 - 0.074 | 51.69 \pm 2.18 |
| Crickets/N3 | 0.1000 | 0.090 - 0.100 | 70.96 \pm 1.50 |
| Mealworm/L1 | 0.0130 | 0.010 - 0.016 | 51.25 \pm 4.07 |
| Mealworm/L2 | 0.0320 | 0.029-0.036 | 58.16 \pm 1.82 |
| Mealworm/L3 | 0.0650 | 0.059-0.072 | 79.14 \pm 2.94 |

part observed in our dependent variable was linked to the fixed terms in our model. Prey weight significantly affected GPT ($\chi^2 = 54.97$, $P < 0.01$). GPT was negatively correlated to prey weight ($R = 0.95$): the heavier the prey, the more time food remained in the digestive tract. Furthermore, prey species did affect significantly GPT ($\chi^2 = 56.43$, $P < 0.01$): GPT was longer for mealworms than for crickets. Finally, the interaction between prey species and their weight was significant and positive ($\chi^2 = 4.49$, $P = 0.034$). This finding indicates that feeding on heavier prey had a stronger effect on GPT for mealworms than for crickets (Fig. 2).

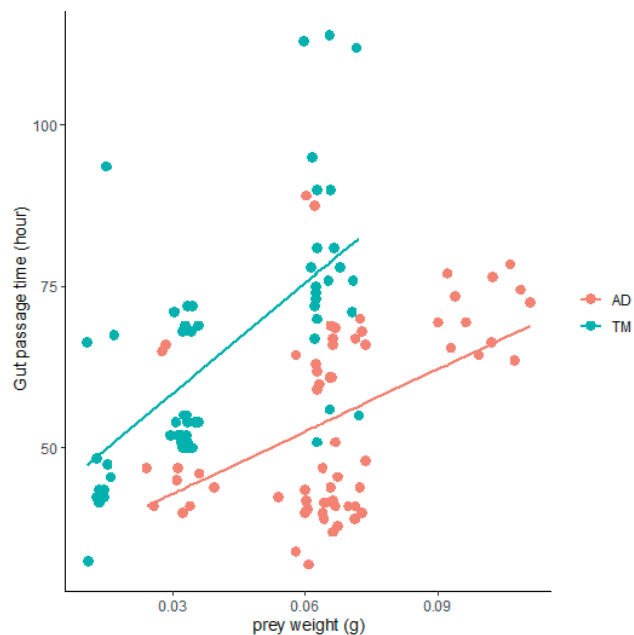


Fig. 2. Variation in the gut passage time (GPT) in hour as a function of increased weight of prey consumed by the Atlas day gecko *Quedenfeldtia moerens*, (TM) for mealworm as prey and (AD) for house cricket as prey. Solid lines represent the regression line for each prey species predicted by linear mixed model.

DISCUSSION

Digestion, a crucial function for nutrient absorption, rules energy acquisition (Karasov et al., 2011). There are many parameters affecting the digestive performance in lizards and, as mentioned above, gut passage time (GPT) is one of them (Pafilis et al., 2016; Sagonas et al., 2017; Karameta et al., 2017b). The latter may be affected by many intrinsic and extrinsic factors such as temperature, length of the gastrointestinal tract, body size and age (Van Damme et al., 1991, Du et al., 2000; Pafilis et al., 2016; 2007; Sanabria et al., 2020). In this study we found that GPT is also influenced by prey weight and prey species.

Gut passage time is a general indicator of the digestive process that measures the time that food remains in the gastrointestinal tract, a period which is decisive for digestive efficiency (Pafilis et al., 2007). Interestingly, though the impact of prey species and prey weight is known to influence overall digestion (Johnson and Lillywhite, 1979; Starck and Beese, 2001), the (presumable) impact of the aforementioned prey features on GPT has not been investigated. In this study, we selected two species typically used in captive lizard breeding (*Tenebrio molitor* and *Acheta domestica*) that have been analyzed in previous studies, thus allowing a comparative framework (Pafilis et al., 2016; Sanabria et al., 2020; Miller et al., 2013).

According to our results, GPT was significantly longer after the consumption of *T. molitor* than of *A. domestica* (Table 1). This finding could be attributed to the different energy content and chemical composition of the two prey species. Indeed, previous studies focusing on the nutritional composition of invertebrate prey found that TM larvae contained more than double metabolizable energy (2056 Kcal/Kg) than AD nymphs (949 Kcal/Kg) (Finke, 2002; 2015). Additionally, TM larvae have been reported to be richer in fat and proteins (134 g/Kg and 187 g/Kg, respectively) than AD nymphs (33 g/Kg and 154 g/Kg, respectively). In contrast, AD nymphs had higher water content than TM larvae (77.1% vs 61.9%). Furthermore, lizards fed with mealworms ingested significantly more energy, had significantly higher food conversion efficiencies, higher daily gains in mass, and greater total growth in mass than lizards fed on crickets (Rich and Talent, 2008). It seems that the nutrient and energy rich TM meals require more time compared to AD to get effectively absorbed. The observed difference in GPT between the two prey species could be explained by an adaptive strategy of the focal gecko to nutrient and energy rich prey. TM larvae contain more energy than AD nymphs, so lizards increase GPT to maximize the gain of this energy.

Prey weight also had an impact on GPT. Higher prey weights resulted in increased GPT in both cases of the two tested prey species. There was a linear correlation between the prey weight classed and GPT, with L1 and N1 (the lighter weight classes) resulting in lower GPTs and L3 and N3 (the heavier weight classes) inducing higher GPTs (Fig. 2). This should come as no surprise. Animals need more time to digest larger meals (Karasov and Del Rio, 2007) and thus the increase of prey weight dictates a considerable prolongation of GPT. The extra time provided by the longer GPTs offer gastric enzymes more time to act on ingested food and thus absorb more nutrients and energy (Alexander et al., 2001).

The GPT values found here are comparable with those reported in previous studies. Mean GPT for AD varies between 48.2 and 70.96 hours, while the respective values for TM are somewhat higher (51.25-79.14 hours). GPT may vary with the family: in lacertids, GPT receives values between 36-85 hours (Zhang and Ji, 2004; Vervust et al., 2010; Sagonas et al., 2015; Pafilis et al., 2016), in cordylids between 20-32 hours (McConnachie and Alexander, 2004), in skinks between 45-74 hours (Du et al., 2000) and in agamids between 67-86 hours (Karameta et al. 2017a). Those differences should be attributed to different body sizes and also distinct phylogenetic histories. More studies on sphaerodactylids will enrich the respective literature and give the opportunity for a comparative approach in saurian digestion.

Temperature greatly affects digestion and GPT represents no exception (Van Damme et al., 1991; Pafilis et al., 2007). Gut passage time decreases with increasing temperature (Du et al., 2000; Pafilis et al., 2007). Our experiment took place exclusively in 25°C, hence we cannot assess the impact of temperature on digestion under different feeding regimes (prey species and weight). In a future study we plan to assess this very important aspect of digestion process.

To conclude, this study shows that prey species and weight affected gut passage time in *Q. moerens*. More studies on digestive efficiency, including other variables such as temperature and apparent digestive performance would be valuable. Moreover, a comparative study between lizards from different populations will shed more light on the ecology of this Moroccan endemic diurnal gecko.

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A contribution to the knowledge on the diet and food preferences of *Darevskia praticola* (Reptilia: Lacertidae)[§]

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Abstract. The Meadow lizard (*Darevskia praticola* s.l.) is one of the more poorly-studied lizard species in Europe, and no detailed data on its diet is available. We investigated a total of 180 faecal samples of *D. praticola* s.l. from two locations in Bulgaria, and conducted a comparison between sex and age groups (adult males, adult females, and immatures). In addition, the correlations between the consumed prey and the available resources were also analysed. Food selectivity was analysed by comparing the faecal samples with pit-fall trap samples on the basis of abundance of prey items from particular operational taxonomic units (OTUs). Results indicate that the diet of the Meadow lizard contains mainly arthropods (insects and spiders) and the most abundant prey items belong to Araneae, Auchenorrhyncha, and Coleoptera. According to the used electivity indices none of the OTUs are highly preferred by *D. praticola* s.l., but Formicidae are the most avoided OTU for all sex/age groups. Differences in food preferences can be found between adults and immatures, while differences among males and females seem to be insignificant. The lack of clear differentiation between males and females could be a result of their similar size and locomotor ability. In conclusion, our results reveal that *Darevskia praticola* s.l. is a generalist and it shows no food specialization due to its narrow spatial niche.

Keywords. Faecal samples, keratophagy, trophic niche, saurophagy, Sauria.

INTRODUCTION

Dietary studies play a key role in understanding lizard ecology and knowledge on feeding ecology is of crucial importance in establishing the interactions among species. European lizards from the family Lacertidae feed on a wide variety of arthropods and therefore they could be considered generalist predators which do not exhibit well-defined patterns of prey selection. Nevertheless, there are data which show that (at least) some lacertid species have precisely defined patterns of food selection (e.g., Díaz, 1995; Carretero, 2004). In the last decades, numerous studies on food preferences and trophic ecology of lacertids were conducted (Arnold, 1987; Carret-

ero and Llorente, 1993; Capula and Luiselli, 1994; Pérez-Mellado et al., 2011; Crovetto and Salvidio, 2013; Mamou et al., 2016, 2019), although there are still many gaps in dietary research in some species and/or regions.

The Meadow lizard (*Darevskia praticola* s.l.) occurs only in SE Europe and its distribution is limited to parts of NE Serbia, S Romania, Bulgaria, NE Greece, European Turkey, SW Russia, and NW Georgia (Sillero et al., 2014). The taxonomic status of the Meadow lizard populations from the Balkans is still not fully clarified (Doronin and Ljubisavljevic, 2014; Freitas et al., 2016; Saberi-Pirooz et al., 2018). Moreover, *D. praticola* is one of the least-studied European lizard species in regards of ecology and especially trophic niche. In Bulgaria, this species has a

widely but very sporadic distribution, from the sea level up to about 1100 m a.s.l., yet is missing from the south-western part of the country (Stojanov et al., 2011).

Stugren (1984) summarised all available data for *D. praticola* and found that quantitative analyses of the food composition were lacking. Some data on the diet are presented for the Eastern (sub)species (e.g. Terentyev and Chernov, 1949; Bannikov et al., 1971; 1977), but the trophic spectrum of *D. praticola* s.l. from the Balkans and adjacent areas remains unstudied.

The aim of the present study was to document the diet and feeding preferences of *D. praticola* s.l. in Bulgaria, including possible intraspecific variation. In that sense, the following work hypotheses were formulated: 1) considering what is found in other lacertids, immatures should be unable to eat large prey items, therefore their trophic spectrum should be narrower than that of adults; 2) considering what is found in regard to microhabitat choice and sexual size dimorphism of *Darevskia praticola* s.l., there should be no substantial differences between sexes in their food preferences.

MATERIAL AND METHODS

Study area

For the sampling, we chose two sites in western Bulgaria: the first site was situated at the east coast of the Ogosta Reservoir, 3.5 km from the town of Montana (43.3739° N, 23.2086° E, 180-240 m a.s.l.), and the second was situated in the Sredna Gora Mts., near Gabrovnitsa Village (42.2602° N, 23.9208° E, 430-570 m a.s.l.). According to “World-Clim v.2” (Fick and Hijmans, 2017) the annual mean temperature is 11.3 °C for Ogosta and 10.6 °C for Gabrovnitsa, and the annual precipitation is respectively 624 and 568 mm (the values are extracted from the respective freely available GIS-layers with original resolution $\approx 1 \text{ km}^2$ cell). More detailed descriptions of the studied sites are given by Vacheva et al. (2020).

Sampling

For the purpose of the study, we used a faecal samples analysis: a non-invasive method which, despite of some limitations (e.g., impossibility for prey recognition in such taxonomic level as by direct analysis of the stomach content), provides adequate results in dietary studies (Bombi and Bologna, 2002; Luiselli et al., 2011; Pérez-Mellado et al., 2011). Lizards were captured in 2013, 2014 and 2016 in Ogosta and in 2017 and 2018 for Gabrovnitsa. A total of 53 field days were conducted, as follows:

Ogosta – 28 days and Gabrovnitsa – 25 days. Lizards were captured by hand and were measured (snout-vent length, SVL) with a transparent ruler to the nearest 1 mm. For each captured lizard, sex and age class were recorded. Age was not determined directly but estimated from body size and sexual secondary characters, so two age groups were defined: adults (SVL > 45 mm) and immatures (SVL between 24-44 mm). All of the captured lizards were placed separately in plastic boxes until defecation and after that, released at the site of capture. Faecal samples from each lizard were preserved in separate test tubes with ethanol for further examination under stereoscopic microscope (magnification 10-40X). Invertebrate remnants were identified to the lowest possible systematic level (in most cases to the level of Order). Collected invertebrates (both from the faecal and trap samples) were categorized with regards to their hardness (hard, intermediate, soft) and evasiveness (sedentary, intermediate, evasive) in accordance with Verwajen et al. (2002) and Vanhooydonck et al. (2007).

Food resources were evaluated by pit-fall traps, which is a widely used method in similar studies (see Vacheva and Naumov, 2020 and references therein). A total of 24 pit-fall traps were exposed (10 meters apart) in four different habitat types (river bed, meadow, deciduous forest and the ecotone between the meadow and the forest). This was done only in Gabrovnitsa for 23 and 17 days in spring, and 16 and 23 days in summer for 2017 and 2018 respectively. Collected invertebrates were identified to the lowest possible taxonomic level. We use the term “operational taxonomic unit” (abbreviated as OTU) instead of the term “taxon” for the invertebrates from both faecal samples and traps, because here the individual taxa are considered without taking into account their rank.

Statistics

Taxonomic diversity in the diet of *D. praticola* s.l. was analysed by Rényi's index family (diversity profiles), which is considered one of the most useful methods for ordering communities according to their diversity (see Tóthmérész, 1995). The significance of differences in diversity between the separate samples (adult males, adult females, and immatures) was assessed by a permutation test, based on the diversity indices of Shannon (H) and Simpson (1-D).

Food selectivity was analysed by comparing the faecal samples with trap samples on the basis of abundance of individuals from particular OTUs (standardized toward total number of individuals in the sample). The electivity was described by the indices of Ivlev (E) and Vanderploeg and Scavia (E*) (see Ivlev, 1961 and Vander-

ploeg and Scavia, 1979, respectively). Both indices take values from -1 to +1, where the positive values indicate that the respective component is preferred, and the negative – it is avoided (for a detailed review of the electivity indices see Lechowicz, 1982).

Spearman's rank correlation coefficient was used to test for correlation between abundance and frequency of the prey items. A Chi-square test was used for the comparison between sexes and between age groups, regarding the categories of evasiveness and hardness of the nutritional components.

Calculations of the diversity indices, as well as statistical tests, were done using PAST 3.21 (Hammer et al., 2001). The electivity indices were calculated in Microsoft Excel (2010) after manual input of the respective formulas.

RESULTS

A total of 180 faecal samples from *D. praticola* s.l. were collected – 31 from Ogosta and 149 from Gabrovitsa. Among them, 136 from adults (70 male and 66 female) and 44 immatures. The distribution of the material from the faecal samples of *Darevskia praticola* from Ogosta and Gabrovitsa is presented in Appendices 1 and 2. The identifiable invertebrate remnants could be attributed to 622 individual specimens: 100 from Ogosta and 522 from Gabrovitsa (Appendix 3). The average number of invertebrates found in the faecal pellets was 3.46 (3.23 for Ogosta and 3.74 for Gabrovitsa), and the maximum was 15.

A total of 23 OTUs were identified in the faecal samples, and most of them were the same for both study sites. In Gabrovitsa more OTUs were observed – 22, in contrast to Ogosta where only 15 OTUs were observed (Appendices 1 and 2). Dermaptera, Formicidae, Gastropoda, Mecoptera, Myriapoda, Pseudoscorpiones, and Scorpiones were recovered only from Gabrovitsa, while Hemiptera were found only in the samples from Ogosta. Among all of the OTUs, the most abundant and frequent for Ogosta were Araneae and Coleoptera, as well as Blattodea but only by frequency of occurrence, while in Gabrovitsa predominant by both number and frequency were Araneae, Auchenorrhyncha, and Insecta indet. (Fig. 1). The correlation between abundance and frequency of occurrence of OTUs for the two sites was positive and with very high level of statistical significance (Table 1).

The total number of OTUs registered in the pit-fall traps at Gabrovitsa was 25. Most abundant OTUs were Formicidae, Aranea, and Coleoptera (Appendix 3). According to the electivity indices, none of the OTUs were highly preferred by *D. praticola* s.l. (Table 2). The highest values for both indices were observed for Blat-

todea for males, for Insecta larvae for females and for Hymenoptera (excl. Formicidae) in immatures. On the other hand, with lowest values of the indices (close to -1) were Formicidae for all of the three sex/age groups (the index values for immatures are not presented in the table).

According to the Rényi's profiles (Fig. 2) the highest diversity of the diet was observed in males, and likewise diversity in the diet of adults was higher than in immatures. Statistically significant differences between adult males and immatures were established for the sample from Gabrovitsa with respect to the Shannon index (Table 3); the number of registered OTUs in adults was 22, while for immatures this was only 13. While the total number of OTUs was lower for Ogosta, this could be due to the lower sample size, and a total of 13 OTUs were observed in adults, compared to only 9 in immatures. OTUs presented only in adults were Acari, Dermaptera, Gastropoda, Hemiptera, Heteroptera, Isopoda, Mecoptera, Myriapoda, Pseudoscorpiones and Scorpiones. None of these OTUs were present in immatures only. The total number of observed OTUs in males was 21, while in females this was 19; for Ogosta there were 11 and 8 OTUs respectively, and for Gabrovitsa, where the sample size was larger – 20 and 18 respectively. OTUs observed only in males were Isopoda, Hemiptera, Dermaptera, and Scorpiones, while in females Lepidoptera, Mecoptera, and Myriapoda, but represented by single items.

Regarding the evasiveness of the prey, the highest values in faecal samples at both sample sites were sedentary prey items, and in terms of hardness, soft prey items were consumed more often (Table 4). The results of Chi-square test did not show statistically significant difference between all age/sex groups, in regards to neither evasiveness nor hardness of the prey items (Table 5).

In addition, parts of ingested tails and finger were discovered in the faecal samples. Cases of saurophagy were established in two adult males and one female from Gabrovitsa, i.e., in 2.01% of the samples from Gabrovitsa and 1.66% of total sample size. Keratophagy (the consumption of shed skin) was observed in two adults – a male and a female, or 1.34% of the samples from Gabrovitsa and 1.11% from the total sample size. Non-organic matter (grit) was recorded in three individuals, and plant matter was recorded in nine adults (five males and four females), which presents 5% from the total sample size (Appendix 2).

DISCUSSION

Our results suggest that *D. praticola* s.l. feeds mainly on arthropods, like many other lacertids, with insects

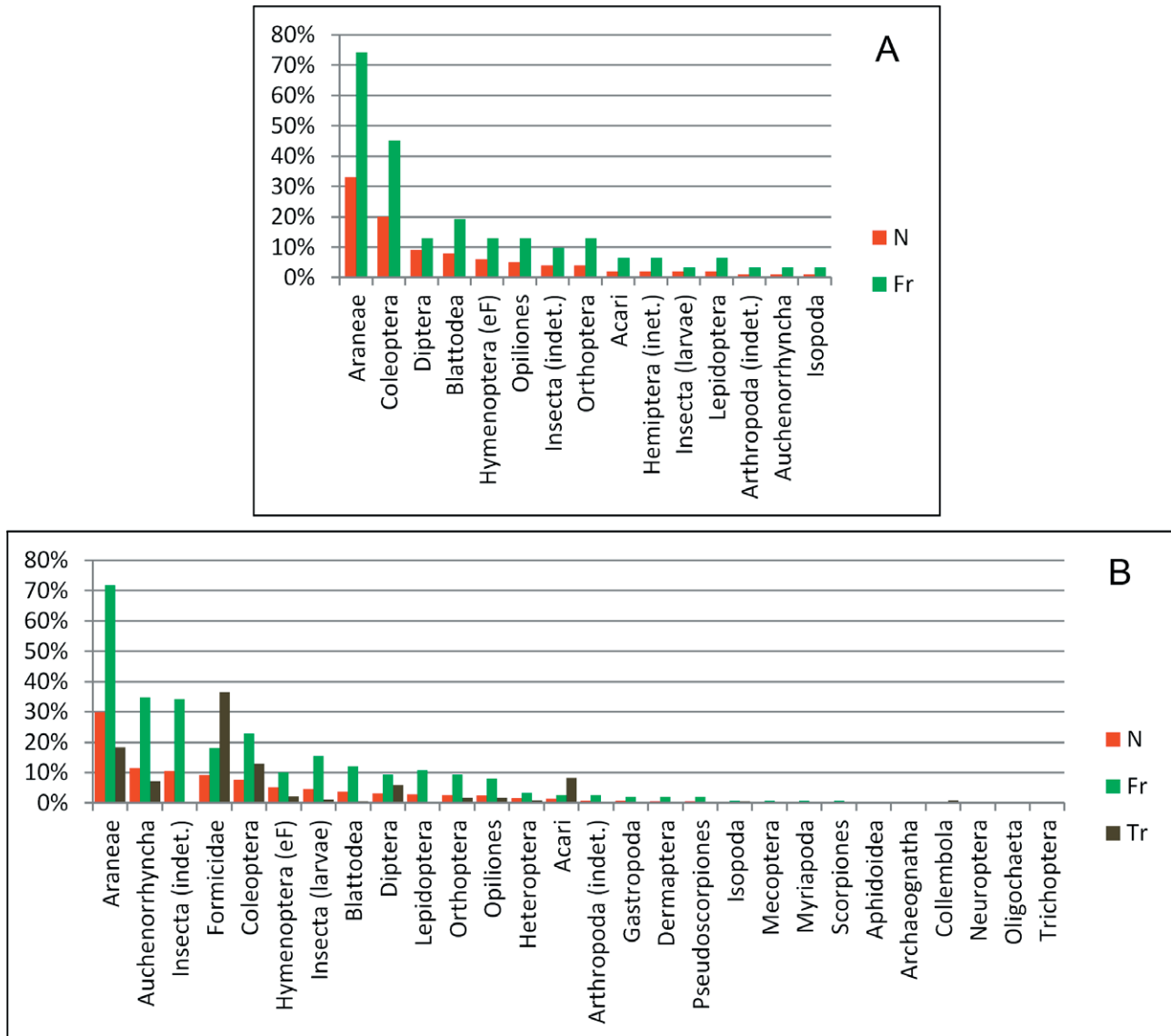


Fig. 1. Percentage of the invertebrates by OTUs according to: total number of specimens in the faecal samples of *D. praticola* (N); number of faecal samples in which the OTU occurs (Fr); total number of specimens, collected by pitfall traps (Tr). OTUs are in descending order according to the values of N.

being the predominant group – more than 60% of the total items recovered from the faecal pellets. Seven groups (Araneae, Auchenorrhyncha, Coleoptera, Insecta indet., Formicidae, and other Hymenoptera) composed more than 70% of the consumed prey, and among them the most abundant food source were spiders (more than 30%.) We also recorded OTUs that could be described as “dangerous prey”, such as Dermaptera, Myriapoda, and Scorpiones, which were present with single individuals and in adults only.

In the available literature there are no detailed data about diet and food preferences in *D. praticola* s.l. In a

few sources, a brief description of the most common prey was provided: Terentyev and Chernov (1949) state that the food of *Darevskia praticola* s.l. consists mainly of beetles (about 50%), orthopterans, arachnids, and dipterans. Bannikov et al. (1971) assign small insects, spiders, earthworms, molluscs and other invertebrates as prey to the Meadow lizards, specifying that among insects, small beetles, ants, orthopterans, leafhoppers, caterpillars, earwigs, aphids, as well as woodlice were the most consumed (Bannikov et al., 1977). The above mentioned has been confirmed by other authors (e.g., Orlova and Tertyshnikov, 1979; Tertyshnikov, 2002) and some anecdotal

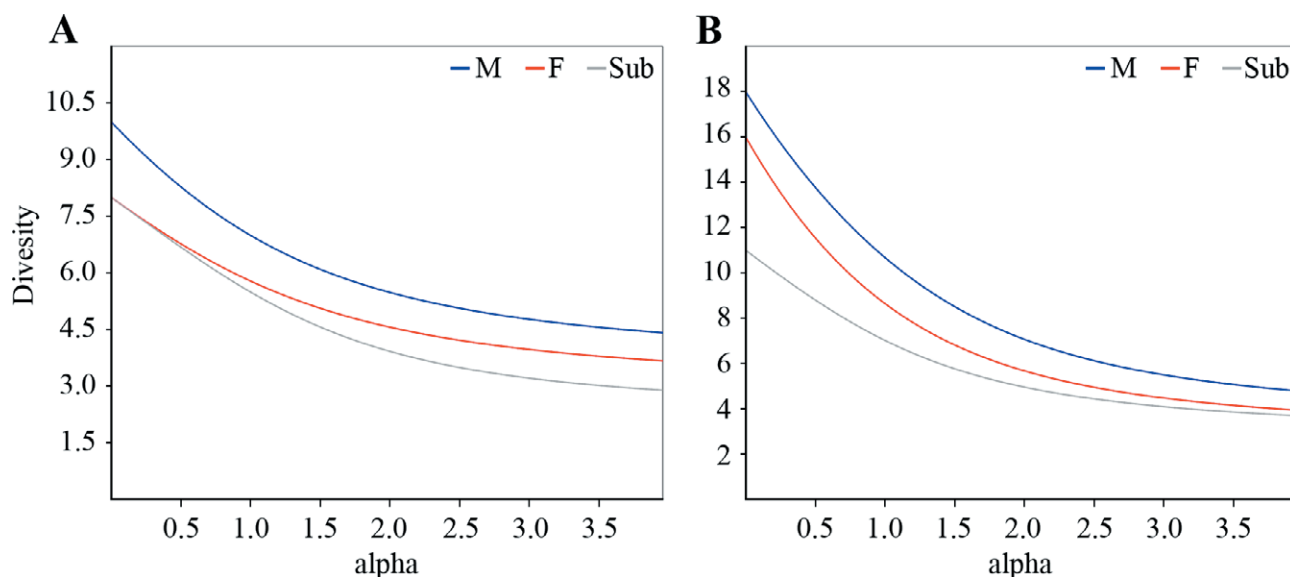


Fig. 2. Diversity profiles of the diet in males (M), females (F), and immatures (Sub) of *D. praticola* according to the abundance of OTUs in the faecal samples from Ogosta (A) and Gabrovitsa (B).

Table 1. Correlation (Rho) between abundance and frequency of OTUs in the faecal samples of male (M), female (F) and immature (Sub) *Darevskia praticola*, and its statistical significance (P).

| | | Rho | P |
|------------|-----|------|--------|
| Ogosta | M | 0.84 | 0.0023 |
| | F | 0.77 | 0.0476 |
| | Sub | 0.89 | 0.0179 |
| Gabrovitsa | M | 0.97 | 0.0000 |
| | F | 0.92 | 0.0000 |
| | Sub | 0.83 | 0.0014 |

data are reported in Stugren, 1984. Bischoff (1976) points out that the meadow lizard, like its relatives, preys on all edible invertebrates that can be overwhelmed.

Differences in food preferences were found between adult males and immatures, and the diet of adults in general was more diverse than that of immatures. It could be due to size limitation of the immatures (i.e., impossibility to consume large invertebrates), but also could be (at least partially) a result of the bias in terms of sample size differences (much smaller in immatures). In adults, the established lack of clear differentiation between males and females could be a result of their similar size and locomotor ability.

In term of evasiveness of the prey, less mobile prey categories were predominant, which can be explained by the fact, that the Meadow lizard is ground-dwelling and comparatively slow-moving species (Arnold, 1987).

Table 2. List of the most abundant ($r > 5\%$) OTUs from the faecal samples of males (M), females (F), and immatures (Sub) of *Darevskia praticola* according to the electivity indices of Ivlev (E) and Vanderploeg & Scavia (E*); r = percentage in the faecal samples, p = percentage in the pitfall traps.

| | | OTU | r | p | E | E* |
|-----|------------|------------------|--------|---------|---------|---------|
| M | | Blattodea | 5.19% | 0.55% | 0.8084 | 0.3087 |
| | | Hymenoptera (eF) | 5.19% | 2.17% | 0.4103 | -0.3516 |
| | | Araneae | 27.36% | 18.39% | 0.1961 | -0.5402 |
| | | Auchenorrhyncha | 8.49% | 7.14% | 0.0865 | -0.6147 |
| | | Coleoptera | 5.19% | 12.81% | -0.4234 | -0.8497 |
| | | Formicidae | 12.74% | 36.60% | -0.4837 | -0.8695 |
| F | | Insecta (larvae) | 6.02% | 1.22% | 0.6630 | 0.0611 |
| | | Araneae | 31.94% | 18.39% | 0.2694 | -0.4307 |
| | | Auchenorrhyncha | 12.04% | 7.14% | 0.2555 | -0.4428 |
| | | Coleoptera | 9.72% | 12.81% | -0.1369 | -0.7038 |
| | Formicidae | 7.87% | 36.60% | -0.6461 | -0.9061 | |
| Sub | | Hymenoptera (eF) | 6.38% | 2.17% | 0.4927 | 0.3822 |
| | | Auchenorrhyncha | 17.02% | 7.14% | 0.4091 | 0.2891 |
| | | Araneae | 30.85% | 18.39% | 0.2532 | 0.1213 |
| | | Coleoptera | 8.51% | 12.81% | -0.2016 | -0.3286 |

Regarding the hardness of the prey, predominant were soft prey categories. In view of the relatively small head size in comparison to body size (personal data), *D. praticola* s.l. probably avoids highly chitinized invertebrates.

Cannibalism and saurophagy in general, has been observed more often in island populations, where it could be caused by high lizard density and scarce food

Table 3. Diversity indices of the diet in males (M), females (F), and immatures (Sub) of *Darevskia praticola*, and the statistical significance of the differences between them (Permutation P).

| Index value | | Permutation P | | | | |
|-------------|-----|---------------|------|------------|------|-------|
| | | Ogosta | | Gabrovitsa | | |
| Simpson 1-D | M | 0.82 | 0.86 | M vs. F | 0.29 | 0.36 |
| | F | 0.78 | 0.82 | M vs. Sub | 0.07 | 0.19 |
| | Sub | 0.75 | 0.80 | F vs. Sub | 0.42 | 0.72 |
| Shannon H | M | 1.95 | 2.37 | M vs. F | 0.17 | 0.14 |
| | F | 1.76 | 2.16 | M vs. Sub | 0.09 | 0.002 |
| | Sub | 1.70 | 1.95 | F vs. Sub | 0.71 | 0.1 |

Table 4. Division of the invertebrates per categories of evasiveness (E1, E2, and E3) and hardness (H1, H2, and H3) as a percentage of all of the identified invertebrates in the faecal samples of male (M), female (F), and immature (Sub) *Darevskia praticola*.

| | | Ogosta | | | Gabrovitsa | | |
|-------------|----|--------|--------|--------|------------|--------|--------|
| | | M | F | Sub | M | F | Sub |
| Evasiveness | E1 | 44.68% | 48.00% | 55.00% | 53.68% | 60.82% | 63.29% |
| | E2 | 25.53% | 24.00% | 10.00% | 24.21% | 22.16% | 15.19% |
| | E3 | 29.79% | 28.00% | 35.00% | 22.11% | 17.01% | 21.52% |
| Hardness | H1 | 59.57% | 68.00% | 80.00% | 60.53% | 56.70% | 54.43% |
| | H2 | 8.51% | 0.00% | 0.00% | 3.68% | 3.61% | 2.53% |
| | H3 | 31.91% | 32.00% | 20.00% | 35.79% | 39.69% | 43.04% |

Table 5. Chi-square test for the differences between male, female, and immature *Darevskia praticola* in regards to evasiveness and hardness of the prey items.

| | | Ogosta | Gabrovitsa |
|----------|----------|-------------|------------|
| | | Evasiveness | χ^2 |
| | df | 4 | 4 |
| | P | 0.71 | 0.32 |
| Hardness | χ^2 | 5.49 | 1.53 |
| | df | 4 | 4 |
| | P | 0.24 | 0.82 |

resource (Pérez-Mellado and Corti, 1993; Castilla and Van Damme, 1996; Cooper et al., 2015), while it is rare in continental populations (Simović and Marković, 2013). Cases of saurophagy were more frequent in males, as males often display more aggressive behaviour to other conspecifics (Castilla, 1995), and the presence in females mentioned here is interesting. On the base of the pholidosis, we determined that the remnants of the consumed lizard parts in the faecal samples of *D. praticola* s.l. belong to representatives of Lacertidae family, but because of the presence of two other syntopic lacertids in Gabrovitsa (*Lacerta viridis* (Laurenti, 1768) and *Podar-*

cis muralis (Laurenti, 1768)), we can suggest only saurophagy, as far as there are no direct evidence for cannibalism. Until now, cases of (partial) saurophagy, were not established for *Darevskia praticola* s.l., and this is the first observation to our knowledge. The only known record of saurophagy in another member of the *Darevskia* genus was mentioned for an adult female *Darevskia brauneri* (Méhely, 1909) that fed on a juvenile *Lacerta agilis* (Golynsky and Doronin, 2014).

Another interesting feeding behaviour, keratophagy (the ingestion of shed skin), was observed for the first time in *D. praticola* s.l. Keratophagy was previously known for only four lacertids (Mitchell et al., 2006 and references therein) but in more detailed dietary study it was recorded for the Viviparous lizard *Zootoca vivipara* (Lichtenstein, 1823) (see Vacheva, 2018; Vacheva and Naumov, 2020), where keratophagy was present in more than 9% of the samples, as well as in two other lacertids (*Podarcis muralis*, and *Lacerta viridis*), hence this event seems to be more common than previously thought and probably has more complex and important evolutionary significance.

The Meadow lizard is a species with very limited spatial niche and it is a typical forest inhabitant, strongly associated with deciduous forests (mostly oak forests)

(Vacheva et al., 2020). As an active and effective thermoregulator, with a preferred temperature close to the lower limit of mean body temperatures in comparison to other European lacertids (Ćorović and Crnobrnja-Isailović, 2018), it has to choose suitable thermal microhabitats. In that sense, the observed low food specialization can be explained mainly by the narrow spatial niche and the species can be categorized as a generalist in regards to its prey choice.

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APPENDIX 1

Distribution of the material from the faecal samples of *Darevskia praticola* from Ogosta per OTU (M, F, and Sub: males, females and immatures; Evas.: evasiveness category, E1, E2, and E3 refer to sedentary, intermediate, and evasive, respectively; Hard.: hardness category, H1, H2, and H3 refer to soft, intermediate, and hard, respectively; N: number of identified specimens; Fr: number of the faecal samples in which OTU occurs; eF: except Formicidae).

| OTU | Evas. | Hard. | M | | F | | Sub | | Total | |
|---------------------|-------|-------|----|-----|---|-----|-----|-----|-------|-----|
| | | | N | Fr. | N | Fr. | N | Fr. | N | Fr. |
| Acari | E1 | H1 | 1 | 1 | 1 | 1 | 0 | 0 | 2 | 2 |
| Araneae | E1 | H1 | 15 | 12 | 9 | 5 | 9 | 6 | 33 | 23 |
| Arthropoda (indet.) | | | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| Auchenorrhyncha | E1 | H3 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| Blattodea | E3 | H1 | 2 | 2 | 3 | 1 | 3 | 3 | 8 | 6 |
| Coleoptera | E2 | H3 | 12 | 8 | 6 | 4 | 2 | 2 | 20 | 14 |
| Diptera | E3 | H1 | 6 | 2 | 1 | 1 | 2 | 1 | 9 | 4 |
| Hemiptera (indet.) | | | 2 | 2 | 0 | 0 | 0 | 0 | 2 | 2 |
| Hymenoptera (eF) | E3 | H3 | 3 | 1 | 2 | 2 | 1 | 1 | 6 | 4 |
| Insecta (indet.) | | | 4 | 3 | 0 | 0 | 0 | 0 | 4 | 3 |
| Insecta (larvae) | E1 | H1 | 0 | 0 | 2 | 1 | 0 | 0 | 2 | 1 |
| Isopoda | E1 | H2 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| Lepidoptera | E3 | H1 | 0 | 0 | 1 | 1 | 1 | 1 | 2 | 2 |
| Opiliones | E1 | H1 | 4 | 3 | 0 | 0 | 1 | 1 | 5 | 4 |
| Orthoptera | E3 | H2 | 4 | 4 | 0 | 0 | 0 | 0 | 4 | 4 |

APPENDIX 2

Distribution of the material from the faecal samples of *Darevskia praticola* from Gabrovitsa per OTU (M, F and Sub: males, females and immatures; Evas.: evasiveness category, E1, E2, and E3 refer to sedentary, intermediate, and evasive, respectively; Hard.: hardness category, H1, H2, and H3 refer to soft, intermediate, and hard, respectively; N: number of identified specimens; Fr: number of the faecal samples in which OTU occurs; eF: except Formicidae OPI: other prey items).

| OTU | Evas. | Hard. | M | | F | | Sub | | Total | |
|---------------------|-------|-------|----|-----|----|-----|-----|-----|-------|-----|
| | | | N | Fr. | N | Fr. | N | Fr. | N | Fr. |
| Acari | E1 | H1 | 7 | 4 | 0 | 0 | 0 | 0 | 7 | 4 |
| Araneae | E1 | H1 | 58 | 41 | 69 | 46 | 29 | 20 | 156 | 107 |
| Arthropoda (indet.) | | | 2 | 2 | 1 | 1 | 1 | 1 | 4 | 4 |
| Auchenorrhyncha | E1 | H3 | 18 | 17 | 26 | 22 | 16 | 13 | 60 | 52 |
| Blattodea | E3 | H1 | 11 | 9 | 7 | 7 | 2 | 2 | 20 | 18 |
| Coleoptera | E2 | H3 | 11 | 11 | 21 | 16 | 8 | 7 | 40 | 34 |
| Dermaptera | E2 | H1 | 3 | 3 | 0 | 0 | 0 | 0 | 3 | 3 |
| Diptera | E3 | H1 | 8 | 7 | 5 | 4 | 3 | 3 | 16 | 14 |
| Formicidae | E2 | H3 | 27 | 15 | 17 | 8 | 4 | 4 | 48 | 27 |
| Gastropoda | E1 | H3 | 1 | 1 | 3 | 2 | 0 | 0 | 4 | 3 |
| Heteroptera | E2 | H1 | 5 | 4 | 3 | 1 | 0 | 0 | 8 | 5 |
| Hymenoptera (eF) | E3 | H3 | 11 | 8 | 10 | 5 | 6 | 2 | 27 | 15 |
| Insecta (indet.) | | | 20 | 19 | 21 | 20 | 14 | 12 | 55 | 51 |
| Insecta (larvae) | E1 | H1 | 9 | 8 | 13 | 13 | 2 | 2 | 24 | 23 |
| Isopoda | E1 | H2 | 2 | 1 | 0 | 0 | 0 | 0 | 2 | 1 |
| Lepidoptera | E3 | H1 | 7 | 8 | 4 | 4 | 4 | 4 | 15 | 16 |
| Mecoptera | E2 | H1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 |
| Myriapoda | E2 | H1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 |

| OTU | Evas. | Hard. | M | | F | | Sub | | Total | |
|----------------------|-------|-------|---|-----|---|-----|-----|-----|-------|-----|
| | | | N | Fr. | N | Fr. | N | Fr. | N | Fr. |
| Opiliones | E1 | H1 | 4 | 4 | 6 | 5 | 3 | 3 | 13 | 12 |
| Orthoptera | E3 | H2 | 5 | 5 | 7 | 7 | 2 | 2 | 14 | 14 |
| Pseudoscorpiones | E1 | H1 | 2 | 2 | 1 | 1 | 0 | 0 | 3 | 3 |
| Scorpiones | E1 | H1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 |
| OPI (cannibalism) | | | | 1 | | 1 | | 1 | | 3 |
| OPI (keratophagy) | | | | 1 | | 1 | | 0 | | 2 |
| OPI (grit) | | | | 3 | | 0 | | 0 | | 3 |
| OPI (plant material) | | | | 5 | | 4 | | 0 | | 9 |

APPENDIX 3

Distribution of the material from the pitfall traps in Gabrovitsa per OTU (Evas.: evasiveness category, E1, E2, and E3 refer to sedentary, intermediate, and evasive, respectively; Hard.: hardness category, H1, H2, and H3 refer to soft, intermediate, and hard, respectively; eF: except Formicidae).

| OTU | Evas. | Hard. | N |
|------------------|-------|-------|------|
| Acari | E1 | H1 | 832 |
| Aphidoidea | E1 | H1 | 22 |
| Araneae | E1 | H1 | 1839 |
| Archaeognatha | E1 | H1 | 9 |
| Auchenorrhyncha | E1 | H3 | 714 |
| Blattodea | E3 | H1 | 55 |
| Coleoptera | E2 | H3 | 1281 |
| Collembola | E1 | H1 | 83 |
| Dermaptera | E2 | H1 | 4 |
| Diptera | E3 | H1 | 586 |
| Formicidae | E2 | H3 | 3661 |
| Gastropoda | E1 | H3 | 16 |
| Heteroptera | E2 | H1 | 81 |
| Hymenoptera (eF) | E3 | H3 | 217 |
| Insecta (larvae) | E1 | H1 | 122 |
| Isopoda | E1 | H2 | 57 |
| Lepidoptera | E3 | H1 | 27 |
| Mecoptera | E2 | H1 | 1 |
| Myriapoda | E2 | H1 | 42 |
| Neuroptera | E2 | H1 | 3 |
| Oligochaeta | E1 | H1 | 10 |
| Opiliones | E2 | H1 | 168 |
| Orthoptera | E3 | H2 | 168 |
| Pseudoscorpiones | E1 | H1 | 2 |
| Trichoptera | E3 | H1 | 2 |

First report on two loggerhead turtle (*Caretta caretta*) nests in the Aeolian Archipelago (Southern Italy)

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Abstract. The Aeolian Archipelago (Southern Tyrrhenian Sea, Italy) hosts important foraging/overwintering habitats for Mediterranean loggerhead sea turtles (*Caretta caretta*), although nesting sites have never been documented. This study reports the data of two nesting events occurred in summer 2019 at Stromboli and Lipari islands. A hatchling success of 20.69 % (18 hatchlings from 87 eggs) was recorded at Stromboli, while a complete hatchling unsuccessful characterised the Lipari nest, where 111 eggs were deposited. Data acquired during the monitoring of the nests suggest that combined factors, mainly temperature, beach morphology, and sand composition, could be the causes for the low success of these nesting events.

Keywords. Anoxic conditions, Lipari, Mediterranean Sea, Stromboli, temperature.

INTRODUCTION

The Aeolian Archipelago (Sicily, Italy), composed of 7 islands and located in the Southern Tyrrhenian Sea (Italy) (Fig. 1), is of volcanic origin with both extensive neritic and oceanic habitats within short distances (Favalli et al., 2005), which provide optimal foraging and overwintering grounds for both immature and adult loggerhead turtles (Blasi et al., 2016; Blasi and Mattei, 2017; Blasi et al., 2018) and fall within the historical nesting range of loggerhead turtle, although the 1960s quotations were not supported by documented data (Mingozzi et al., 2007).

Italy hosts regular nesting events along the Ionic coasts of the southern Calabria and in the Pelagian Islands (Linosa and Lampedusa; Mingozzi et al., 2008).

Irregular nesting events are also reported on the coasts of Sicily, Sardinia, Apulia and the Ionic coasts of Basilicata and Calabria. However, in recent years, a significant increase in the numbers of nests along the Italian coasts has been reported, with 30-40 nests estimated per year up to 70 nests recorded in 2018, through a survey carried out on the Ionian coasts of Calabria, facing the Messina strait (Mingozzi et al., 2007) and in Sicily (Casale et al., 2012).

In Sicily there are numerous suitable coasts for loggerhead turtle nesting; nesting events are also occasionally reported by tourists or local people. For example, in 2011, seven nests were reported along the coasts close to Palermo and on the southern Sicily (Casale et al., 2012). Even though many potential nesting sites are not ade-

guately monitored and consequently the actual nesting level and distribution in several areas remain partially unknown.

Here we report data on two nesting events by loggerhead turtles at Stromboli and Lipari islands (Aeolian Archipelago) in summer 2019. Our data represent the first official documentation showing that the Aeolian Archipelago could host irregular nesting events, and suggest a higher monitoring and conservation efforts to increase the chance of positive hatchings of these sites.

MATERIALS AND METHODS

Nesting sites

Two loggerhead turtle nests were surveyed and monitored during summer 2019 at Stromboli and Lipari islands, respectively. These two islands are stratovolcanoes, built by alternating hard lava flows and pyroclastic deposits of different sizes (ashes, pumices, scoriae, lithic fragments, and volcanic bombs). Stromboli, characterized by a continuous volcanic activity during the last 2,000 years, has a basaltic nature, with abundance of dark Fe-Mg minerals (Rosi et al., 2013). Its activity generated beaches composed of large (meters) basaltic rock blocks mixed to abrasive textures of sand and pebbles, with sizes up to few tens of centimetres. The dark colour of this substrate encourages the adsorption of the thermal infrared solar radiation, with surface daytime summer temperatures that can exceed 50 °C. The volcanic products of Lipari Island are more acidic, i.e., with major abundances of whitish silica. Solid wastes of pumice extraction from a close coastal quarry, presently inactive, are transported to the beach (Anzidei et al., 2017). Furthermore, during the last years, artificial replenishments of the beach were carried out, causing dramatic changes in its original morphology and lithological nature.

The first loggerhead turtle nested on the 21st of June at 7:35 AM (GMT+1) at Scari beach, in the north-east of Stromboli Island (15.2428°E, 38.8034°N WGS84), about 1 Km N of the main harbour and 14 m from the seashore (Fig.1). The site is characterized by a berm composed of large-sized pebbles, with a slope of 16.9°, located seaward of a dumping site. An anchor buoy field is present in its immediate neighbourhood and a sailing boat (about 50' long) was stranded in front of the nest few days after its emplacement, and stayed there for the whole incubation period. The nesting event was reported by two tourists and lasted about 2 hours.

The second loggerhead turtle nested on the 5th of July at 9:00 PM (GMT+1) in the heavily urbanised Canneto beach, in the north-east of Lipari Island (14.9617°E,

38.4937°N WGS84), at its inner end (Fig. 1). The turtle was disturbed by people with light and noise and consequently multiple nesting attempts were made before the final site was chosen. Facilities and roads run along the coast and, during the summer season, many tourists overcrowd the beaches all day long for the presence of bathhouses. A runoff channel is located in the proximity of the nesting site.

Nests monitoring and data collection

Standard fences were constructed to protect both sites immediately after the nesting events (Nooren and Claridge, 2002). A 24-hour monitoring was provided for each nest by Filicudi Wildlife Conservation volunteers during the whole incubation period, for checking sand temperatures and preventing predator/human intrusions. Two ONSET USB data loggers, equipped with 12-bit temperature smart sensors, were provided by the Istituto Nazionale di Geofisica e Vulcanologia (INGV); temperature sensors were buried at 10 and 40 cm depths in the proximity of the nests, and acquired data every 30 minutes in order to provide non-aliased hourly data. A via-radio remote Reolink IP RCL-410 W (4.0 MP) night camera was installed at Stromboli (courtesy of Attiva Stomboli Association). The duration of incubation period for both nests was predicted using the recorded mean temperature during the middle third of the incubation period as reported by Kaska et al. (1998) and other authors (Reid, 2005; Reid et al., 2009; Houghton and Hays, 2001; Godley et al., 2001).

The hatchling phase was continuously monitored with at least two operators for each nest, equipped with red filter headlights. During the hatchling phase at Stromboli, date and time of the emerging events were recorded and body size measurements (SCL, SCW, SPL, SPW, and weight) of hatchlings were collected, with a calibre and a scale, following standard protocols (Bolten et al., 1993; Nooren and Claridge, 2002). Dead hatchlings were stored in tubes provided with 70% ethanol solution, encoded with ID number, date, and time of collection. At the end of each emergence event, the hatchlings were left free inside the fenced area for a few minutes, to allow the imprinting process. Afterwards, considering that lights near to the beach and obstacles on the sand are a considerable risk to the hatchlings (Demetropoulos and Hadjighristophorous, 1995), they were placed in a basket filled with sand, which was transported offshore by boat, and then released directly into the sea. As a matter of fact the light might attract them in the wrong direction or slow down the race to the sea extending the period hatchlings remain on land. Moreover, obsta-

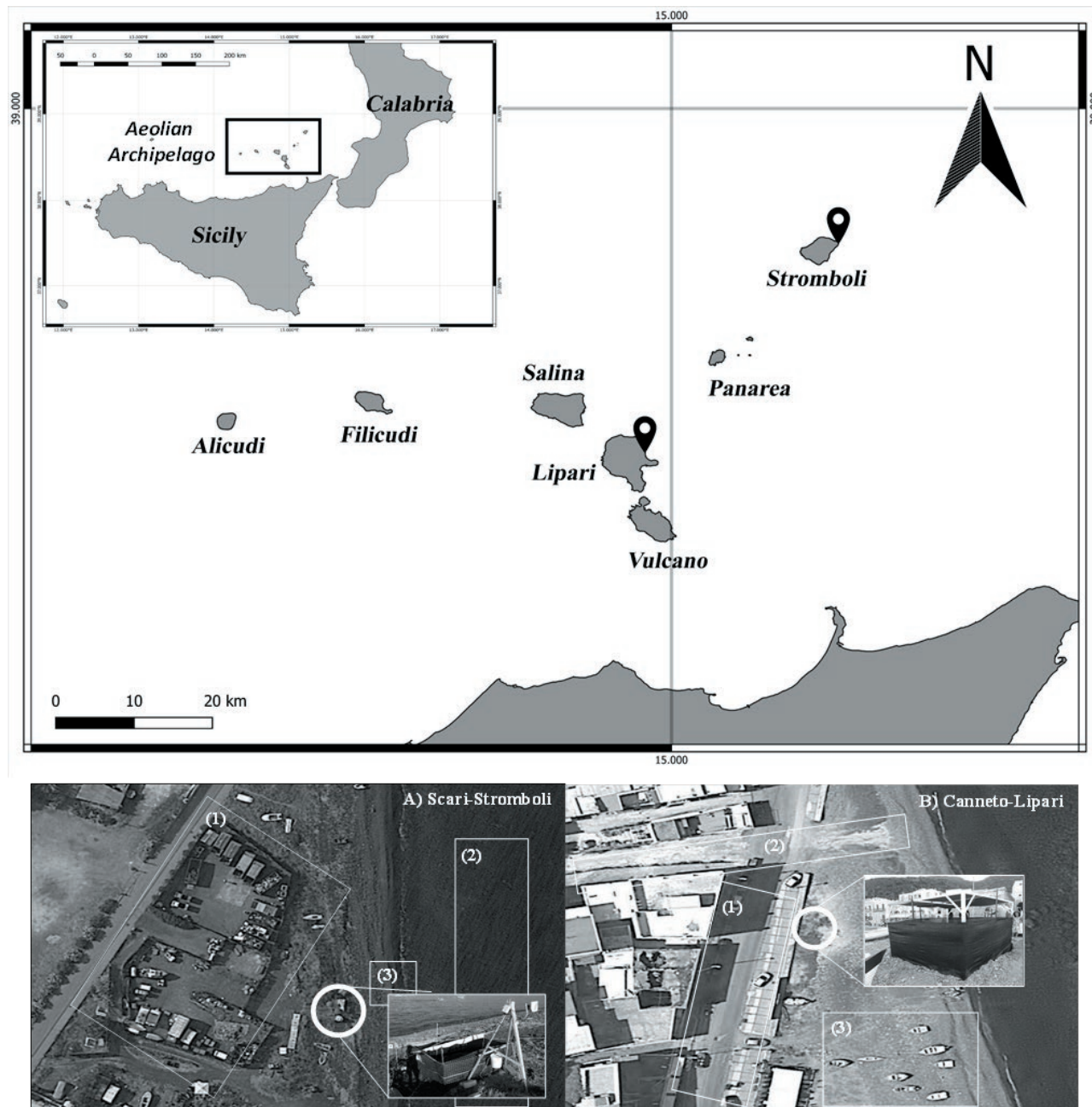


Fig. 1. On the top, location of study area and loggerhead turtle nests. On the lower left (A), particular of the Scari-Stromboli nest with in evidence (1) the dumping site, (2) the buoy field, and (3) the sailing boat stranded on the beach. On the lower right (B), particular of the Canneto-Lipari nest with in evidence (1) the cemented road (2) the runoff channel and (3) the boat storage. The nest removable protective coverages were indicated for both nests.

cles on the sand surface and in front of nest could also may extend the time to arrive at sea for hatchlings on the beaches. Finally, the longer hatchlings reaminding on land the higher is the risk of predation as well (Demetropoulos and Hadjighristophorous, 1995). The features of the beach and the set of circumstances did not

allow to let the hatchlings spontaneously reach the sea. The beach was characterized of large rocks, and pieces of bamboo cane. Furthermore, in front of the nest, there is an area where many sailing boats are in the harbour and during the night, a sailboat ran around on the beach. At the end of the last hatching phase, fixed at 72 hours

(Demetropoulos and Hadjighristophorous, 1995) from the last emergence event, the nest was excavated. After excavation, unhatched eggs were counted, weighed, and the developmental stage of embryos assessed according to standard classification (Miller, 1985), using the methodology stated by Kobayashi et al. (2017). Three embryogenetic classes were used in this study: a) $\leq 21^{\text{st}}$ stages, b) between 22^{nd} - 29^{th} stages, and c) $\geq 30^{\text{th}}$ stages, which included pipping and not emerged hatchlings (for detailed embryonic stage description see Miller, 1985). Finally, each unhatched/hatched egg/embryo was stored in a plastic bag, encoded with an ID number, time, and date of collection, and immediately frozen at -20°C . Core drill samples for sand analyses and nest measurements were taken, including nest minimum and maximum depths and distance from the sea.

RESULTS

Temperature monitoring

Temperature data acquired at 10 cm and 40 cm depths are reported for both nests (Fig. 2A). In the Stromboli nest the temperature at 10 cm depth was over the upper threshold for ideal egg maturation (32°C) during the majority of the study period. In particular, higher values were reported on the 11^{th} and 12^{th} of July and since the 17^{th} of July, every afternoon. On the other hand, temperatures $< 26^{\circ}\text{C}$ were never recorded, with the exception of the 17^{th} of July. At 40 cm the temperature regime was more stable, with a daily oscillation not higher than 1°C , one order of magnitude less than that one observed at 10 cm. An approximately 0.5°C temperature passing of the maximum threshold was observed in the period between the 29^{th} of July and the 4^{th} of August, after which temperature oscillations remained permanently below this limit. In addition, 2 peaks over the maximum threshold of temperature were recorded on the 15^{th} and the 16^{th} of July respectively, as a consequence of a rainfall event of 1 mm. The minimum temperatures recorded at 40 cm depth were over the lower threshold (26°C) during the entire period, except for a few days in the middle of July.

Temperatures at Lipari showed lower variations (Fig. 2B). The maximum daily oscillation was $< 5^{\circ}\text{C}$, at the end of August and at 10 cm depth, and a few decimals of $^{\circ}\text{C}$ at 40 cm. Values over the upper threshold were recorded only after the 17^{th} of August at 10 cm depth. The lower temperature values recorded were between 29 and 31.5°C at 40 cm depth, and between 27 and 30°C at 10 cm.

Hatchlings

At Stromboli 87 eggs were found in the nest (Table 1). The nest had a width of 15 cm (maximum distance between two eggs) and a depth of 18 cm and 33 cm to top and bottom of the eggs chamber, respectively. The incubation period ranged 46 days in Stromboli with a hatchling duration of 46-51 days. Particularly, 3 hatchling events were recorded and 2 excavations performed for a total of 18 emerged hatchlings (20.69%):

- on the 6^{th} of August, between 9:00 PM and 11:46 PM, with 6 emerging hatchlings,
- on the 7^{th} of August, at 2:30 PM, with a single emerging hatchling immediately dead, probably for the high surface temperature (about 39°C):
- on the 8^{th} of August, between 5:56 PM and 8:25 PM, with 8 emerging hatchlings, one of which died shortly after;
- on the 10^{th} of August, at 11:00 PM, after two days since the last emersion, we cautiously excavated the most surficial portion of the nest, finding other two alive hatchlings stuck between basaltic stones;
- on the 11^{th} of August, at 11:00 PM, after another day without any activity, we continued the excavation discovering the last alive hatchling blocked in the sand.

The remaining 69 eggs (79.31%) hosted unhatched embryos at $\leq 21^{\text{st}}$ embryonic stages (Table 1).

The Lipari nest was excavated at the 54^{th} day of incubation, on the 28^{th} of August at 8:03 PM, since no emergences had occurred several days after the predicted incubation period (i.e., 45-50) (Kaska et al., 1998; Reid, 2005; Godley et al. 2001), finding 111 eggs with unhatched embryos. Twenty-one eggs (18.92%) contained embryos at $\leq 21^{\text{st}}$ developmental stages, 70 eggs (63.06%) had embryos at 22^{nd} - 29^{th} stages, and 16 eggs (14.41%) had embryos at $\geq 30^{\text{th}}$ stages (Table 1), 11 of them (9.91%) had pipped (stage 31a) (Table 1). Additionally, 4 not-emerged hatchlings (stage 31b) were found dead at the upper part of the nest (3.60%). The nest had a width of 19 cm (maximum distance between two eggs) and a depth of 16 cm and 35 cm to top and bottom of the eggs chamber, respectively.

Average body size measurements of hatchlings for both nests are reported in Table 2.

DISCUSSION

This study is the first quantitative documentation of two nesting events of loggerhead turtle in the Aeolian Archipelago, with 18 hatchlings (20.69%) from 87

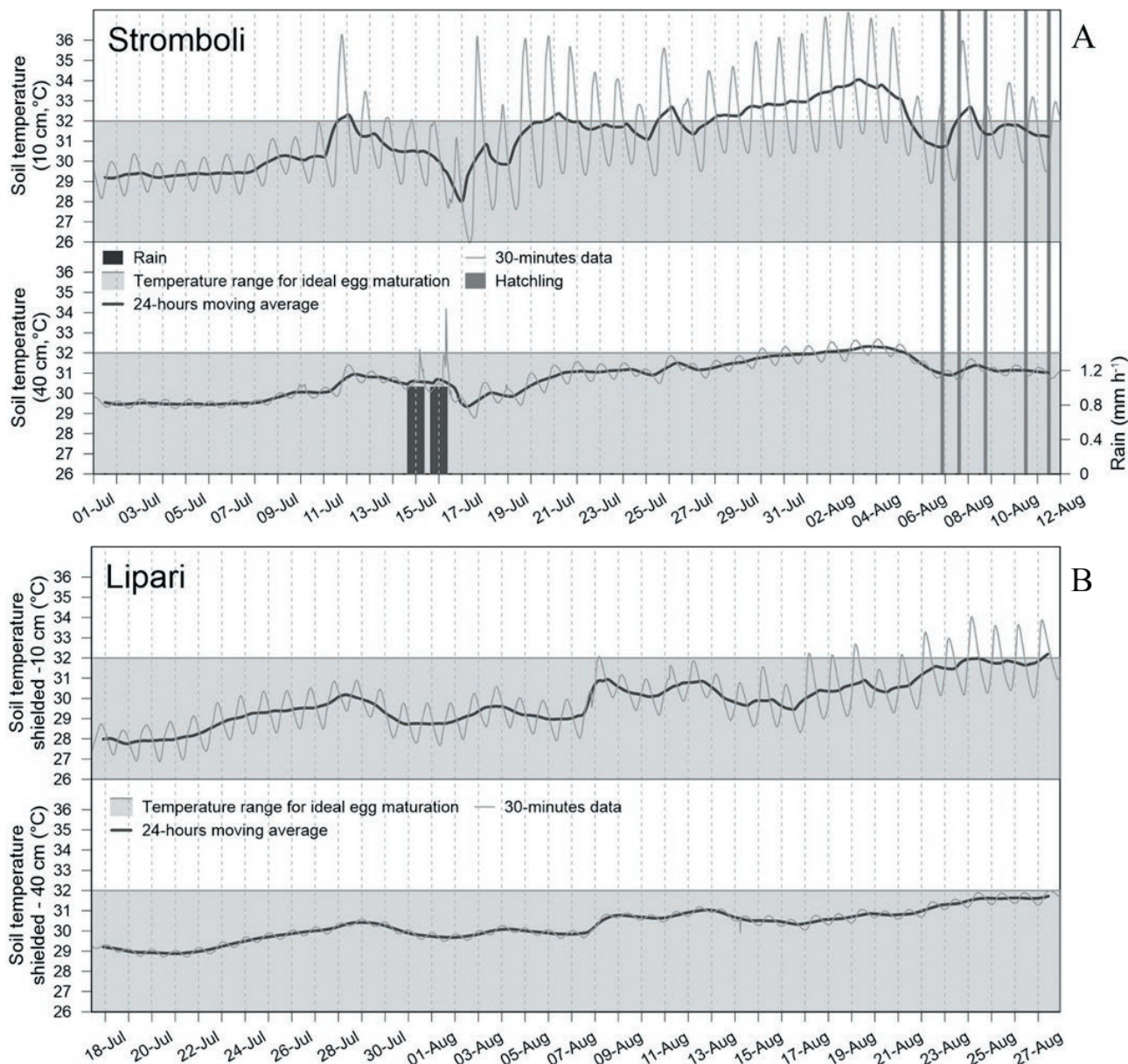


Fig. 2. Nest temperature at 10 cm and 40 cm depths recorded at Stromboli (A) and Lipari (B) during the nesting periods. Ideal temperature range for egg maturation, hatchlings and rainfall events are also reported for Stromboli.

eggs at Stromboli and no successfully released hatchlings at Lipari (111 eggs). Both nests were laid during the seasonal period of maximum frequency for the species in Italy and in the Mediterranean Sea (Giacoma et al., 2011). Similarly, the incubation period and the clutch size fall within the normal range for the species (Giacoma et al., 2011).

Different reasons could be at the base of the scarce hatchling success at Stromboli and its total unsuccess at Lipari.

In the case of Stromboli, the high temperatures recorded inside the nest, due to the color and composition of sand, could have been the main reason of the low percentage of hatchlings. The nest showed temperatures at 10 cm in depth over the upper threshold for ideal egg maturation (32 °C), with particular reference to the later incubation period. Conversely, temperatures were never below the lower threshold. Studies on nests with similar temperature ranges report on a low emergence success (Chu et al., 2008; Read et al., 2012), especially during the

Table 1. Percentage of hatchlings and embryos at different development stages (Miller, 1985) for Stromboli and Lipari nests.

| | Stromboli (%) (N=87) | Lipari (%) (N=111) |
|-----------------------|----------------------|--------------------|
| Hatchlings | | |
| Successfully released | 18.40 | 0 |
| Pre-emergence death | 0 | 3.60 |
| Post-emergence death | 2.29 | 0 |
| Embryos | | |
| ≤ 21 | 79.31 | 18.92 |
| 22-29 | 0 | 63.06 |
| ≥ 30 | 0 | 14.41 |

Table 2. Average morphometric data for Stromboli and Lipari hatchlings. SCL = straight carapace length; SCW = straight carapace width; SPL = straight plastron length; SPW = straight plastron width (Bolten et al., 1993).

| Morphometrics data | Mean (±SD) Stromboli | Mean (±SD) Lipari |
|--------------------|----------------------|-------------------|
| SCL (mm) | 39.57 ± 3.73 | 37.26 ± 2.64 |
| SCW (mm) | 30.31 ± 4.35 | 27.48 ± 3.43 |
| SPL (mm) | 30.32 ± 5.19 | 26.96 ± 6.19 |
| SPW (mm) | 29.41 ± 4.11 | 23.36 ± 1.23 |
| Weight (gr) | 14.28 ± 1.27 | 11.6 ± 2.79 |

last days of incubation (Matsuzawa et al., 2002; Maulany et al., 2012). Finally, the presence of basaltic products mixed to sand in the nests could have influenced escape success (i.e., 3 hatchling events and 3 blocked hatchlings) and duration (from 46-51 days) of hatchling phase (Foley et al., 2006).

At Lipari nest temperature was always within the thresholds for ideal egg maturation, so different reasons should be invoked for explaining the complete hatchling unsuccess. Possible explanations could be found in the absorption of parassites/contaminants from the material constituting the partially artificial beach (Alava et al., 2006), favored by a rainfall event during the middle third of the incubation period (Foley et al., 2006), or in anoxic conditions (Margaritoulis, 2005; Lolavar and Wyneken, 2015) due to the presence of the very fine particulate created by the mechanical crushing of the pumice.

From this study, we have learned that the Aeolian Archipelago may ideally host irregular nesting sites for loggerhead turtles. A higher monitoring and conservation effort is recommended for these sites to increase the chance of positive hatchings.

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Threatened and extinct amphibians and reptiles in Italian natural history collections are useful conservation tools

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Abstract. Natural history museums are irreplaceable tools to study and preserve the biological diversity around the globe and among the primary actors in the recognition of species and the logical repositories for their type specimens. In this paper we surveyed the consistency of the preserved specimens of amphibians and reptiles housed in the major Italian scientific collections, and verified the presence of threatened species according to the IUCN Red List, including the Extinct (EX), Extinct in the Wild (EW), Critically Endangered (CR), Endangered (EN), and Vulnerable (VU) categories. Altogether, we analyzed 39 Italian zoological collections. We confirmed the presence of one extinct reptile (*Chioninia coctei*) and five extinct or extinct in the wild amphibian species (*Atelopus longirostris*, *Nectophrynoides asperginis*, *Pseudophilautus leucorhinus*, *P. nasutus*, and *P. variabilis*). Seven CR amphibians, fourteen CR reptile species and the extinct skink *C. coctei* are shared by more than one institution. Museums which host the highest number of threatened and extinct amphibian species are respectively Turin (17 CR and 1 EX), Florence (13 CR and 1 EX), and Trento (15 CR and 1 EW), while for reptiles the richest museums are those from Genoa (15 CR and 1 EX), Florence (11 CR and 1 EX), and Pisa (7 CR). Finally, we discussed the utility of natural history museums and the strategies to follow for the implementation of their functionality.

Keywords. Biodiversity, collections, conservation, herpetology, IUCN categories, natural history museums.

INTRODUCTION

Biodiversity collections hosted in natural history museums, universities and other institutions are important cultural, scientific heritages, irreplaceable resources, and useful tools for a great array of studies to enhance biodiversity understanding and conservation. These include systematic investigations, taxonomic revisions, species descriptions, epidemiology, anatomy, historical reconstructions (e.g., about collectors and the institutions housing their collections, as well as about the history of science in general), but also many more science-outreach purposes, such as educational activities, and exhibitions (Bakker et al., 2020).

The origin and the history of biological – in particular zoological - “voucher specimens” or, simply, “vouchers” (Schilthuizen et al., 2015) housed in scientific collections, are rather heterogeneous, since they may result from various activities, e.g., field surveys with collecting, sampling for scientific research, recovery of dead animals (e.g., on roads, from zoological parks, captive breeders, etc.), direct purchases from dealers (for collections increase, exhibits and education), and others (Funk et al., 2005). Data associated with vouchers may also vary

substantially across periods, going from just a rough species determination (as it was often the case in the past, especially for specimens obtained from commercial dealers), to an exhaustive set of information, including place and date of collection, collectors, donors, ecological and behavioral parameters, etc.

The different kind of preservation techniques may also affect the degree of scientific, expository and utility of specimens, going from traditionally naturalized / preserved specimens used for display, to “ad hoc” prepared series for scientific research, accompanied by tissue samples, photographs, parasitological/epidemiological specimens, etc. (Lorch et al., 2021). Particular and precious vouchers hosted in collections of museums are the so-called «type specimens», i.e., preserved individuals upon which new taxa are described, including, among the others, holotypes, paratypes, lectotypes, and syntypes (Dubois, 2017).

Natural history museums (hereafter NHMs or museums) are considered the heirs of the 16th-17th century “Wunderkammern”, also known as “cabinets of curiosities” (Butler et al., 1998; McCarter et al., 2001). The collections of “mirabilia”, “naturalia” and “exotica” (as natural history objects were sometimes labeled in the

past) were accumulated over the time by naturalists and “savants”. In a first phase of museums’ life, these objects were mainly used to solicit and address the wonders and varieties of nature, at those times still largely unknown. Until then, objects were at the same time the origin and the pulsing heart of these collections and early museums, as well as one of the few ways to discover and describe nature (Findlen, 1996).

Nowadays, the ultimate profile of scientific collections can be drawn looking at what happened in medium-large and national museums (Suarez and Tsutsui, 2004). These institutions, which are true documenting centers and repositories of the world’s geo- and biodiversity, manage specimens and associated materials that often represent unique evidence of species distribution and evolution patterns (Clemann et al., 2014). The information gathered from extinct species is irreplaceable and lost forever: the International Union for Conservation of Nature (IUCN) declared 160 animal and plant species extinct between 2010 and 2019, although recent estimates suggest that this estimate may be as high as one thousand species per year (Ceballos et al., 2015; Shivanna, 2020).

NHMs are relevant in preserving specimens and other materials, and this is gradually becoming one of the modern museums’ top finalities. Indeed, vouchers of extinct species are useful reservoirs of information, crucial to understand the reasons and history of their extinction. At the same time, the availability of samples belonging to threatened species is an important and irreplaceable source of data for better understanding the conservation threats (e.g., Reed and Shine, 2002).

As reported elsewhere (Alberch et al., 1994; Bakker et al., 2020), NHMs should be primarily considered as research, documentation and divulgation centers, since their role in biodiversity discovery and nature valorization is central, and useful in showing variations in abundance, flora and fauna (Ewers-Saucedo et al., 2021). Natural history and biodiversity museums are also the logical repositories for vouchers of species becoming rarer, and in some cases extinct (Buckingham et al., 2021). This allows to keep track of biological changes since preserved specimens are excellent scientific resources representing unique means through which such species can be studied for their morphology, ecology, genetics, and other traits. In addition, they can be used in comparative studies, e.g., with extant species, not only for taxonomic and phylogenetic studies, but also to unveil ecological and life history traits (Figueirido and Janis, 2011).

Notwithstanding, while the advancement in our understanding of nature and the drafting of a life catalogue of our planet are still badly needed and considered as a humankind priority mission, in many cases NHMs

themselves are increasingly facing severe problems of identity and survivorship, due not only to economic reasons but also to a generalized shift and diffused amnesia of their original missions (Boero, 2010; Andreone et al., 2014, 2022; Andreone, 2015; Ceriaco et al., 2021). On the other hand, we also believe that it is imperative to reconcile the research/collection and outreach/education components within NHMs, and that vouchers and scientific collections can be efficiently used for this aim.

In the course of two national projects focused on the valorization of natural history collections – namely “VertEx (Vertebrata Extincta)” and “Estinzioni” (Extinctions) (Nicolosi et al., 2013, 2019) – we evaluated the consistency of vertebrate collections in Italian NHMs, with the aim to define the conservation status and IUCN Red List placement of the housed voucher specimens. Hopefully, the identification of threatened and extinct species within museum collections is useful to address scientists and the public to understand and contrast the rarefaction and disappearance of our biodiversity, with dedicated temporary expositions, books, postcards, and gadgets.

So far, in the present paper we focused our attention on amphibians and non-avian reptiles, two vertebrate groups often treated together in both research and the traditional imagery, which represent a major component of museum collections. Here, we also give a general overview of the overall Italian herpetological/museological patrimony, about ten years after the first comprehensive work (Mazzotti, 2010) and provide indications on their conservation assessment.

MATERIAL AND METHODS

We selected the major Italian herpetological collections, basing upon data provided by the National Association of Scientific Museums (Associazione Nazionale dei Musei Scientifici - ANMS), associated projects, e.g., CollMap (Vomero, 2013), and previously published contributions (Mazzotti, 2010). Basing upon feedbacks from curators and referring institutions, we gathered useful information from 39 natural history collections (managed by public museums, universities, and/or a few private bodies), which replied positively to our request and provided relevant data (Table 1). A few NHMs were excluded since, although known for possessing herpetological specimens, they did not reply, or did not provide sufficiently complete information.

The existence of published/unpublished catalogues and/or lists of species/specimens housed in each collection was assured by the relative curator/referent, as well as through bibliography [(Carmagnola: Boano and Del-

Table 1. List of the Italian natural history museums contributing with collections data (abbreviations of provinces are reported between parentheses; TAEI = taxonomic auto-evaluation index; total number of species of amphibians and reptiles preserved in the collection is reported). Museums accompanied by an asterisk (*) are those having a herpetologist as curator.

| Used acronym and official museum denomination | Municipality (Province) | Management type | TAEI | Species number | |
|---|----------------------------------|-----------------|------|----------------|----------|
| | | | | Amphibians | Reptiles |
| TO Museo Regionale di Scienze Naturali * | Turin (To) | Region | 3 | 593 | 855 |
| TO-DB Museo di Storia Naturale "Don Bosco" | Turin (To) | High school | 2 | 12 | 49 |
| TO-C Museo Civico di Storia Naturale * | Carmagnola (To) | Municipality | 4 | 69 | 374 |
| CN-A Museo Civico "Federico Eusebio" | Alba (Cn) | Municipality | 4 | 8 | 9 |
| CN-B Museo Civico "Craveri" di Storia Naturale | Bra (Cn) | Municipality | 4 | 10 | 53 |
| VC-V Museo di Storia Naturale "Pietro Calderini" | Varallo Sesia (Vc) | Foundation | 4 | 2 | 20 |
| VCO-D Civico Museo di Storia Naturale "G. G. Galletti" | Domodossola (VCO) | Municipality | 3 | 28 | 46 |
| GE Museo Civico di Storia Naturale "Giacomo Doria" * | Genoa (Ge) | Municipality | 4 | 590 | 1450 |
| MI Museo di Storia Naturale * | Milan (Mi) | Municipality | 4 | 116 | 637 |
| PV Museo di Storia Naturale dell'Università * | Pavia (Pv) | University | 4 | 100 | 310 |
| PV-V Civico Museo di Scienze Naturali | Voghera (Pv) | Municipality | 4 | 6 | 16 |
| BG Museo Civico di Scienze Naturali | Bergamo (Bg) | Municipality | 4 | 28 | 153 |
| SO-M Museo Civico di Storia Naturale * | Morbegno (So) | Municipality | 4 | 10 | 16 |
| TV Museo Zoologico "G. Scarpa" | Treviso (Tv) | Diocese | 4 | 83 | 285 |
| VR Museo di Storia Naturale * | Verona (Vr) | Municipality | 4 | 99 | 263 |
| VI Museo Naturalistico Archeologico di Vicenza | Vicenza (Vi) | Municipality | 1 | 12 | 23 |
| PD Museo di Zoologia dell'Università | Padova (Pd) | University | 3 | 75 | 115 |
| VE Museo di Storia Naturale "G. Ligabue" * | Venice (Ve) | Foundation | 4 | 77 | 170 |
| RO Fondazione Museo Civico di Rovereto | Rovereto (Tn) | Foundation | 4 | 11 | 41 |
| TN MUSE - Museo delle Scienze * | Trento (Tn) | Province | 4 | 185 | 170 |
| PN Museo Civico di Storia Naturale | Pordenone (Pn) | Municipality | 3 | 1 | 23 |
| UD Museo Friulano di Storia Naturale * | Udine (Ud) | Municipality | 4 | 153 | 63 |
| TS Museo Civico di Storia Naturale * | Trieste (Ts) | Municipality | 4 | 78 | 360 |
| PR Museo di Storia Naturale dell'Università | Parma (Pr) | University | 2 | 17 | 75 |
| MR Museo di Zoologia e Anatomia Comparata dell'Università | Modena (Mo) / Reggio Emilia (Re) | University | 3 | 37 | 112 |
| BO Collezione di Anatomia Comparata, Sistema Museale di Ateneo | Bologna (Bo) | University | 4 | 4 | 53 |
| FE Museo Civico di Storia Naturale * | Ferrara (Fe) | Municipality | 4 | 77 | 95 |
| AN-O Museo di scienze naturali "Luigi Paolucci" | Offagna (An) | Municipality | 3 | 10 | 12 |
| FI Museo di Storia Naturale dell'Università * | Florence (Fi) | University | 3 | 627 | 1268 |
| PI Museo di Storia Naturale dell'Università * | Calci di Pisa (Pi) | University | 2 | 158 | 582 |
| GR Museo di Storia Naturale della Maremma | Grosseto (Gr) | Municipality | 3 | 3 | 14 |
| LI Museo di storia naturale del Mediterraneo | Livorno (Li) | Province | 3 | 6 | 31 |
| SI Museo di Storia Naturale dell'Accademia dei Fisiocritici | Siena (Si) | Foundation | 3 | 26 | 95 |
| RM Museo Civico di Zoologia * | Rome (Rm) | Municipality | 4 | 168 | 194 |
| RM-S Società Romana di Scienze Naturali | Rome (Rm) | Association | 4 | 43 | 117 |
| LE Museo di Storia Naturale del Salento * | Calimera (Le) | Municipality | 4 | 29 | 77 |
| NA Centro Museale Centro Musei delle Scienze Naturali dell'Università | Naples (Na) | University | 2 | 35 | 72 |
| BA Museo di Zoologia "Lidia Iaci" dell'Università | Bari (Ba) | University | 4 | 25 | 71 |
| PA Museo di zoologia "Pietro Doderlein" dell'Università | Palermo (Pa) | University | 2 | 32 | 85 |

mastro, 1990; Sindaco, 1990); (Turin: Elter, 1982; Gavetti and Andreone, 1993; Andreone et al., 2007); (Domodossola: Andreone et al., 2005); (Genoa: Doria et al., 2002);

(Milan: Leonardi et al., 1995; Scali, 1996; Blackburn and Scali, 2014); (Varese: Danini and Baratelli, 2000); (Morbegno: Zuffi, 1990); (Padua: Centis, 2004); (Udine: Lap-

ini, 1984); (Trieste: Bressi, 1996); (Florence: Lanza et al., 2005, 2006); (Ferrara: Mazzotti and Miserocchi, 2009, 2010); (Rome: Capula et al., 2011; Crucitti et al., 2017, 2021); (Naples: Maio et al., 2004)].

We used as taxonomic references “Amphibian Species of the World” (Frost, 2021), “AmphibiaWeb” (AmphibiaWeb, 2021), and “The Reptile Database” (Uetz et al., 2021). Exhaustiveness and correctness of the reported information is warranted directly by curators of the museums in this study. Taken into account that the degree of past and ongoing curatorial activity and taxonomic revisions are rather variable among institutions and since it was impossible to revise all the collections, we asked to provide a “taxonomic auto-evaluation index” (TAEI), as follows: 1 (lowest taxonomic accurateness and/or collection without a proper revision), 2 (collection revised for the 30-40%), 3 (collection revised for the 60-70%), 4 (highest taxonomic accurateness, with both the amphibian and reptile collections fully revised).

Finally, we carried out a conservation assessment for each species, including attribution of threat categories according to the IUCN Red List (IUCN, 2021).

RESULTS

The examination of the Italian herpetological collections produced a catalogue including more than 1400 species and 67 families of amphibians, and more than 2500 species and 80 families of reptiles (respectively 1418 and 2513 species).

Of the amphibians, 257 (18.1%) belong to a threat category. Of these species, 5 (1.9%) are Extinct (EX), 47 (18.3%) Critically Endangered (CR), 100 (38.9%) Endangered (EN), and 105 (40.9%) Vulnerable (VU). Regarding reptiles, 210 (8.3%) belong to a threat category: one species (0.5%) is considered EX, 32 (15.2%) CR, 74 (35.2%) EN, and 103 (49.1%) VU (Fig. 1). Museums and collections were quite heterogeneous, varying in size and finalities. Overall, the NHMs hosting the greatest number of threatened species of amphibians and reptiles (> 50 species of each group) are Genoa (174 in total), Florence (166 in total), and Turin (161 in total) (Fig. 2).

Amphibians hosted in the Italian institutions belong to 7 Gymnophiona, 52 Anura, and 8 Urodela families, while reptiles are represented by 13 Testudines, one Rhyncocephalia, three Crocodylia, 38 Sauria, and 25 Ophidia families (Appendix I). Species assessed as CR constitute respectively the 0.56% and 0.28% of the amphibian and reptile world fauna, on the basis of the global numbers provided by AmphibiaWeb (8384

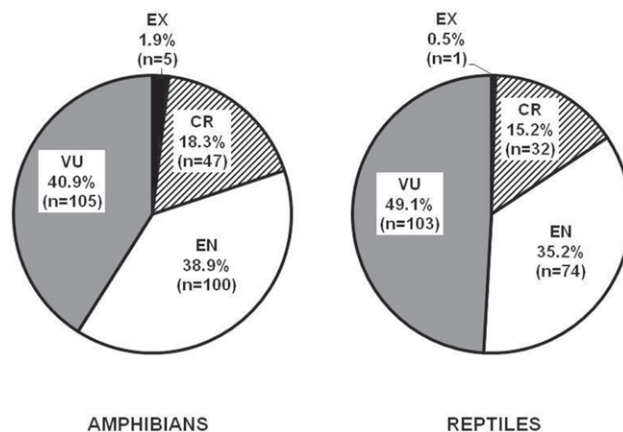


Fig. 1. Percentage of threatened species of amphibians (left) and reptiles (right) in the different IUCN categories.

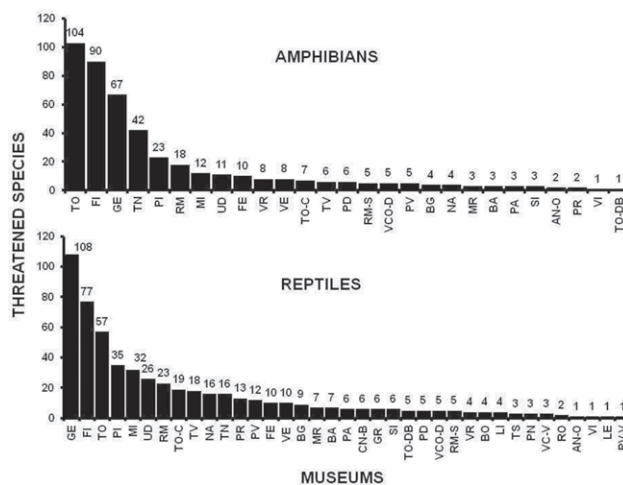


Fig. 2. Presence of threatened species in Italian museums for amphibians (above) and reptiles (below). Museum abbreviation as in Table 1.

amphibians on 25th October 2021) and Reptile Database (11570 reptiles on May 2021; Uetz et al., 2021).

Preserved CR amphibians include five urodeles and 46 anurans (Table 2). *Atelopus longirostris*, *Nectophrynoides asperginis*, *Pseudophilautus leucorhinus*, *P. nasutus*, and *P. variabilis* are currently assessed as EW or EX. About reptiles, we found respectively 32 CR and one EX species, *Chioninia coctei* (Table 3). Four CR species are loricates, 14 chelonians, 12 saurians, and one snake. Fourteen CR reptile species and the extinct *C. coctei* are shared by more than one collection.

NHMs hosting the highest number of high threatened and extinct amphibian species are respectively Turin (17 CR / 1 EX), Florence (13 CR / 1 EX), and Trento (15

Table 2. List of Critically Endangered (CR), Extinct (EX) and Extinct in the Wild (EW) amphibian species housed in Italian natural history museums and their occurrence in the analysed collections. Extinct species are given in bold. Museums are reported according to the abbreviations provided in Table 1.

| Species | IUCN Family | Museums | Species | IUCN Family | Museums |
|--|-------------------|----------------|---|--------------------|---------|
| <i>Hynobius abei</i> | CR Hynobiidae | FI | <i>Nectophrynoides paulae</i> | CR Bufonidae | TN |
| <i>Hynobius okiensis</i> | CR Hynobiidae | FI | <i>Nectophrynoides poyntoni</i> | CR Bufonidae | TN |
| <i>Chiropterotriton magnipes</i> | CR Plethodontidae | RM | <i>Nectophrynoides wendyae</i> | CR Bufonidae | TN |
| <i>Thorius pennatulus</i> | CR Plethodontidae | FI | <i>Werneria mertensiana</i> | CR Bufonidae | TN |
| <i>Pseudoeurycea goebeli</i> | CR Plethodontidae | GE | <i>Wolterstorffina parvipalmata</i> | CR Bufonidae | TN |
| <i>Latonia nigriventer</i> | CR Alytidae | FI | <i>Craugastor fleischmanni</i> | CR Craugastoridae | TO |
| <i>Arthroleptis nikeae</i> | CR Arthroleptidae | TN | <i>Craugastor lineatus</i> | CR Craugastoridae | FI |
| <i>Atelognathus patagonicus</i> | CR Batrachylidae | FI | <i>Craugastor milesi</i> | CR Craugastoridae | GE |
| <i>Callulina hanseni</i> | CR Brevicipitidae | TN | <i>Hyloxalus vertebralis</i> | CR Dendrobatidae | GE |
| <i>Callulina kanga</i> | CR Brevicipitidae | TN | <i>Isthmohyla debilis</i> | CR Hylidae | RM |
| <i>Callulina laphami</i> | CR Brevicipitidae | TN | <i>Hyperolius davenporti</i> | CR Hyperoliidae | TN |
| <i>Callulina meteora</i> | CR Brevicipitidae | TN | <i>Pleurodema somuncureense</i> | CR Leptodactylidae | FI, TO |
| <i>Callulina shengena</i> | CR Brevicipitidae | TN | <i>Boophis ankarafensis</i> | CR Mantellidae | TO |
| <i>Callulina stanleyi</i> | CR Brevicipitidae | TN | <i>Boophis tsilomaro</i> | CR Mantellidae | TO |
| <i>Altiphrynoides osgoodi</i> | CR Bufonidae | FI | <i>Boophis williamsii</i> | CR Mantellidae | TO |
| <i>Atelopus boulengeri</i> | CR Bufonidae | TO | <i>Guibemantis diphonus</i> | CR Mantellidae | TO |
| <i>Atelopus cruciger</i> | CR Bufonidae | TO | <i>Guibemantis punctatus</i> | CR Mantellidae | TO |
| <i>Atelopus ignescens</i> | CR Bufonidae | FI, PD, PI, TO | <i>Mantella milotympnum</i> | CR Mantellidae | TO |
| <i>Atelopus longirostris</i> | EX Bufonidae | FI | <i>Mantidactylus pauliani</i> | CR Mantellidae | TO |
| <i>Atelopus varius</i> | CR Bufonidae | PI, TO | <i>Platypelis karenae</i> | CR Microhylidae | TO |
| <i>Churamiti maridadi</i> | CR Bufonidae | TN | <i>Rana holtzi</i> | CR Ranidae | FI, TO |
| <i>Incilius cristatus</i> | CR Bufonidae | FI | <i>Pseudophilautus leucorhinus</i> | EX Rhacophoridae | GE |
| <i>Leptophryne cruentata</i> | CR Bufonidae | GE | <i>Pseudophilautus nasutus</i> | EX Rhacophoridae | TO |
| <i>Nectophrynoides asperginis</i> | EW Bufonidae | TN | <i>Pseudophilautus variabilis</i> | EX Rhacophoridae | GE, PI |
| <i>Nectophrynoides laticeps</i> | CR Bufonidae | TN | <i>Rhinoderma rufum</i> | CR Rhinodermatidae | TO |
| | | | <i>Telmatobius laticeps</i> | CR Telmatobiidae | FI, TO |

CR / 1 EW), while for reptiles are Genoa (15 CR / 1 EX), Florence (11 CR / 1 EX), and Pisa (7 CR). A large part of the CR amphibian and reptile species (26) comes from a single museum/collection (Florence). The most shared CR species are, among the amphibians, *Atelopus ignescens*, which is present in four collections, and, among the reptiles, *Eretmochelys imbricata* and *Gavialis gangeticus*, respectively hosted by fourteen and eleven institutions.

A particular consideration should be deserved for the case of the axolotl *Ambystoma mexicanum*. This urodele species is present in 16 of the examined collections, and, according to the IUCN Red List, it should be assessed as CR. Anyhow, since it is likely that most of the preserved specimens derive from captive and laboratory strains and given the impossibility of determining whether these animals (especially the historical ones) introgressed with *Ambystoma tigrinum* (Torres-Sánchez, 2020), we decided not to consider them within the list of threatened taxa housed in Italian museums.

DISCUSSION

Extinct and threatened species in scientific collections

The presence in natural history collections of amphibian and reptile species included within the IUCN's threatened categories gives the opportunity to unveil aspects otherwise difficult to obtain in the wild. As an example, the availability of a sufficiently large voucher series of some frogs of the genus *Mantella* from Madagascar allowed to investigate their fecundity (Tessa et al., 2009), age structure (Andreone et al., 2011) and, successively, to use these parameters to draw a general model exploitation method (Andreone et al., 2021).

Differently from collecting for food, traditional medicine, fashion market, handicraft production, and other purposes, which are clearly recognized as relevant threats affecting rare and localized species (especially for the high number of traded individuals), a reasoned collecting of scientific vouchers is unlikely to be or become an

Table 3. List of Critically Endangered (CR) and Extinct (EX) species of reptiles and natural history museums where they are preserved. Extinct species are given in bold. Museums are reported according to the abbreviations provided in Table 1.

| Species | IUCN | Family | Museums |
|--------------------------------------|------|----------------|---|
| <i>Crocodylus intermedius</i> | CR | Crocodylidae | PI, PR, RM |
| <i>Crocodylus rhombifer</i> | CR | Crocodylidae | FI, GE, GR, MI, VCO-D, |
| <i>Crocodylus siamensis</i> | CR | Crocodylidae | FI, GE, MI, NA, PI, PV, RM, TO, TO-DB, TV |
| <i>Mecistops cataphractus</i> | CR | Crocodylidae | PI, TO |
| <i>Gavialis gangeticus</i> | CR | Gavialidae | FI, GE, MI, NA, PI, PR, PV, RM, TO, TO-DB, TV |
| <i>Eretmochelys imbricata</i> | CR | Cheloniidae | BG, BR, FI, GE, LI, MI, PI, PR, PV, TO, TV, UD, VCO-D, VE |
| <i>Lepidochelys kempii</i> | CR | Cheloniidae | BG, GE |
| <i>Dermatemys mawii</i> | CR | Dermatemydidae | TV |
| <i>Batagur baska</i> | CR | Geoemydidae | GE |
| <i>Batagur dhongoka</i> | CR | Geoemydidae | GE |
| <i>Batagur kachunga</i> | CR | Geoemydidae | GE |
| <i>Batagur trivittata</i> | CR | Geoemydidae | BG, GE |
| <i>Cuora trifasciata</i> | CR | Geoemydidae | FI, UD |
| <i>Heosemys depressa</i> | CR | Geoemydidae | GE |
| <i>Astrochelys radiata</i> | CR | Testudinidae | FI, MI, NA, PI, RM, SI, TO, UD |
| <i>Chelonoidis porteri</i> | CR | Testudinidae | RM |
| <i>Geochelone platynota</i> | CR | Testudinidae | GE |
| <i>Psammobates geometricus</i> | CR | Testudinidae | FI, PI, PV, RM |
| <i>Testudo kleinmanni</i> | CR | Testudinidae | GE |
| <i>Hemidactylus bouvieri</i> | CR | Gekkonidae | CN-B, GE |
| <i>Lygodactylus williamsi</i> | CR | Gekkonidae | TN |
| <i>Conolophus marthae</i> | CR | Iguanidae | RM |
| <i>Acanthodactylus beershebensis</i> | CR | Lacertidae | FI |
| <i>Acanthodactylus harranensis</i> | CR | Lacertidae | FI, RM-S, TO-C |
| <i>Eremias pleskei</i> | CR | Lacertidae | TO-C |
| <i>Erythrolamprus cursor</i> | CR | Lacertidae | GE |
| <i>Gallotia simonyi</i> | CR | Lacertidae | GE |
| <i>Podarcis raffonei</i> | CR | Lacertidae | FE, FI, PA, PD, RM |
| <i>Liolaemus rabinoi</i> | CR | Liolaemidae | FI |
| <i>Chioninia coctei</i> | EX | Scincidae | FI, GE, PA, TO, TV |
| <i>Mabuya mabouya</i> | CR | Scincidae | FI, MI |
| <i>Pseudoacantias menamainty</i> | CR | Scincidae | TO |
| <i>Spondylurus culebrae</i> | CR | Scincidae | GE |
| <i>Atheris matildae</i> | CR | Viperidae | TN |

extinction cause (Rocha et al., 2014). We stress that specimen collection for scientific purposes is usually limited to a few vouchers, whose capture and collecting need to be authorised by national authorities and regulated by legislation. At the same time, we believe that the collecting activity of vouchers still remains crucial and should be maintained, as stressed by Dubois (2003, 2010, 2017), to document not only the presence of rare and threatened species, but also biological parameters and represents an unsurpassed source of scientific data that are only in part exploited. Finally, it is often crucial to witness the presence of a species at a confirmed geographic locality, possibly integrated with further evidences, such as eDNA,

acoustic recordings, photographs, and footage. This is particularly important, as the collecting of series of specimens is also useful for conservation purposes, e.g., to identify negative trends in populations, especially in current times which are featured by dramatic changes of climatic and environmental parameters (e.g., Hoffmann et al., 2010; Hou et al., 2021).

Threatened and extinct species of amphibians and reptiles in Italian collections

Some of the analysed Italian NHMs turned out to be especially relevant due to the high number of species

and specimens housed in the scientific collections under their care, and on the amount of threatened species. Most of the extinct species present in these institutions were likely collected during general collecting activities and/or obtained in exchange from other scientists/institutions. In the case of the Kihansi spray toad *Nectophrynoides asperginis*, a species from Tanzania extinct in the wild due to the spread of the chytrid fungus (Channing et al., 2006), the specimens were obtained in the context of structured multi-year research (Menegon et al., 2004; Msuya and Mohamed, 2019). The giant Cape Verde skink *Chioninia coctei* is present in a few Italian museums, which are Florence, Genoa, Palermo, Turin, and Treviso. Of special relevance are the live individuals (around forty) imported by the herpetologist Mario G. Peracca at the end of the 19th century, and currently hosted in Turin (Andreone and Gavetti, 2007, 2010). Such a conspicuous purchase was made through an animal dealer, and was accompanied by the concurrent importation of some other rare or iconic live herps, i.e., *Andrias japonicus*, *Aldabrachelys gigantea*, *Astrochelys radiata*, *Iguana iguana*, and *Sphenodon punctatus* (Andreone and Gavetti, 1998). Peracca also made some interesting observations on the skink natural history, and then exchanged some individuals with other naturalists of his time, such as G. Scarpa in Treviso (Andreone et al., 2010). After Peracca's death some of these skinks were donated (as other animals) to the Turin Museum, which in fact was not the commissioner for the collecting of a rare and threatened species, but just its final repository.

With respect to amphibians, the institution hosting the highest number of CR species is the Turin Museum, with 17 taxa, eight of which originated from the collecting surveys carried out during field-work in Madagascar (Andreone et al., 2005, 2021), followed by MUSE - Museo delle Scienze in Trento, with 15 species from Tanzania and other eastern African countries. Further remarkable species available in Turin come from Latin America, mostly due to the activity of the Italo-Argentinean herpetologist J. M. Cei (Cei, 1993). Florence and Genoa are also the NHMs holding the highest number of CR reptile species. This highlights the importance of active research in the constitution of study collections. The CR amphibian species housed in Trento originated from systematic field research carried out over the past 20 years in the forests of the Eastern Afromontane Ark (Menegon et al., 2008).

Many natural history museums supported, among their institutional activities, survey works in unexplored or marginally explored areas of our planet. In particular, this was one of the ultimate aims of middle-large museums, where collections were usually regarded as vouch-

er repositories (Grimaldi and Engel, 2007; Engel et al., 2021), much less for smaller museums where the education aspects are usually prioritised.

Is scientific voucher collecting still a needed practice?

In Italy, many museums supported collecting activities in the past, but only a few ones pursue research and specimens collecting, especially overseas. In fact, in the 19th century many naturalists gravitating around Italian museums were engaged to explore the World and to collect new materials (Mazzotti, 2011), such as A. Borelli, E. Festa, and F. De Filippi in Turin, L. D'Albertis, G. Doria, and L. Fea in Genoa, O. Beccari and E. H. Giglioli in Florence, G. Scortecci in Milan, O. Antinori in Perugia, and many others, who mirrored the adventures and travels of Victorian naturalists, contributing to discover new species and describing the still unknown world. The beginning of 20th century, however, coincided with a decrease in such activities in most of the Italian museums: the systematic and taxonomic zoology and botany that fed those travels were largely left behind, often considered useless and subsidiary to the newly affirmed organismic biology. At the same time, museums were often seen as mere repositories and/or expositive locations, and much less (or no more) as research centers (Fischer, 2015). For these reasons too, many ancient collections were neglected and rarely utilised for either scientific or educational purposes (Ceriaco et al., 2021).

To better frame this situation, it should also be remembered that many Italian museums were, and still are, managed by local administrations, such as municipalities, provinces, and regions. This often led to a difficult balance between the expositive/outreach finality and research/collection components, in particular since museums were often nested within culture or education departments and only rarely associated with research and/or environmental ones. Therefore, while research was progressively relegated to a subsidiary activity, most natural history museums acquired a prominent expositive function, sometimes detaching the physiological link between these "souls". Only a few museums appear to have escaped this trend, such as those of Turin (Andreone, 2013), Trento (Menegon et al., 2008), Verona (Latella and Zorzin, 2018), and Florence (Van Lien et al., 2014), whose personnel was able to carry out recent oversea research.

Taking into consideration that many (16) of the analysed Italian NHMs have herpetologists as curators, it is worth to verify whether their presence is accompanied by a better knowledge of amphibian and reptile taxonomy in their collections. The TAEI varied from 1 (one collection) to 4 (22 collections), with a mean value of $3.41 \pm$

0.82, thus indicating that most of these collections were revised recently. This happened mostly for small collections, which were objectively easier to be studied and catalogued, and usually limited to Italian/European faunas.

Since most of these collections are not formed recently, the majority of Italian collections are increasingly becoming historical, with recent acquisitions largely due to occasional specimen collections. We consider this a heavy bias, since it means a loss of taxonomic expertise which may have a negative impact on the increase and valorization of scientific collections.

Andreone et al. (2014), following a proposal by Minelli (2013, 2015), suggested that, in absence of a traditional national museum, a “diffuse network” or “meta-museum” could be a solution to manage the Italian scientific collections in a joint way, also to share resources and personnel. Although little was done to accomplish this proposal so far, this is still an option to be taken into serious consideration together with the possibility of creating a centralized coordination hub. Considering the present fragmentation and the scarce connection among museums, it is first of all important that all Italian natural history collections are increasingly revised and digitalized by each museum, hopefully using shared protocols. This would be enhanced by the establishment of a national strategy that encompasses the coordination and resource distribution as a priority objective.

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APPENDIX I

List of amphibian and reptile families conserved in the Italian natural history collections

AMPHIBIA

GYMNOPHIONA – Caeciliidae, Dermophiidae, Herpeliidae, Ichthyophiidae, Scolecomorphidae, Siphonopidae, Typhlonectidae. ANURA – Alsodidae, Alytidae, Arthroleptidae, Ascaphidae, Batrachylidae, Bombinatoridae, Brachycephalidae, Craugastoridae, Brevicipitidae, Bufonidae, Calyptocephalellidae, Centrolenidae, Ceratobatrachidae, Ceratophryidae, Conrauidae, Eleutherodactylidae, Cycloramphidae, Aromobatidae, Dendrobatidae, Dicroglossidae, Heleophrynidae, Hemiphractidae, Hemisotidae, Hylidae, Hylodidae, Hyperoliidae, Leiopelmatidae, Leptodactylidae, Mantellidae, Megophryidae, Micrixalidae, Microhylidae, Limnodynastidae, Myobatrachidae, Nyctibatrachidae, Odontophrynidae, Pelobatidae, Pelodryadidae, Pelodytidae, Petropedetidae, Phrynobatrachidae, Phyllomedusidae, Pipidae, Ptychadenidae, Pyxicephalidae, Ranidae, Ranixalidae, Rhacophoridae, Rhinodermatidae, Rhinophrynidae, Scaphiopodidae, Telmatobiidae. URODELA – Ambystomatidae, Amphiumidae, Hynobiidae, Plethodontidae, Proteidae, Rhyacotritonidae, Salamandridae, Sirenidae.

REPTILIA

TESTUDINES - Emydidae, Testudinidae, Geoemydidae, Platysternidae, Trionychidae, Chelydridae, Dermatemydidae, Kinosternidae, Cheloniidae, Dermochelyidae, Chelidae, Pelomedusidae, Podocnemididae. RHYNCHOCEPHALIA – Sphenodontidae. CROCODYLIA – Crocodylidae, Gavialidae, Alligatoridae. SAURIA – Agamidae, Chamaeleonidae, Corytophanidae, Crotaphytidae, Dactyloidae, Hoplocercidae, Iguanidae, Leiocephalidae, Leiosauridae, Liolaemidae, Opluridae, Phrynosomatidae, Polychrotidae, Tropiduridae, Gekkonidae, Carphodactylidae, Diplodactylidae, Eublepharidae, Phyllodactylidae, Sphaerodactylidae, Pygopodidae, Cordylidae, Gerrhosauridae, Scincidae, Xantusiidae, Gymnophthalmidae, Lacertidae, Teiidae, Anguinae, Diploglossidae, Xenosauridae, Amphisbaenidae, Blanidae, Rhineuridae,

Trogonophidae, Helodermatidae, Varanidae, Dibamidae.
OPHIDIA – Acrochordidae, Cyliodrophiidae, Uropeltidae, Loxocemidae, Pythonidae, Xenopeltidae, Boidae, Colubridae, Atractaspididae, Cyclocoridae, Lamprophiidae, Psammophiidae, Pseudaspidae, Elapidae, Anomalopidae, Gerrhopilidae, Typhlopidae, Leptotyphlopidae, Xenotyphlopidae, Aniliidae, Homalopsidae, Pareidae, Tropidophiidae, Viperidae, Xenodermidae.

Re-description of external morphology and factors affecting body and tail shape of the stone frog tadpoles

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Abstract. Ecological studies testing the preponderance of environmental filters on ontogeny to explain the variation in tadpole morphology are scarce for Neotropical anurans. We used tadpoles of the stone frog *Limnomedusa macroglossa* (Alsodidae): (1) to assess the variation in body and tail shape; (2) to examine the effect of streamlet depth and allometry on tadpole shape, and (3) to re-describe and compare the tadpole external morphology with closely related species. We obtained the body shape and size from 150 tadpoles. The re-description was based on 57 qualitative and 24 quantitative characters, from 19 tadpoles between stages 30 and 37 and 31 to 37, respectively. Allometry was the major factor influencing the lateral view of body shape: smaller tadpoles had round bodies and eyes and nostrils positioned more laterally in comparison with larger ones. Thus, the power of ontogenetic variations reported here makes the tadpole developmental “climax” period a questionable concept that deserves additional attention. The depth gradient of streamlets also affected the shape: in shallower environments, the tadpoles presented a decrease in height of the body, fins and tail muscles, and an increase in body width. These results may indicate adaptations allowing better swimming performance in lotic environments with intense water flow. The external morphological characterization of *L. macroglossa* presented here differed from that previously reported, mainly due to coloration, body shape, nostril, anal tube, tail, shape and position of nostrils and snout. Additionally, we presented unknown traits for this species, making comparisons with closely related species within the Alsodidae family.

Keywords. Anuran larvae, Alsodidae, geometric morphometrics, allometry, streamlet depth.

INTRODUCTION

Morphology is one of the main factors that influenced, perhaps all, aspects of tadpole biology (McDiarmid and Altig, 1999). For instance, the establishment of a species in a given habitat is largely influenced by morphological features such as type of oral disc, shape of body, fin presence (McGill et al., 2006; Lavorel et al., 2007; Queiroz et al., 2015). Therefore, morphological traits are a useful

character in understanding their phylogenetic, taxonomic, ecomorphological, evolutionary and functional aspects (McDiarmid and Altig, 1999; Borteiro and Kolenc, 2007; Barrasso et al., 2013; Pezzuti et al., 2016).

One of the principal uses of morphology is to help in the species description. Descriptions of the external morphology of South American tadpoles are available at least since 1899, most of them revised by Cei (1980). Although these early descriptions are a valuable source

of information for several species (the only one for some of them), they usually included on a single or few individuals, without morphometrics and detailed illustrations, thus limiting intra and interspecific morphological comparisons (Borteiro and Kolenc, 2007), even the taxonomic identification (Rojas et al., 2018). Not surprisingly, re-descriptions of the morphology of the tadpoles are increasing in the last few years (Borteiro and Kolenc, 2007; Proverte et al., 2012; Barrasso et al., 2013; Iop et al., 2015; Pezzuti et al., 2016; Rojas et al., 2018). Usually based in linear measurements, only a few studies have described the shape of tadpoles obtained with geometric morphometric methods (Klingenberg, 2011) as an additional factor to morphological diagnosis (Haad et al., 2011; Pezzuti et al., 2016). Consequently, multivariate measures of size and shape of body structures used as diagnostics characters in tadpoles are still unknown to several species.

This is the case of *Limnomedusa macroglossa* (Alsodidae) Duméril and Bibron 1841, in which the larval description is quite brief and based solely on one individual from Uruguay (Ceï, 1980). Besides of intraspecific morphological variation, essential measurements of both body and tail structures are missing (e.g., spiracle length, dorsal membrane height, nostril format, anal tube position). Moreover, the shape and position of some diagnostic characters in tadpoles (e.g., shape of fins, nostril shape and mouth size) are completely unknown for this species. Therefore, a re-description of tadpoles of *L. macroglossa* is necessary to accurately describe all these features, including intraspecific variation.

The genus *Limnomedusa* Fitzinger 1834 is monospecific (Blotto et al., 2013) and has been included (together with *Alsodes* Bell 1843 and *Eupsophus* Fitzinger 1843) in the family Alsodidae (Pyron and Wiens, 2011; Frost, 2020). The phylogenetic placement of *L. macroglossa* is historically controversial (Frost, 2020). The rapid frog *L. macroglossa* inhabits rocky streams in southern Brazil, Uruguay, northeast Argentina and northern Paraguay (Maneyro and Carreira, 2012; Frost, 2020). The oviposition period of *L. macroglossa* occurs between September and November, and larval recruitment, from September to February (Kaefer et al., 2009). Previous studies have found tadpoles in puddles formed on rocks in the stream bed (Kwet and Lingnau, 2010; Maneyro and Carreira, 2012) or back waters (Kaefer et al., 2009).

In this study, we used geometric morphometric procedures to quantify the body and tail shape and to test whether allometry and water depth affect the shape variation of tadpoles. We expect that allometry is not strong and that environmental variables (such as deeper streamlets) are more influential in form, since tadpoles

are phenotypically plastic organisms in response to the environment within the developmental “climax” period (Grosjean, 2005; Xavier Jordani et al., 2019). According to the Altig and Johnston (1989) guild hypothesis’ for tadpoles, lotic forms have more massive tail muscle than lentic forms, and the largest muscles are associated with lowest fins (Altig and McDiarmid, 2006). In fact, Rivera-Correa and Faivovich (2020) described the larvae of *Hyloscirtus antioquia* and showed morphological characters commonly associated with lotic habitats are depressed body, low fins, long tail, well-developed tail musculature, and oral disc with many labial tooth rows. Although we expected this general morphological pattern in *L. macroglossa*, in deeper microhabitat, we also expected to find globular forms and higher fins when compared with shallow habitats where individuals will tend to be more depressed forms and low fins, due to the difference in hydrodynamics present in these environments. Additionally, we re-described the external morphology of the tadpole of *L. macroglossa*, presenting comparisons with closely related species.

MATERIALS AND METHODS

Data collection

We collected data from 150 tadpoles of *L. macroglossa* housed in the herpetological collections of the Universidade Federal de Santa Maria, Brazil (ZUFISM). Tadpoles were collected in the Area de Proteção Ambiental do Ibirapuitã (APA) (30°51’57,41”S; 55°38’59,63”W northernmost limit and 29°57’20,52”S; 55°40’16,80”W southernmost limit), anesthetized with lidocaine 0.1% and fixed in 10% formalin solution. Tadpoles were sampled in 13 streamlets during the daytime, using a collecting net with a long handle and a 3 mm metallic mesh (see details in Bolzan et al., 2016; Fig. 1). The sampling effort consisted by one single full scan along a 100 m section of each streamlet channel. The distance among streamlets varied from 2.97 to 90.36 km (36.79 ± 19.35; mean ± SD). The water depth was measured using a tape measure (five measures along streamlet channels) and varied from 12.4 to 40 cm (18.24 ± 5.27; mean ± SD). Tadpole coloring observations were recorded during field activities at APA do Ibirapuitã and municipalities of São Sepé, Santo Cristo, and Itaara.

Morphological measures for the larval re-description

We based the re-description on 19 tadpoles with developmental stages (Gosner, 1960) ranging from 31 to

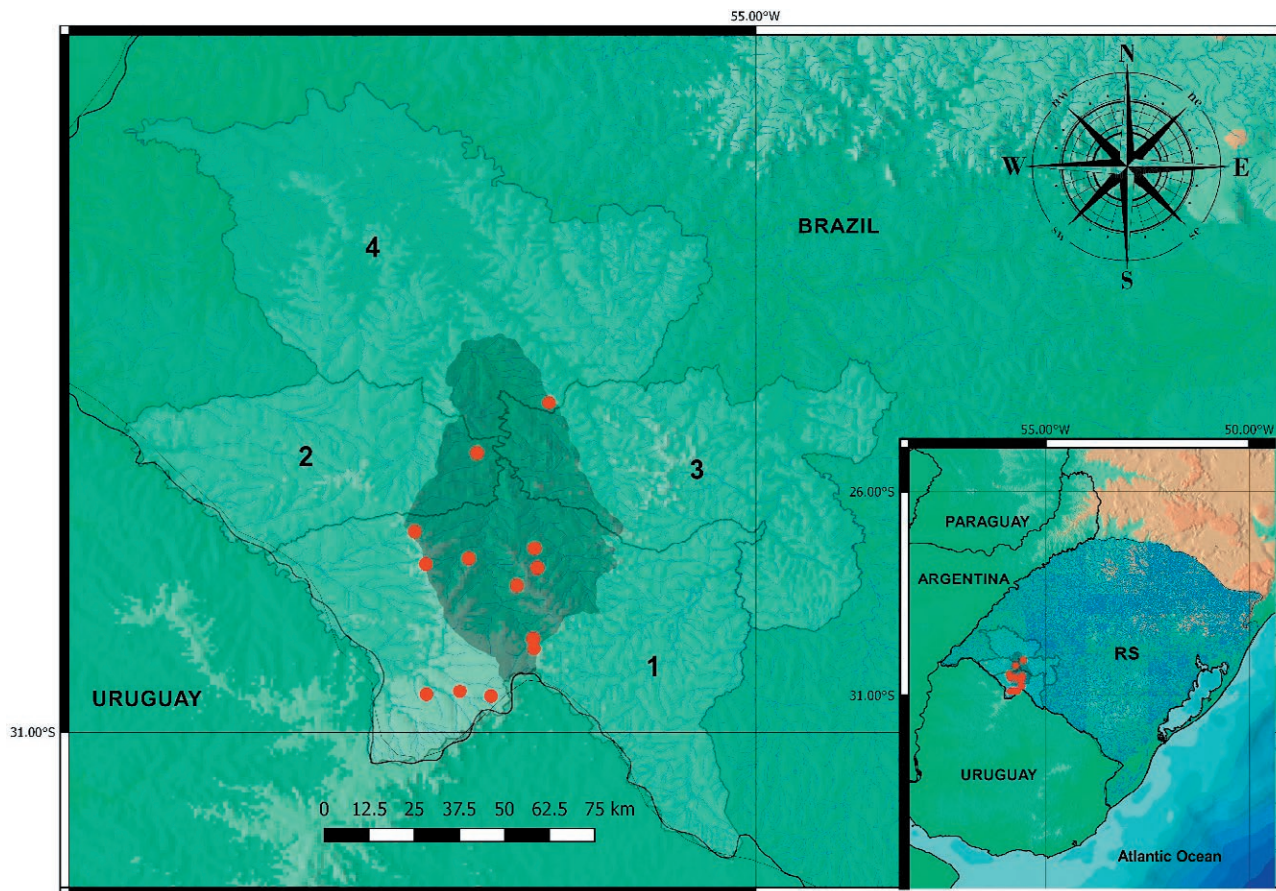


Fig. 1. Distribution of the 13 streamlets in the Environmental Protect Area of the Ibirapuitã and surroundings, where tadpoles of *Limnomedusa macroglossa* were collected. The grey area represents boundaries of the APA of the Ibirapuitã encompassing four Brazilian municipalities in the state of Rio Grande do Sul (RS): Alegrete (4), Rosário do Sul (3), Quaraí (2), and Santana do Livramento (1).

37. Fifty-seven qualitative (Table S1) and 19 quantitative measurements were recorded (Table 1; Fig. 2), according to Lavilla and Scrocchi (1986), McDiarmid and Altig (1999) and Altig (2007). For the tail length (TL) and body length (BL) measures we used a digital caliper (0.01 mm precision), while the others were recorded under a stereoscopic lens (0.07 mm precision), except for the upper jaw sheath width (UJSW), upper jaw sheath height (UJSH), lower jaw length (LLJ) and lower jaw height (HLJ) measurements, for which we used a lens with 1.5 mm precision. Coloration and natural history aspects were described based on field observations.

Geometric morphometric variation

We obtained 2-dimension (2D) images of both left-lateral and dorsal body view from 150 tadpoles of *L. macroglossa*. By using a geometric morphometric approach, one of us (BSG) digitized 15 landmarks and 2 semilan-

dmarks on lateral, and 9 and 5 in dorsal view to capture the left-lateral and dorsal body shape (Fig. 3; Table S2). The landmarks and semilandmarks were digitized using TPSDig2 ver. 2.26 (Rohlf, 2015). In lateral view, we did not include landmarks in the posterior tip of the tail due to damage, predator marks and deformities observed in some specimens, which should imply errors during the digitization of landmarks or semilandmarks and posterior comparisons.

Only tadpoles between stages 30 and 37 were included (Gosner, 1960), which represents stages within the developmental “climax” period when ontogenetic variation is expected to be low and changes in tadpole’ body parts are expected isometrics (Grosjean, 2005).

After digitization, the landmark and semilandmarks coordinates of each view were superimposed applying the Generalized Procrustes Analysis (GPA, Rohlf and Slice, 1990). GPA generates a new set of coordinates, the Procrustes coordinates, the tadpole’s body shape

Table 1. Quantitative measures (in mm) of 19 individuals *L. macroglossa* between the stages 31-37, collected in the APA of Ibirapuitã, Rio Grande do Sul state, Brazil. Measures using 0.7 mm increase: BH – Body height, BW – body width, BL - Body length, TL - Total length, ND – nostril diameter, IOD – Interorbital distance, NSD – nostril-snout distance, ESD – eye-snout distance, IND – internasal distance, SL – spiracle length, WOS – width of the opening of the spiracle, SH –spiracle height, TMH – height of the tail musculature, TMW – tail musculature width, DMH – dorsal membrane height, VMH - ventral membrane height, HM -height of the mouth, WB - width of the mouth. Using 1.5 mm increase: UJSW – upper jaw sheath length, UJSH – upper jaw sheath height, LLJ – lower jaw length and HLJ – lower jaw height. Average and standard deviation are shown. Stage and number of individuals are in main row.

| Measure | Stage 31, n=2 | Stage 32, n=2 | Stage 33, n=2 | Stage 34, n=1 | Stage 35, n=4 | Stage 36, n=7 | Stage 37, n=1 |
|---------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| TL | 31.11 ± 0.26 | 38.21 ± 4.16 | 34.51 ± 1.63 | 38.25 | 42.46 ± 5.77 | 45.01 ± 3.86 | 60.22 |
| BL | 11.14 ± 0.35 | 13.38 ± 1.32 | 11.80 ± 0.09 | 12.82 | 16.0 ± 0.61 | 16.01 ± 1.5 | 20.43 |
| BW | 7.21 ± 0.1 | 7.9 ± 1.14 | 7.2 ± 0.1 | 8.29 | 10.14 ± 0.78 | 9.36 ± 1.23 | 14.43 |
| TMW | 2.42 | 3.01 ± 0.58 | 2.35 ± 0.3 | 3.29 | 4.14 ± 0.81 | 3.77 ± 0.46 | 5.86 |
| BH | 5.57 ± 0.4 | 6.29 ± 0.40 | 5.35 ± 0.3 | 6.43 | 8.28 ± 0.45 | 7.63 ± 0.86 | 11.14 |
| DMH | 2.28 ± 0.4 | 2.72 ± 0.59 | 2.14 | 2.43 | 2.64 ± 0.14 | 3.00 ± 0.29 | 4.43 |
| TMH | 2.71 ± 0.2 | 3.35 ± 0.50 | 2.71 ± 0.2 | 3.57 | 4.14 ± 0.5 | 3.95 ± 0.35 | 6.00 |
| VMH | 1.57 ± 0.2 | 1.62 ± 0.32 | 1.57 ± 0.4 | 1.43 | 1.71 ± 0.11 | 1.77 ± 0.11 | 2.29 |
| IOD | 1.64 ± 0.3 | 1.7 ± 0.42 | 1.64 ± 0.1 | 1.86 | 2.50 ± 0.34 | 2.18 ± 0.33 | 2.86 |
| IND | 1.71 ± 0.2 | 1.62 ± 0.32 | 1.57 ± 0.2 | 1.71 | 1.78 ± 0.24 | 1.87 ± 0.12 | 1.71 |
| ESD | 2.64 ± 0.1 | 2.88 ± 0.96 | 2.92 ± 0.1 | 3.00 | 3.85 ± 0.42 | 3.75 ± 0.28 | 5.00 |
| NSD | 1.14 | 1.38 ± 0.26 | 1.42 | 1.43 | 1.89 ± 0.29 | 1.79 ± 0.21 | 2.43 |
| ED | 1.28 | 1.31 ± 0.16 | 1.35 ± 0.1 | 1.43 | 1.53 ± 0.07 | 1.71 ± 0.11 | 2.14 |
| ND | 0.35 ± 0.1 | 0.36 ± 0.09 | 0.28 | 0.43 | 0.46 ± 0.07 | 0.40 ± 0.09 | 0.43 |
| SL | 1.42 | 1.6 ± 0.15 | 1.21 ± 0.3 | 1.43 | 1.75 ± 0.42 | 1.59 ± 0.30 | 2.00 |
| SW | 0.92 ± 0.1 | 0.92 ± 0.31 | 0.85 ± 0.2 | 0.86 | 1.28 ± 0.16 | 1.18 ± 0.22 | 1.43 |
| WOS | 0.57 | 0.55 ± 0.22 | 0.57 | 0.71 | 0.92 ± 0.24 | 0.73 ± 0.22 | 1.14 |
| SH | 2.64 ± 0.5 | 2.99 ± 0.41 | 2.14 ± 0.2 | 2.43 | 3.07 ± 0.44 | 2.85 ± 0.56 | 4.43 |
| HM | 1.52 ± 0.16 | 1.5 ± 0.30 | 1.92 ± 0.3 | 2.71 | 2.28 ± 0.26 | 2.59 ± 0.25 | 2.71 |
| WM | 3.14 ± 0.28 | 3.57 ± 0.2 | 4.21 ± 0.70 | 5.14 | 4.89 ± 0.41 | 5.30 ± 0.61 | 5.71 |
| HLJ | 0.13 | 0.13 | 0.16 ± 0.04 | 0.20 | 0.20 | 0.21 ± 0.05 | 0.27 |
| LLJ | 0.96 ± 0.04 | 1.06 ± 0.09 | 0.9 ± 0.33 | 1.2 | 1.36 ± 0.34 | 1.44 ± 0.16 | 1.67 |
| UJSH | 0.26 | 0.33 | 0.3 ± 0.04 | 0.33 | 0.31 ± 0.03 | 0.4 ± 0.06 | 0.53 |
| UJSW | 1.23 ± 0.14 | 1.5 ± 0.23 | 1.43 ± 0.14 | 1.33 | 1.63 ± 0.47 | 1.73 ± 0.28 | 2.33 |

variables. Size was obtained as the centroid size; i.e., the square root of the sum of squared distances between each landmark and the configuration centroid (Bookstein, 1989).

We visualized the shape variation between individuals through a Relative Warp Analysis (RWA, analogous to Principal Component Analysis). To test for allometry, we regressed shape on log-transformed centroid size with a Procrustes ANOVA. This analysis was implemented using the function `procD.lm` in the R package `geomorph` (Adams et al. 2021; R Core Team 2020). We explored the influence of depth of the streamlets on the shape of tadpoles by implementing a Multivariate Regression Analysis. The geometric morphometric analyses were performed using the `tpsRelw` and `tpsRegr`, respectively (Rohlf, 2015).

RESULTS

Shape variation in lateral and dorsal view

The first two RWA axes summarized 57.7% of total variation of body shape in lateral view. The tadpoles with mostly positive scores on RW1 (39.64%) exhibited a proportionally smaller and more depressed body, eyes closer to nostrils, and more slender tail (fin and tail muscles), compared to the other tadpoles, with negative scores on RW1. The main variation in RW2 (18.06%) is associated with both fin and body height. On the negative scores, tadpoles have proportionally taller fins (ventral and dorsal) as well as taller and globular bodies in relation to the individuals occupying positive scores (Fig. 4A).

In dorsal view the first two RWA axes cumulatively explained 58.83% of total variation. The RWA segregated tadpoles proportionally more compressed laterally

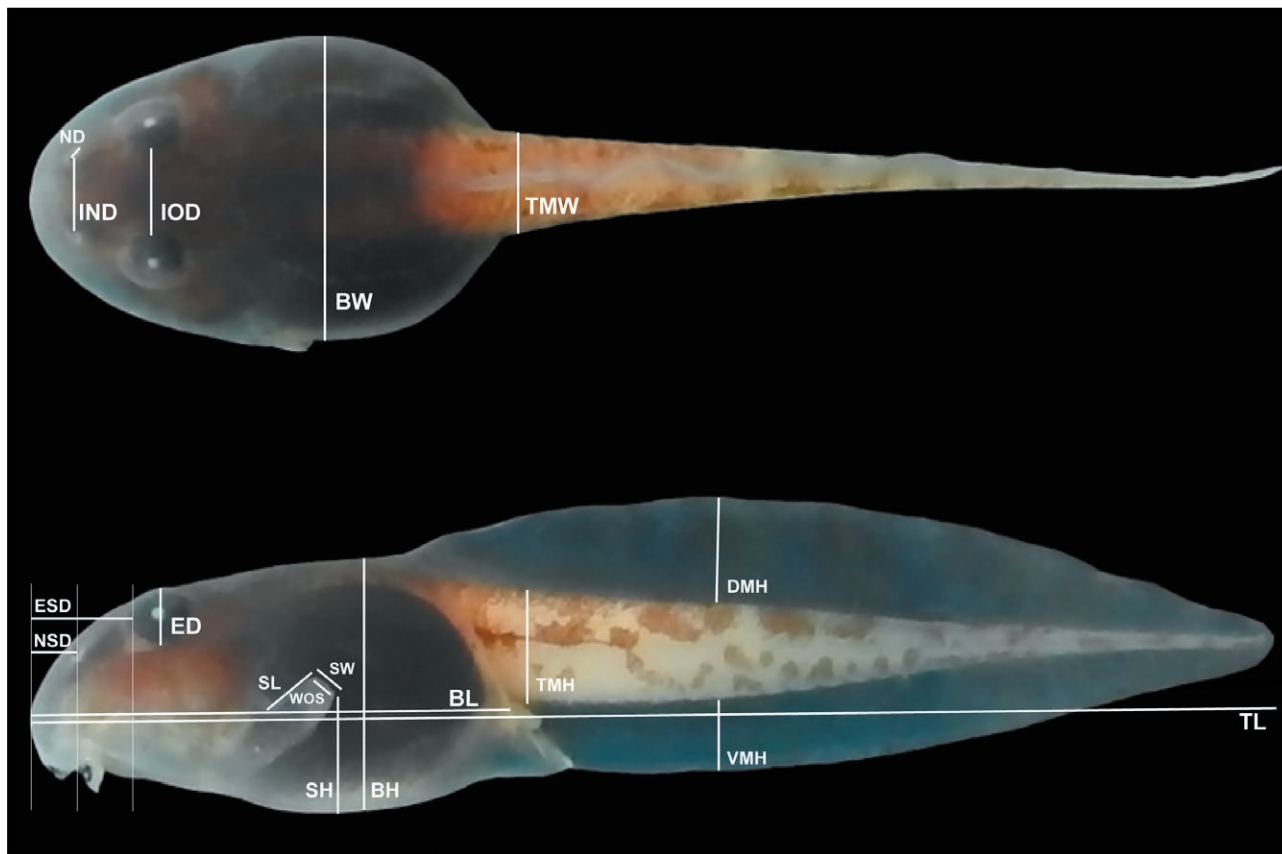


Fig. 2. Representation of the measures carried out in the larvae of *Limnomedusa macroglossa* used for description of external morphology in dorsal and lateral view (sensu Lavilla and Scrocchi (1986), McDiarmid and Altig (1999) and Altig (2007)): BH = body height, SH = spiracle height, TMH = tail musculature height, VMH = ventral fin height, DMH = dorsal fin height, BL = body length, SL = spiracle length, TL = total length, ED = eye diameter, ND = nostril diameter, IND = internal distance, IOD = interorbital distance, ESD = eye-snout distance, NSD = nostril-snout distance, WOS = spiracle aperture width, SW = spiracle width, BW = body width, TMW = tail musculature width.

and with eyes and nostrils positioned closer to the edges of the body in the positive scores of RW1 (35.46%). In RW2 (23.37%), at the negative scores, tadpoles had proportionally smaller eyes and nostrils positioned closer to the snout and the body was more globular in the middle third of the tadpole in comparison to those at the positive scores at RW2 (Fig. 4B).

Size showed a weak influence on the body shape of tadpoles (lateral view: $R^2 = 0.07$, $F = 11.756$, $P < 0.05$; dorsal view: 0.05% of the variation; $F = 1.40$; $P > 0.05$).

Smaller tadpoles had round bodies, eyes and nostrils positioned more laterally, while larger tadpoles had more oval-shaped bodies with dorsal eyes and nostrils (Fig. 5A).

Water depth influenced the shape variation in both views (lateral: 2.87% of the variation; $F = 4.38$; $P < 0.05$; dorsal: 2% of the variation; $F = 2.89$; $P < 0.05$). In lateral view, the general height of the body, the ventral and dorsal fins, and the tail muscles increased with water depth, while the position of eyes and nostrils becomes more

dorsal. In dorsal view, the body becomes more elongated (Fig. 5B). We don't find correlation between body shape and oxygen dissolved, pH, or temperature ($P > 0.05$).

External morphology re-description

The body of *L. macroglossa* tadpoles is ovoid in dorsal view and depressed globular in lateral view (BH/BW: 0.79), representing one third of the total length (BL/TL: 0.35). The snout is elongated oval in dorsal view and round in lateral view; the small oval nostrils (ND: 0.40 ± 0.08 ; mean \pm SD) with thin edges are dorsolateral and equidistant from the snout and eyes (NSD: 1.67 ± 0.35 and ESD: 3.50 ± 0.66). The internasal distance (IND: 1.77 ± 0.19) is smaller than that of the interocular distance (IOD: 2.11 ± 0.44). The eyes are dorsal (ED: 1.54 ± 0.24), with dorsolateral orientation. The spiracle is sinistral, long, cylindrical, with a wide opening (WOS: 0.74 ± 0.21 , free, short and

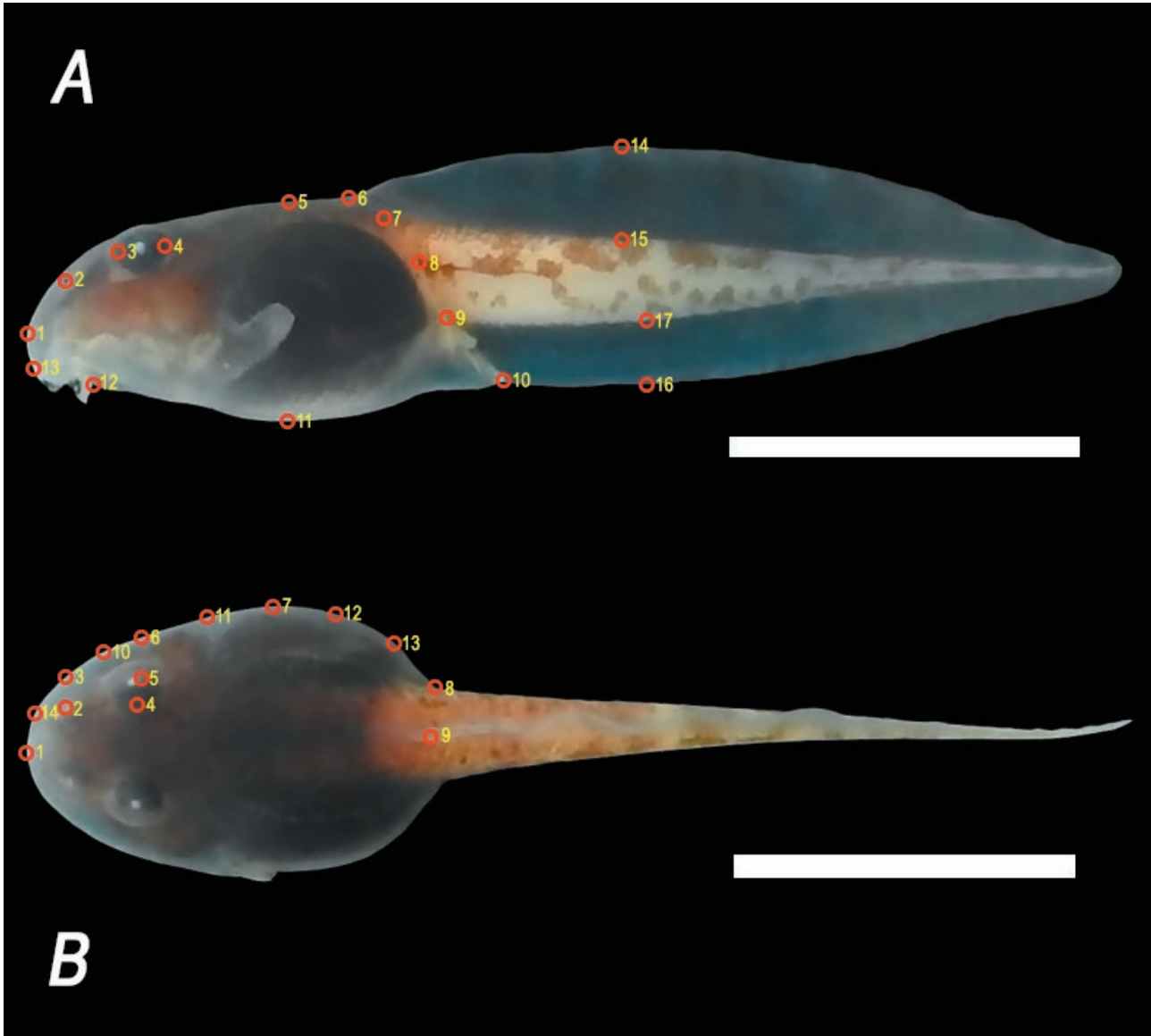


Fig. 3. Position of the landmarks and semilandmarks on the lateral (A) and dorsal view (B) of the tadpole of *Limnomedusa macroglossa*. Landmark and semilandmarks descriptions are in Table S2.

posterodorsally oriented tip) ($SH: 2.89 \pm 0.61$). The anal tube is long, connected to the ventral fin, medially positioned, and with a dextral opening. In dorsal view, the width of the tail muscles is greater than one third of body width ($TMW: 3.55 \pm 0.94$, $BW: 9.11 \pm 1.82$), with a gradual tapering, and a wide rounded tip in lateral view. Dorsal and ventral fins are low and parallel to the body ($DMH: 2.77 \pm 0.54$, $VMH: 1.70 \pm 0.22$). The dorsal fin gently starts at the junction of the tail and body. The oral disc is proportionally large ($WM/BW: 0.50$ and $HM/BL: 0.14$), anteroventral, laterally emarginated, with a broad dorsal gap, a double row of elongated papillae, and dispersed lateroven-

tral and laterodorsal submarginal papillae. The superior jaw has a ventral recess and width four times greater than its height ($UJSW/UJSH: 4.56$). The lower jaw is V-shaped and wider than the taller ($LLJ/HLJ: 6.73$). The serrations of the jaws are long with a narrow base. The oral formula is $2(2)/3(1)$, where P3 is slightly smaller than P2 and P1 (Fig. 6). The side-line system is not visible.

Colouration

In live animals, the dorsal region of the body is grey-brown, the lateral portion of the body is golden brown,

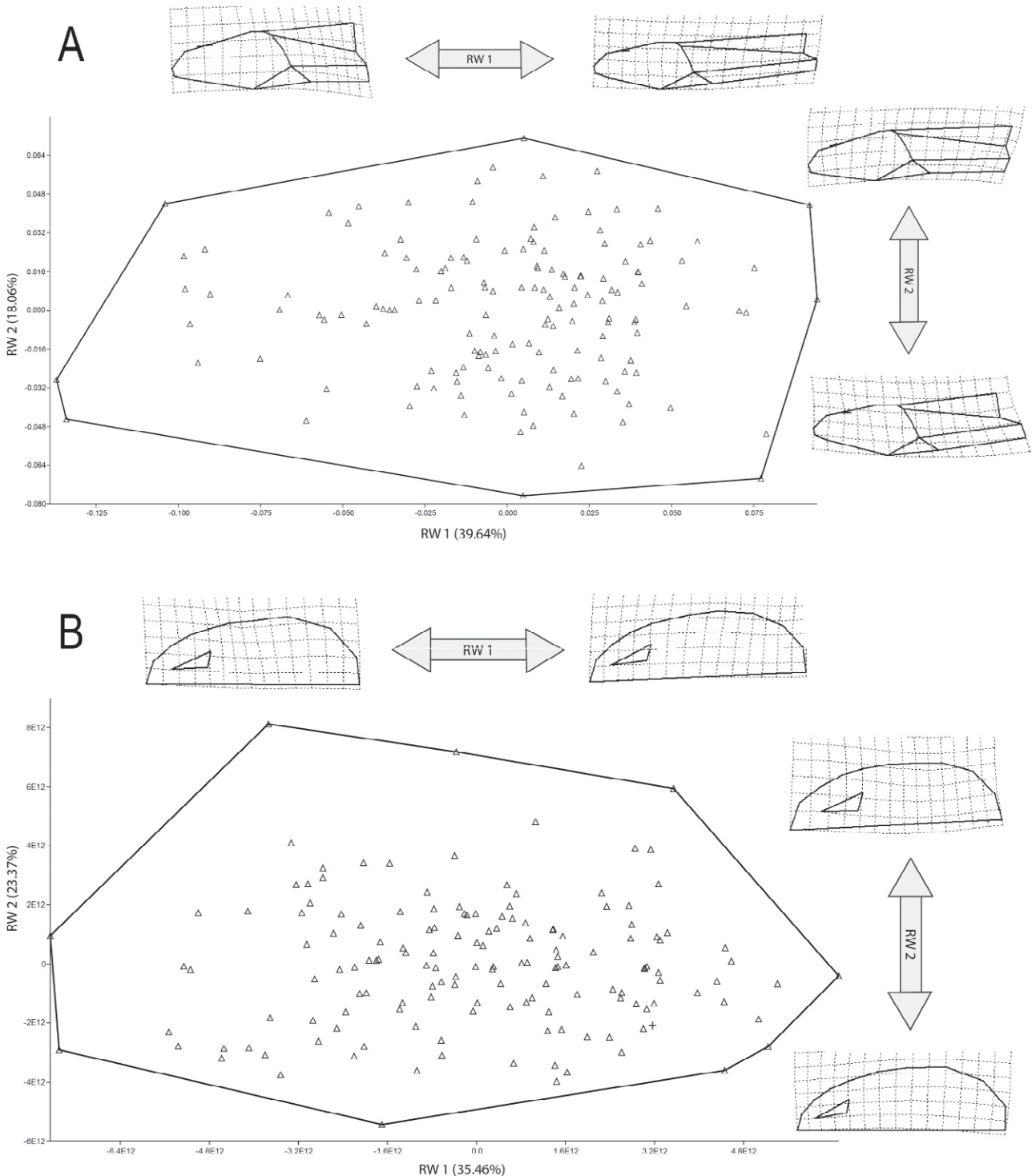


Fig. 4. Scatter plot of RW1 vs. RW2. The deformation grids demonstrate the average deformation of the shape in lateral view (A) and dorsal view (B), at the positive and negative scores of the Relative Warp axes for tadpoles of *Limnomedusa macroglossa*.

with darker shades around the nostrils and eyes. The iris is golden with a vertical pupil. In ventral view, silver pigmentation is observed in the abdominal region, but

decreases around the oral disc. Tail muscles are yellowish with golden brown pigmentation throughout their length, mainly in the dorsal region. Fins are translucent, with

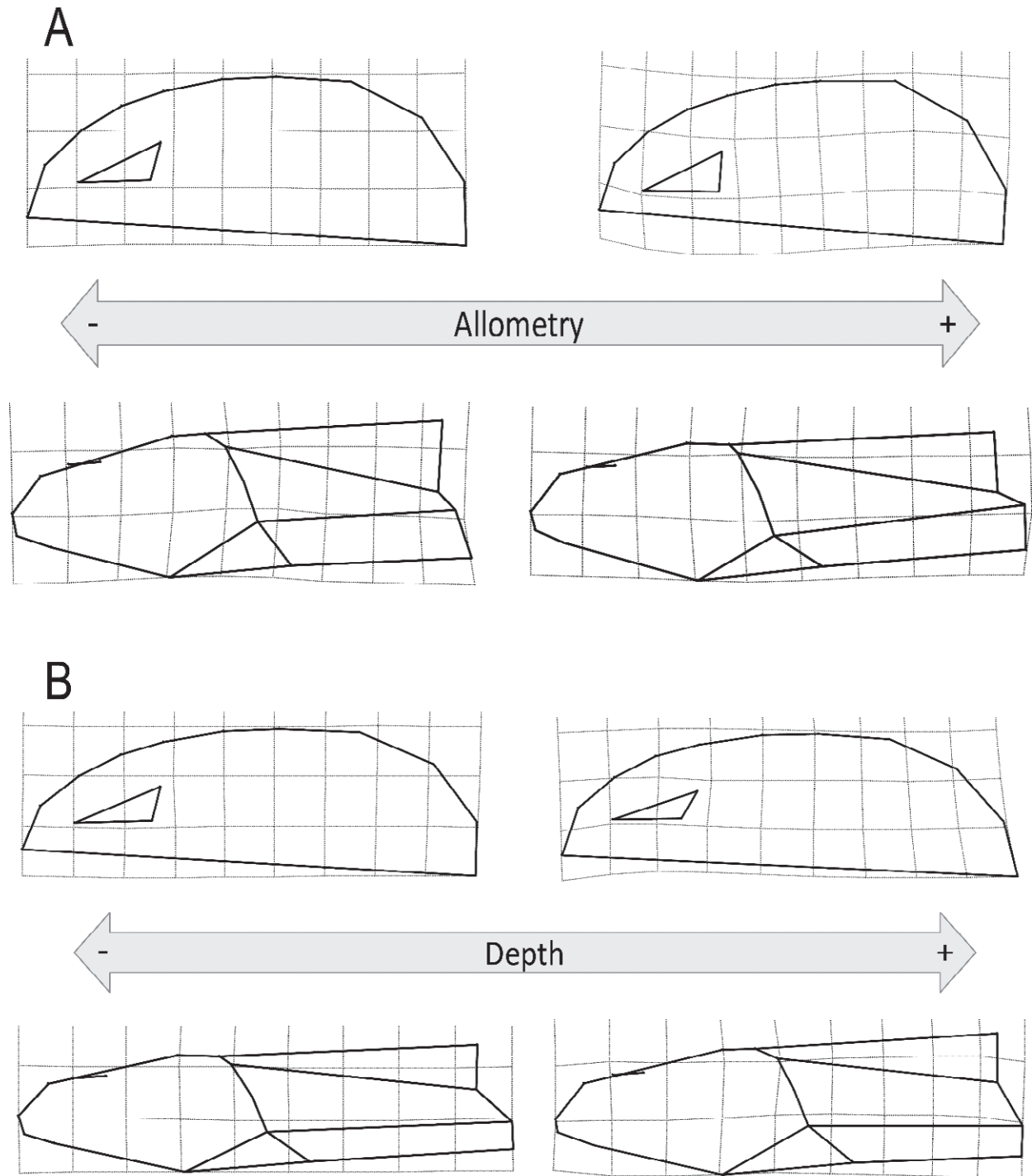


Fig. 5. Shape deformations related to the multivariate regression going from the most extreme negative score (left) to the most positive scores (right). In (A) deformations are from the effect of allometry. In (B) from the depth influence in the lateral and dorsal views of tadpoles of *Limnomedusa macroglossa*.

clusters of evenly scattered melanophores. After fixing in 10% formalin, the colour of the body changes to a greyish brown and the eyes become black.

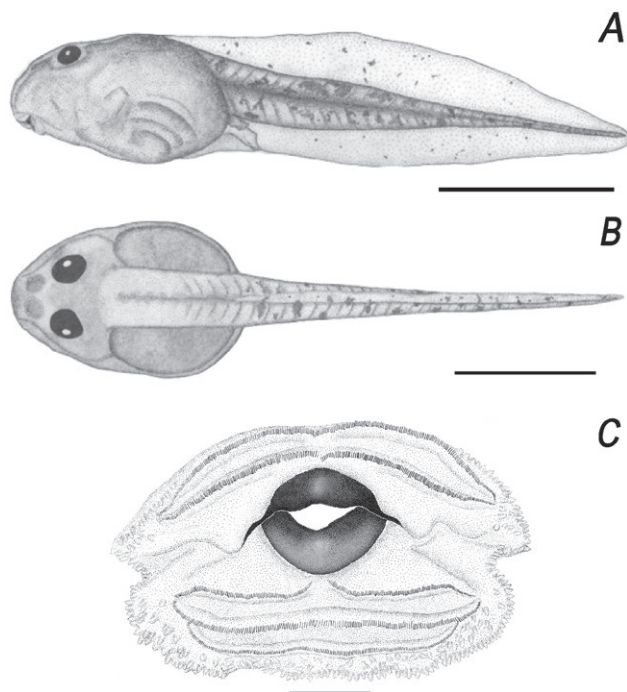


Fig. 6. Tadpole of *Limnomedusa macroglossa* of stage 33: (A) in lateral view (scale 1 cm); (B) in dorsal view (scale 1 cm); (C) Oral disc (scale 1mm). Drawings by B.S.G.

DISCUSSION

Shape variation in lateral and dorsal view

This study provides the first description of the body and tail shape of *L. macroglossa* tadpoles. The shape variation is mainly explained by allometry for much of the larval period (i.e., between stages 30 and 37). Small tadpoles have a rounder body, eyes and nostrils positioned more laterally and, as the body size increases, the tadpole body becomes more oval with eyes and nostrils more dorsal. Among the several factors that contribute to the morphological variation in anurans, changes in body size (allometric) associated with development have a strong effect on anatomical forms (e.g., Di Cerbo and Biancardi, 2010; Garriga and Llorente, 2012; Acosta and Candioti, 2017). In fact, the effects of allometry on anuran larvae was verified for both external (e.g., Di Cerbo and Biancardi, 2010; Garriga and Llorente, 2012; Acosta and Candioti, 2017) and internal larval morphology (e.g., Larson, 2002, 2004, 2005; Garriga and Llorente, 2012), but was expected to be low within the developmental “climax” period, when changes in tadpole body parts are expected to be isometrics (Grosjean, 2005; Xavier Jordani et al., 2019).

Our results also reveal variation in the shape associated with the depth gradient among streams. In lateral

view, tadpoles vary in height and body shape (i.e., from depressed to high), position of the eyes and nostrils (close to each other or near the sides of the body), height and width of tail muscles (thin or well developed) and fins (high or low). In dorsal view, the variation observed was associated with body shape (e.g., compressed laterally or globular) and position of eyes and nostrils (e.g., lateral or dorsal). These characteristics show the refined responses of the tadpoles to changes in the habitats, especially the tail and body characteristics (Grosjean, 2005; Xavier Jordani et al., 2019), since morphology should provide an optimal swimming performance in an occupied habitat (Pinto and Ávila-Pires, 2004; Marques and Nomura, 2015; Xavier Jordani et al., 2019). We can't exclude the tadpoles autonomy to access different water depths according to your preferences to abiotic variations on streamlets (Warkentin, 1992). According to Johnson et al. (2008) and Arendt (2010), depth is associated with the selective pressure exerted by the lotic environment on organisms, since the water flow is lower in deeper streams, while in shallow streams, currents are more intense. In the latter, a hydrodynamic body shape is advantageous to minimize drag and allow the animal to move (e.g., increase in tail height and width, reduction in body height, and increase in structures for attachment; Arendt, 2010).

External morphology re-description.

The description presented in our study differs from the previously reported, mainly regarding the following aspects: coloration, body shape, nostrils, anal tube, tail, shape and position of nostrils and snout. In the description by Ceï (1980), coloration was briefly characterized as “dorsum and tail with dark round spots”, but it does not mention whether this trait was observed in the live specimen or after being fixed. Body shape, previously reported as “depressed oval”, differed from that observed in the present study (i.e., ovoid shape in dorsal view and rounded depressed in lateral view). In agreement with the described by Ceï (1980), the snout of *L. macroglossa* is round (but only in lateral view), while it has an elongated oval shape in dorsal view. The internasal distance, previously characterized as equidistant from the interocular distance, differs from our findings (i.e., internal distance less than interocular distance). The anal tube, previously described as having a median aperture, differed from that reported here (dextral). Besides, we added information for both the shape and position of the anal tube (long and connected to the ventral fin, respectively). The tail muscles are well developed in lateral and dorsal view, as also previously described, gradually tapering into a round and wide tip.

We added information on the spiracle, which is long cylindrical, ending with a wide opening and free, short, and posterodorsally oriented tip. We described fin shape, emergence angle, and body attachment site, previously unavailable. The pattern of oral formula 2(2)/3(1) agrees with the previously reported, and additional undescribed characters are presented, such as the proportion, position, arrangement, and shape of papillae, as well as oral disc size, and jaw size.

For some groups, morphology is widely used as a parameter to reconstruct phylogenetic relationships among species groups (Marques and Nomura, 2015). Thus, future comparisons between *L. macroglossa* tadpoles and those of the other two genera in the Alsodidae family are relevant to understanding the evolution of the group. Both *Aldoses* and *Eupsophus* are endemic to beech forests (*Notophagus* spp.) in the Patagonian region of Chile and Argentina (Formas and Cuevas, 2017; Frost, 2020; IUCN, 2019) and therefore have a parapatric distribution to *L. macroglossa*. Two modes of obtaining energy have been described for larval development in *Alsodes* and *Eupsophus*: (i) endotrophic tadpoles (i.e., tadpoles obtain energy entirely from maternal energy sources, usually yolk, to become free-living juveniles), and (ii) exotrophic tadpoles (i.e., the energy required for development is ingested by free-living larvae after yolk reserves are depleted; Altig and McDiarmid, 1999). *Eupsophus* larvae differ the most from *L. macroglossa* tadpoles. According to Candiotti et al (2011), the *Eupsophus* species is classified as having endotrophic tadpoles that develop in a nest (Altig and McDiarmid, 1999), and are considered uncommon as both eggs and larvae develop in small dark chambers (= burrows) filled or no by water, near streams or flooded areas. On the other hand, *Alsodes*, despite having exotrophic benthic tadpoles (Formas and Cuevas, 2017) as reported for *L. macroglossa*, use small water-filled cavities near streams for larval development (e.g., *Alsodes vittatus*; Glime and Boelema, 2017) and differ from *L. macroglossa* tadpoles mainly by the smaller oral disc, a single row of submarginal papillae (also arranged in a single row or clustered in the supra-angular region), smaller eyes and larger fins with a rounded tip.

The dissimilarity between *L. macroglossa*, *Eupsophus* and *Alsodes* tadpoles seem related to the still poorly resolved phylogeny for this group. In fact, the phylogenetic placement of *L. macroglossa* is historically controversial (Frost, 2020). A molecular study did not find a particularly close relationship between *Limnomedusa* and the *Eupsophus* + *Alsodes* clade, but rather a proximity to *Cycloramphus* (Cycloramphidae; Blotto et al., 2013). Recently, Sabbag et al. (2018) recovered *L. macroglossa* as taxon sister of Odontophrynidae. Thus, according to

Frost (2020), the inclusion of *Limnomedusa* within Alsodidae is provisional and requires further investigation (but see support to Alsodidae as a monophyletic group by Grant et al., 2017).

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SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at <<http://www-9.unipv.it/webshi/appendix/index.html>> manuscript number 11315

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Preliminary data on the diet of *Chalcides chalcides* (Squamata: Scincidae) from Northern Italy

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Abstract. The diet in skinks is known mainly for extra-European species, especially from Australian ones, where these lizards are represented by a great number of species, while, in comparison, data for species from other continents are scarce. The three-toed skink, *Chalcides chalcides*, is found in a restricted part of northern Africa and in Italy, where it is distributed almost uniformly throughout the peninsula and on the major islands. Although it is well studied for aspects such as morphology and ecology, data concerning trophic preferences are scarce, and available only for the populations of south-central Italy. In this note we report preliminary data about the diet of an Apennine population of the three-toed skink, *Chalcides chalcides*, at the northern boundary of its distribution area. Faecal contents from 20 individuals were collected in June 2015, obtaining an overall sample of 48 prey items. Araneae constituted the most preyed taxon (over 40%), followed by Hemiptera (35,4%) and other prey taxa (Hymenoptera, Coleoptera, and Dermaptera) in much lower percentages. We found no differences between smaller/younger and larger/older individuals in consumed preys. As well as confirming the general trophic predilection of this skink for spiders, we also found some interesting differences in preyed items with studied populations of south-central Italy.

Keywords. Apennines, *Chalcides chalcides*, diet, faecal pellets, Northern Italy, skink.

Diet in lizards is a very dynamic component, since it can be variable over time (Floyd and Jenssen, 1983; Dearing and Schall, 1992). Changes are often seasonal, related to different prey availability and abundance between seasons (Durtsche, 1995). Lizards diet can also vary among sites (Barden and Shine, 1994), since prey availability and abundance may vary geographically too. Lastly, it may be different between sexes, between adults and juveniles and also among morphs (Rocha, 1998; Fialho et al., 2000; Scali et al., 2016).

Skinks diet is known mainly for extra-European species, especially from Australia (Wapstra and Swain, 1996; Duffield and Bull, 1998; Clemann et al., 2004; Shea, 2006;

Pavey et al., 2010), where these lizards are represented by a great number of species, while, in comparison, data for species from other continents are scarce. Skinks are known to be primarily insectivorous, even though some species may include plants in their diet, as shown in the ocellated skink *Chalcides ocellatus* (Kalboussi and Nouria, 2004; Lo Cascio et al., 2008; Carretero et al., 2010).

The three-toed skink, *Chalcides chalcides* (Linnaeus, 1758), is a scincid lizard found in a restricted part of northern Africa (NE Algeria, Mediterranean regions of Tunisia and Libya), and in Italy, where it is distributed almost uniformly throughout the peninsula and on the major islands (Caputo et al., 2010). The northern

boundary of its distribution coincides with the Northern Apennines since the species is almost absent from the Po plain, except for few populations near the Po delta (Caputo et al., 2010). The species shows a snakelike habitus, with reduced tridactyl limbs. The evolution towards limblessness has an adaptive meaning, as suggested by some authors, since it favours the locomotion in grassland habitat (Caputo et al., 1995).

Despite its quite wide range, there is paucity of information regarding some aspects of the biology of the species. This is probably due to its particular lifestyle, and to the consequent elusiveness that makes this reptile difficult to be captured in the field. So far, there is a good amount of information related to morphology and osteology (Caputo et al., 1995, 2000; Greer et al., 1998; Caputo, 2004; Guarino, 2010) and to the biology and ecology of the species (Orsini and Cheylan, 1980; Rugiero, 1997; Caputo and Silvano, 1999; Luiselli et al., 2005). On the contrary, data concerning structure and dynamic of the populations are absent, while those concerning trophic preferences are scarce, and available only for the populations of southern-central Italy. Rugiero (1997) analysed the stomach content of specimens from the surroundings of Rome, while Caputo (2000) studied the diet composition of a population from Molise. The present study aims to collect information about the diet of the three-toed skink in Northern Italy, analysing the faecal pellets of individuals from a population of Northern Apennines.

All data presented here were collected in June 2015, during the breeding period of the species. We sampled a population located in the so-called hilly area of the “Oltrepò Pavese”, in the municipality of Codevilla (44°57'N, 9°4'E; Fig. 1). The site, situated at an altitude of 260 m a.s.l., was characterized by the presence of uncultivated grasslands, surrounded by woodland area represented for the most by *Quercus pubescens* and *Ostrya carpinifolia*. Bushy zones of *Rosa canina* and *Crataegus monogyna* were present at some spots inside the grasslands.



Fig. 1. Map showing the study site in Northern Italy (municipality of Codevilla, province of Pavia). Contour lines (elevation a.s.l. in meters) for Codevilla municipality are also displayed.

Table 1. Biometric variables of the juvenile and adult three-toed skinks ($n = 20$) measured in a population of the Northern Apennines in Italy (municipality of Codevilla, province of Pavia): SVL (Snout-Vent Length), Ta_L (Tail Length), TL (Total Length), HH (Head Height), HW (Head Width), HL (Head Length), W (Weight).

| Code | Capture date | SVL (mm) | Ta_L (mm) | TL (mm) | HH (mm) | HW (mm) | HL (mm) | W (g) |
|-------|--------------|----------|-----------|---------|---------|---------|---------|-------|
| COD01 | 12/6/2015 | 68.7 | 70.0 | 138.7 | 3.4 | 4.5 | 7.4 | 1.6 |
| COD02 | 12/6/2015 | 75.8 | 76.0 | 151.8 | 3.6 | 4.6 | 7.9 | 2.1 |
| COD03 | 13/6/2015 | 80.0 | 81.0 | 161.0 | 3.8 | 4.6 | 7.6 | 2.4 |
| COD04 | 11/6/2015 | 81.5 | 85.0 | 166.5 | 3.3 | 4.7 | 7.9 | 2.2 |
| COD05 | 7/6/2015 | 81.9 | 84.0 | 165.9 | 3.7 | 5.0 | 8.0 | 2.5 |
| COD06 | 1/6/2015 | 83.3 | 91.0 | 174.3 | 3.6 | 4.9 | 8.4 | 3.2 |
| COD07 | 1/6/2015 | 85.0 | 91.0 | 176.0 | 3.8 | 4.6 | 7.8 | 2.8 |
| COD08 | 24/6/2015 | 88.0 | 60.0 | 148.0 | 4.1 | 4.9 | 8.5 | 3.0 |
| COD09 | 10/6/2015 | 88.0 | 95.0 | 183.0 | 4.0 | 4.0 | 8.6 | 3.6 |
| COD10 | 25/6/2015 | 89.9 | 96.0 | 185.9 | 4.1 | 4.9 | 8.6 | 3.5 |
| COD11 | 24/6/2015 | 90.0 | 32.0 | 122.0 | 3.6 | 4.7 | 8.3 | 3.0 |
| COD12 | 24/6/2015 | 93.0 | 98.0 | 191.0 | 4.1 | 4.9 | 8.9 | 3.6 |
| COD13 | 25/6/2015 | 97.4 | 98.7 | 196.1 | 3.9 | 4.6 | 7.8 | 4.5 |
| COD14 | 30/6/2015 | 106.6 | 119.7 | 226.3 | 4.2 | 5.1 | 8.9 | 4.5 |
| COD15 | 24/6/2015 | 126.0 | 139.0 | 265.0 | 5.1 | 6.5 | 11.7 | 10.0 |
| COD16 | 10/6/2015 | 126.0 | 138.0 | 264.0 | 4.6 | 5.7 | 10.2 | 9.8 |
| COD17 | 25/6/2015 | 131.7 | 129.0 | 260.7 | 4.8 | 5.8 | 9.4 | 9.2 |
| COD18 | 10/6/2015 | 138.0 | 138.0 | 276.0 | 4.9 | 5.7 | 10.3 | 12.0 |
| COD19 | 24/6/2015 | 139.0 | 145.0 | 284.0 | 5.5 | 6.3 | 10.5 | 12.3 |
| COD20 | 24/6/2015 | 172.0 | 93.0 | 265.0 | 6.1 | 6.6 | 12.2 | 19.5 |

We caught 20 individuals by hand, searching for them in the grass. Each individual was measured using a digital calliper (accuracy ± 0.1 mm) for snout-to-vent length (SVL), tail, head size (height, width, and length), weighed by a digital scale (accuracy ± 0.1 g) (Table 1), and photographed on the dorsal and ventral pattern. Faecal pellets were usually defecated by lizards immediately after capture, although sometimes they were obtained by applying a slight pressure on the belly of each individual, eliciting defecation. Pellets were preserved in sterile tubes containing 70% alcohol for subsequent analysis. All individuals in our sample were captured once, as assessed by the manual comparison of both biometric measures and photographic images (dorsal pattern, intersection of head and ventral scales, scars). After each sampling session, all individuals were released at the exact point of capture. It was not possible to attribute sex to captured skinks as this species lacks any external sexual dimorphism, except for very large pregnant females (Caputo et al., 2010). In order to tentatively evaluate possible differences in dietary habits between smaller, and consequently younger, individuals and larger/older animals, we separated the 20 skinks into two groups (10 juveniles and 10 adults), based

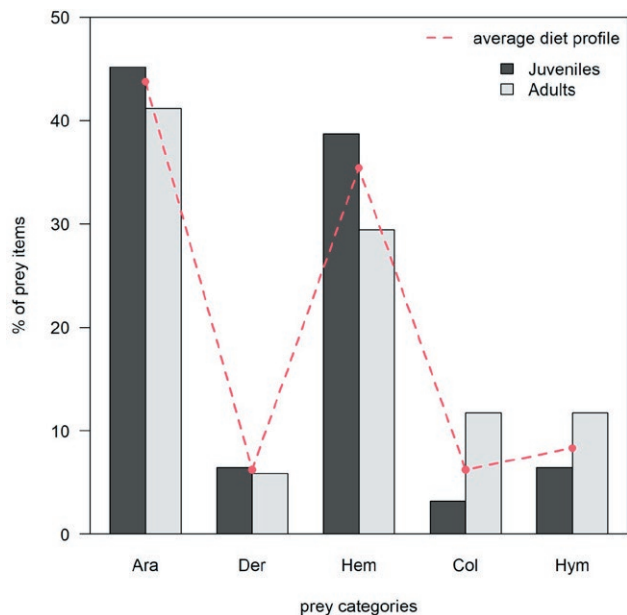


Fig. 2. Percentages of different taxa of preyed items in *Chalcides chalcides* for juveniles and adults, categorized by SVL (< 91 mm: juveniles, n = 10; ≥ 91 mm: adults, n = 10). Prey's legend: Ara – Araneae; Der – Dermaptera; Hem – Hemiptera; Col – Coleoptera; Hym – Hymenoptera.

on minimum size of adult individuals (SVL = 91 mm) as reported in literature (Caputo et al., 2010).

The analysis of faecal pellets is considered to be fully reliable to describe lizard feeding habits (Perez-Mellado et al., 2011; Civantos et al., 2013; Scali et al., 2016). Faeces were dissolved in a Petri dish to separate all prey items, which were identified by using a stereomicroscope by M.P., expert entomologist of the Natural History Museum of Milan (Italy). Where possible, prey items

were recognized at the family taxonomic level, and were grouped at the order level. However, since some soft diet items (e.g., insect larvae, spiders) might not appear in faecal pellets, we carefully searched for body parts of small and soft-bodied prey taxa that are less likely to be digested (Civantos et al., 2013).

Overall, we obtained 48 prey items from a sample of 20 individual faecal pellets (mean ± SE: 2.4 ± 0.3, range: 1-6; 31 from juveniles and 17 from adults). The taxonomic composition of preyed items, with the percentage of contribution of each taxon, is reported in Table 2. Considering the overall small sample size, differences in prey items frequency between juveniles and adults for each prey taxa were tested using χ^2 with Monte-Carlo simulation (1000000 iterations) to obtain reliable P value (Patefield, 1981). The observed frequencies were not significantly different from the expected ones (P = 0.83; Fig. 2), indicating that juvenile and adult diets overlapped. In general, the most present taxon in the three toed-skink diet was represented by Araneae (juveniles: 14; adults: 7), followed by Hemiptera (juveniles: 12; adults: 5). Few items were identified as Coleoptera (juveniles: 1; adults: 2) and Dermaptera (juveniles: 2; adults: 1). It should be stressed that both Coleoptera belonging to Carabidae and Tenebrionidae families and Dermaptera are largely nocturnal, nevertheless they are not uncommon in the diet of diurnal lizards (Vitt and Blackmore, 1991). Formicidae (incidentally, consistently wingless insects), again with a quite small sample, represented the only taxon equally preyed (two prey items for both juveniles and adults). Ants are mostly diurnal, widespread, abundant, easy-to-catch insects, therefore their relative scarcity in the diet suggests they are not among the preferred prey.

The analysis of faecal pellets shows that the most predated invertebrates by the three toed-skink are repre-

Table 2. Prey items (n = 48) of *Chalcides chalcides* from a site of Northern Apennines. Analyses were based on the faecal pellets of 20 skinks (one pellet for each individual). Percentages refer to the number of items for each Suborder and Order of considered taxa with respect to the total of found items.

| Order | Suborder | Family | n | Suborder (%) | Order (%) |
|-------------|---------------|-----------------------|----|--------------|-----------|
| Araneae | Labidognatha | Lycosidae | 3 | 6.25 | 43.75 |
| | Labidognatha | Not determined | 18 | 37.50 | |
| Dermaptera | Forficulina | Anisolabididae | 1 | 2.08 | 6.25 |
| | Forficulina | Not determined | 2 | 4.17 | |
| Hemiptera | Heteroptera | Not determined | 6 | 12.50 | 12.50 |
| | Fulgoromorpha | Issidae | 5 | 10.42 | |
| | “Homoptera” | Not determined | 6 | 12.50 | |
| Coleoptera | Adephaga | Carabidae (larvae) | 1 | 2.08 | 6.25 |
| | Polyphaga | Elateridae (adult) | 1 | 2.08 | |
| | Polyphaga | Tenebrionidae (adult) | 1 | 2.08 | |
| Hymenoptera | Apocrita | Formicidae | 4 | 8.33 | 8.33 |

sented by spiders, since they contributed 43.75% of preyed items. The previous studies, conducted on the trophic preferences of this skink in south-central Italy, led to the same result, with spiders being the most preyed taxon. In the study of Rugiero (1997), based on the analysis of the specimens' stomach content, spiders contributed 42.45% to the diet composition. Caputo (2000) found an even higher contribution, with Araneae contributing up to 51.11% to the diet composition. Further differences with respect to the previous studies are found in the contribution of the other prey taxa. In our study, Hemiptera constituted the second most preyed taxon (35.42%), while both Rugiero (1997) and Caputo (2000) found this group contributed lower percentages to the diet composition (2.83% and 13.33% respectively). Furthermore, we found a higher percentage of Formicidae (8.33%) compared to the studies of Rugiero (4.71%) and Caputo (2.22%, including all Hymenoptera). However, percentages of Formicidae remain quite low, when their abundance at the soil level is considered. This suggests that Formicidae are of quite low value as food for skinks, and only taken as second-choice preys, although not entirely refused. All the other prey taxa we found were present in much lower percentages, such as Coleoptera (6.25% considering both adults and larvae), which, on the contrary, contributed in a significant way both in the population of Rome (18.86%; Rugiero, 1997), and Molise (15.56%; Caputo, 2000). Interestingly, even if with low percentage (6.25%), we firstly detected the presence of Dermaptera in the diet of the three-toed skink, not found in the other Italian populations. Conversely, some taxa were found in south-central Italy, but not in Northern Apennines. For instance, Rugiero (1997) found a strong contribution of Isopoda (15.09%), which were not found nor in our work, nor in that of Caputo (2000). This may be related to a higher aridity of the studied habitats, resulting in a largely nocturnal activity of the quite hygrophilous Isopoda, since their abundance in Rugiero's samples indicates they are not counterselected as preys. The latter author found conversely a rather high contribution of Orthoptera (15.56%), absent both in the present work and in that of Rugiero (1997). Gasteropoda, Blattodea, Diptera were found by Rugiero (1997), even though in very small percentages, while Myriapoda were found by Caputo (2000). None of these taxa were found in our work. However, the differences we found with respect to these studies could be due both to the limited sample sizes in the various surveys and to the fact that none of them considers prey availability.

This study allowed not only to give some preliminary insight about the diet of the three-toed skink in Northern Italy, but also showed no differences in the consumed prey between juveniles and adults. An ontogenetic shift

in diet composition, and thus in trophic preferences, has been reported for skinks, but only for extra-European species (Hall, 1972; Duffield and Bull, 1998; Shea et al., 2009). It represents a very fascinating topic never investigated before for European skink species, so further studies on this or even other species are needed, possibly taking into account larger sample sizes, in order to perform reliable statistical tests.

In conclusion, this study confirms the preference of the three-toed-skink for spiders. As hypothesized by Caputo (2004), this might be due to the particular structure of the teeth of the species, similar to that of the other smaller species of the genus, characterized by a conical longitudinal section, rendering them particularly suitable for preys with a soft body, such as spiders.

Moreover, it is not surprising that adult Coleoptera are scarce in the faecal pellets of the three-toed-skink. Coleoptera are usually preyed by larger species of skinks like *Chalcides ocellatus* and *Chalcides polylepis* (Bons, 1958; Schneider, 1981) which have a stronger bite that easily allows them to crush such hard-bodied preys. The differences we found in the other prey taxa might be due to different factors, such as habitat, climatic conditions or sampling season. This is not uncommon in reptiles that can be at least partially opportunistic in their food choices (Manicom and Schwarzkopf, 2011). However, our findings put light on a basic ecological aspect of the species in its northernmost distribution area and in a particular habitat, the Apennine mountains, never investigated before for skinks.

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The high diversity and phylogenetic signal of antipredator mechanisms of the horned frog species of *Proceratophrys* Miranda-Ribeiro, 1920 (Amphibia: Anura: Odontophrynidae)

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Abstract. Phylogenetic signals indicate the phenotypic similarity of antipredator mechanisms among related species. Herein, we assessed the antipredator mechanisms of the horned frog *Proceratophrys laticeps*, compiled a database including closely phylogenetically-related species, and evaluated their phylogenetic signals. Our dataset comprises 80 records for 13 species of *Proceratophrys*, totaling 11 antipredator mechanisms and 15 variations of these mechanisms. Six antipredator mechanisms show high similarity in the trees' roots within *Proceratophrys* (e.g., aggression, aposematism, camouflage, distress call, immobility, and interrupt calling). Our observations show the first records of antipredator mechanisms for *P. laticeps*, and the first report of interrupt calling for *Proceratophrys* genus, contributing to the knowledge on the behavioural ecology of *Proceratophrys* species, addressing new insights for ecological trait evolution by multiple ancestral states of amphibians.

Keywords. Ancestral trait, anurans, Brownian motion, defensive strategies, evolution, phylogenetic tree.

Observations from closely phylogenetically-related species are often statistically non-independent due to common ancestry (Felsenstein, 1985; Harvey and Pagel, 1991). Shared history leads to the phenotypic similarity among related species under many evolutionary processes (Hansen and Martins, 1996). This phylogenetic dependence in the data can be accounted using various special statistical methods developed for phylogenetic data (e.g., Felsenstein, 1985; Hansen and Martins, 1996; Rohlf, 2001). Phenotypic similarity among related species is known as phylogenetic signal and describes the

tendency of a particular characteristic to be conserved (Blomberg and Garland, 2002). The degree of phylogenetic signal can indicate the weight to which closely related species tend to have similar traits (Blomberg et al., 2003). Phenotypic traits may depend upon for root of a phylogenetic tree or may converge to their tips (Paivone et al., 2010). Moreover, the evolution of these characteristics can be explained by Brownian motion, a process of random genetic drift at a constant rate of evolution and non-directional selection (Diniz-Filho and Vieira, 1998).

Predation is probably the most important selective pressure on the evolution of antipredator mechanism diversity in amphibians (Brodie et al., 1991; Toledo et al., 2007). Anurans display 12 antipredator mechanisms and 28 variations that can be displayed into three phases of defence (i.e., avoid detection, prevent attack, and counter-attack) to respond to the risks imposed by predators (Ferreira et al., 2019). For example, mechanisms such as camouflage and immobility can evade detection by visually oriented predators. Display of aposematic colorations, postures, and escape can prevent attacks. Lastly, mechanisms such as cloacal discharge, secretion release, aggression, and distress call can be displayed in counterattacks to apprehension by the predator (Ferreira et al., 2019). In addition, the sequence and intensity of antipredator mechanisms may be displayed according to the degree of stress imposed by a predator. For example, a single individual can display several antipredator mechanisms during an interaction with a predator (Williams et al., 2000; Lourenço-de-Moraes et al., 2016).

The genus of the horned frog *Proceratophrys* Miranda-Ribeiro, 1920 includes 43 species widely distributed in South America (Frost, 2022). They are characterized by the presence of palpebral appendages and cryptic coloration resembling fallen leaves in decomposition (Prado and Pombal, 2008; Toledo and Haddad, 2009), favouring the display of camouflage and postures such as stretching limbs to avoid detection and prevent possible attacks (Ferreira et al., 2019). In the last decades, studies have recorded the antipredator mechanisms such as camouflage, postures, and aggression for some species of *Proceratophrys* (Toledo et al., 2010; 2011; Peixoto et al., 2013; Mângia and Garda, 2015). However, the phylogenetic origin of antipredator mechanisms of *Proceratophrys* genus is still unknown, and a knowledge gap remains with its evolutionary history (Lande and Arnold, 1983; Price and Langen, 1992). Therefore, herein we evaluated the phylogenetic signal of antipredator mechanisms of *Proceratophrys* species. We hypothesized that antipredator mechanisms of *Proceratophrys* species are purely phylogenetic. We also described the antipredator mechanisms diversity and their variations for *P. laticeps* (Izecksohn and Peixoto, 1981), comparing the antipredator mechanisms diversity among congeners.

We extracted the records of *Proceratophrys* species from the global database of antipredator mechanisms (see Ferreira et al., 2019). We complemented this database with our field observations on *P. laticeps*. For this, we conducted fieldwork on November 2018 and November 2019 in the Estação Biologia Marinha Augusto Ruschi (EBMAR; 19°58'09"S, 40°08'37"W), located in the district of Santa Cruz, municipality of Aracruz, Espírito

Santo state, south-eastern Brazil. We used focal animal sampling (Altmann, 1974) and induced the antipredator mechanisms under field conditions using only fingers lightly touching the back, fore and hind limbs, and snout of the frogs, simulating predator attacks (Lourenço-de-Moraes et al., 2016).

We followed the classification of antipredator mechanisms proposed by Ferreira et al. (2019). After the field observations, the captured males were sacrificed in 3% lidocaine, fixed in 10% formalin, preserved in alcohol 70%, and deposited at the collection of Museu de Biologia Prof. Mello Leitão (MBML 11562, 11882) from Instituto Nacional da Mata Atlântica, municipality of Santa Teresa, Espírito Santo state, Brazil.

For the phylogenetic analysis, we followed the Amphibia phylogeny of Jetz and Pyron (2018) and reconstructed the ancestral character states through maximum-likelihood estimations under stochastic character mapping analysis (SIMMAP; Bollback, 2006), using 1.000 simulations for discrete characters based on the matrix data of antipredator mechanisms. We used the D statistic for the phylogenetic signal analysis (Fritz and Purvis, 2010) to measure phylogenetic signal for discrete attributes. The statistic adds the differences in attributes among sister clades and compares this sum to one generated by the Brownian movement. To compare phylogenies, this difference in the sums is divided by subtracting the sum of the differences simulated randomly about the sum by Brownian motion using 1.000 simulations. We used the packages “phytools” (Revell, 2012) and “caper” (Orme et al., 2018) through the R software (R Core Team, 2017).

We recorded two calling males of *P. laticeps* (SVL: 66.1 and 66.7 mm) on the partially-submerged leaf-litter of a swampy forest. When we approached, they displayed interrupt calling, and when we hand-manipulated them, both displayed other six antipredator mechanisms and nine variations: camouflage (variation: background matching [Fig. 1A]), immobility, posture (variations: body inflation [Fig. 1B], contraction, gland exposure, stretching limbs, death feigning [Fig. 1C]), escape (variations: hide, jump away), aggression (variation: kick), and distress call.

By adding our field observation on *P. laticeps*, the final dataset comprises 80 records on antipredator mechanisms for 13 species of *Proceratophrys* (Table 1), which represents 33% of the species from the genus. We recorded a total of 11 antipredator mechanisms and 15 variations for species of *Proceratophrys*. The mean of antipredator mechanisms displayed by *Proceratophrys* species was 3.8 (min = 2; max = 7). Camouflage was the most displayed antipredator mechanism (n = 13 species; 100%), followed by posture (n = 12 species; 92%), and escape (n = 8 spe-

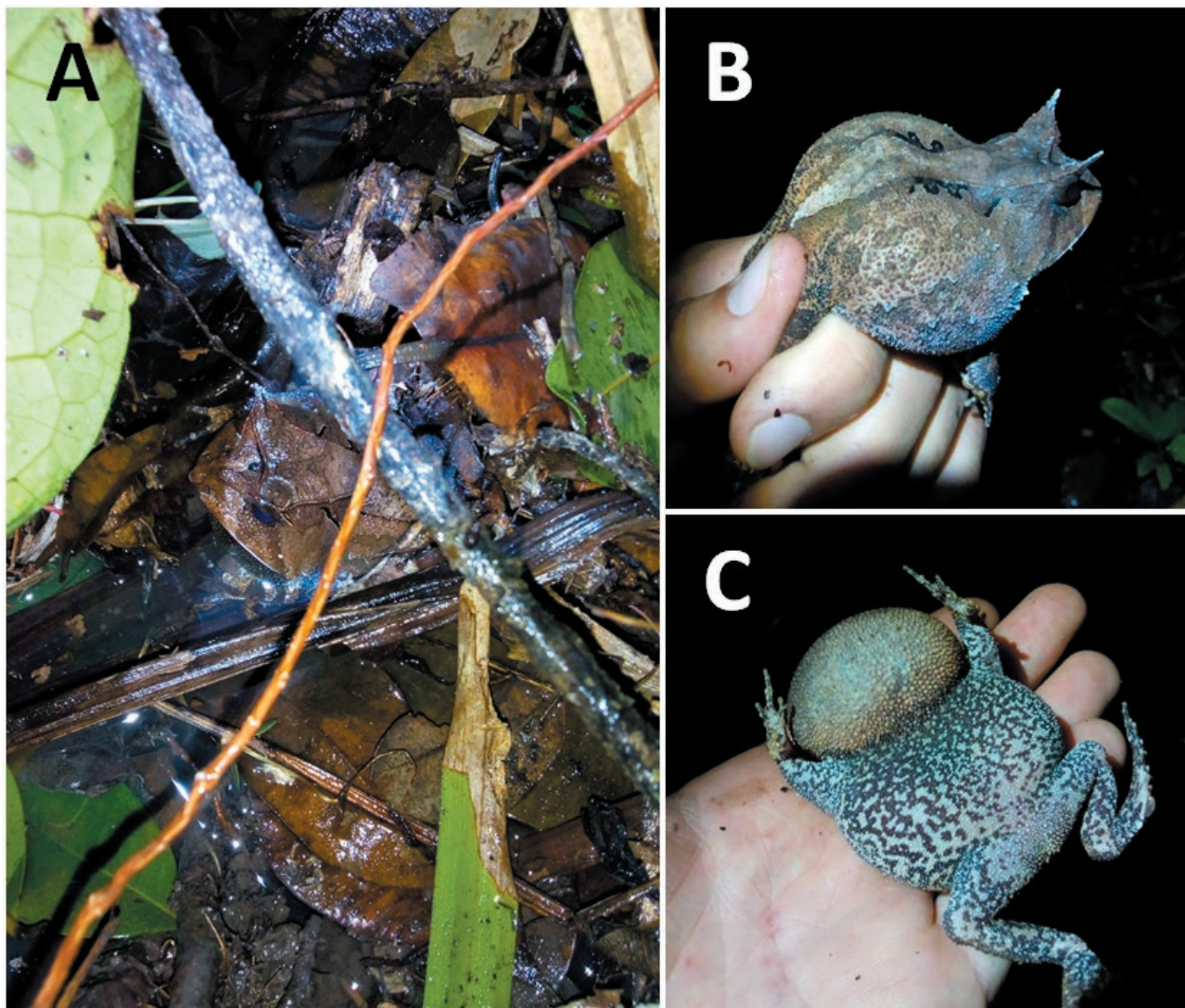


Fig. 1. Antipredator mechanisms displayed by *Proceratophrys laticeps*: A) Camouflage of background matching. B) Posture of body inflation during hand capture. C) Posture of death feigning and body inflation synergistically to distress call.

cies; 62%). Regarding posture, stretching limbs ($n = 8$ species; 62%), body inflation ($n = 7$ species; 54%), and death feigning ($n = 7$ species; 54%) were the most displayed. *Proceratophrys laticeps* ($n = 7$ mechanisms; 64%) displayed the highest number of antipredator mechanisms, followed by *P. boiei* ($n = 6$ mechanisms; 55%), and *P. schirchi* ($n = 6$ mechanisms; 55%). *Proceratophrys schirchi* ($n = 12$ variations; 80%) displayed the highest number of variations, followed by *P. boiei* ($n = 10$; 67%).

The mechanisms of camouflage, immobility, interrupt calling, aposematism, aggression, and distress calls (values > 0.60) have high phylogenetic structure values (Table 2). This result indicates that these mechanisms have high similarity in the trees' roots within *Proceratophrys* (Fig. 2).

On the other hand, the mechanisms of charge, warning sound, and poisonous secretion have prevalent Brownian origin. This result indicates that these mechanisms can occur randomly at phylogenetic trees' tips.

Proceratophrys species display a wide diversity of antipredator mechanisms to avoid detection, prevent attack, and counter-attack. Despite species of *Proceratophrys* are frequently sampled, only 13 (33%) species have description of antipredator mechanisms. Similarly, only four (40%) species of *Odontophrynus* and 13 (27%) species of *Physalaemus* have been tested but displayed several antipredator mechanisms ($n = 11$ and 8, respectively) (Ferreira et al., 2019). The lack of data on antipredator mechanisms is generalized across anurans, and thus we reinforce

Table 1. Antipredator mechanisms recorded for *Proceratophrys* species.

| Species | Antipredator mechanisms | | | | | | | | | | | | | | | | | | | | Ref | |
|-------------------------|-------------------------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|-----|-----------|
| | N | BM | IM | IC | AH | CH | BE | BI | CT | GE | MG | RE | SL | DF | UR | HD | JA | WS | PS | KC | | DC |
| <i>P. appendiculata</i> | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 |
| <i>P. avelinoi</i> | 4 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 |
| <i>P. boiei</i> | 18 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 3,4,5,6,7 |
| <i>P. brauni</i> | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 8,* |
| <i>P. cristiceps</i> | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 9 |
| <i>P. cururu</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 6 |
| <i>P. laticeps</i> | 7 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 7,* |
| <i>P. melanopogon</i> | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5,6,10 |
| <i>P. moehringi</i> | 3 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 |
| <i>P. moratoi</i> | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 6,* |
| <i>P. paviotii</i> | 5 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 7 |
| <i>P. renalis</i> | 2 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7,12 |
| <i>P. schirchi</i> | 6 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 7,13 |
| Total | 53 | 13 | 6 | 1 | 2 | 1 | 2 | 7 | 6 | 5 | 3 | 2 | 8 | 7 | 1 | 3 | 6 | 1 | 1 | 4 | 1 | |

N= number of individuals tested. Antipredator mechanisms (variations): BM = Camouflage (background matching); IM = Immobility; IC = Interrupt calling; HA = Aposematism (hidden); CH = Charge; Posture (BE = Body elevation; BI = Body inflation; CT = Contraction; GE = Gland exposure; MG = Mouth gape; RE = Rear elevation; SL = Stretching limbs; DF = Death feigning; UR = Unken reflex); Escape (HD = Hide; JA = Jump away); WS = Warning sound; PS = Secretion (Poisonous); KC = Aggression (kick); DC = Distress call. Ref = references: 1 = Sazima, 1978; 2 = Lourenço-de-Moraes and Lourenço-de-Moraes, 2012; 3 = Costa et al., 2009; 4 = Toledo and Zina, 2004; 5 = Toledo et al., 2010; 6 = Toledo et al., 2011; 7 = Ferreira et al., 2019; 8 = Solé, 2003; 9 = Mângia and Garda, 2015; 10 = Moura et al., 2010; 11 = Weygoldt, 1986; 12 = Peixoto et al., 2013; 13 = Mónico et al., 2017; * = Present study.

Table 2. Phylogenetic signal of antipredator mechanisms recorded for species of *Proceratophrys*. Bold values indicate significant differences.

| Antipredator mechanisms | Estimated D | Phylogenetic structure | Brownian phylogenetic structure |
|-------------------------|-------------|------------------------|---------------------------------|
| Aggression | 1.753 | 0.721 | 0.193 |
| Aposematism | 8.344 | 0.695 | 0.130 |
| Camouflage | 0.000 | 1 | 0.000 |
| Charge | -1.691 | 0.229 | 0.527 |
| Distress call | 1.732 | 0.841 | 0.122 |
| Escape | 1.199 | 0.548 | 0.115 |
| Immobility | 2.445 | 0.913 | 0.071 |
| Interrupt calling | 8.119 | 0.669 | 0.134 |
| Secretion | -3.581 | 0.136 | 0.853 |
| Posture | 0.767 | 0.523 | 0.278 |
| Warning sound | -4.928 | 0.243 | 0.537 |

the need to induce antipredator mechanisms for all individuals from most species collected in the field.

Proceratophrys laticeps displayed high diversity of antipredator mechanisms often in synergy. Anurans displaying different synergistic antipredator mechanisms may be more successful against predators (Toledo et al.,

2007), probably because of higher effectiveness in signal transmission to predators as observed for two species of *Gastrotheca* (Lourenço-de-Moraes et al., 2016). Probably, *P. laticeps* displays antipredator mechanisms according to researchers' degree of stress during inductions in the field (see Lourenço-de-Moraes et al., 2016). Despite the high diversity of antipredator mechanisms exhibited, *P. laticeps* differed from the congeners only by interrupt calling. Interrupt calling at predator approach aims to avoid giving predators a cue to the anuran location (Ferreira et al., 2019). Only 10 anuran species have been recorded interrupt calling, thus this homoplastic mechanism have evolved independently in Odontophryniidae (Ferreira et al., 2019). The low number of records of interrupt calling is likely a sampling artifact because most researchers do not take notes on frogs that interrupt the calls when approached in the field.

Camouflage is displayed by all species of *Proceratophrys* studied so far. Camouflage is symplesiomorphic in Anura (Ferreira et al., 2019), and showed high phylogenetic structure in *Proceratophrys*, following a purely phylogenetic model. In fact, camouflage is displayed by most odontophryniids that usually have brown coloration resembling the leaf-litter (Sazima, 1978; Ferreira et al., 2019). Camouflage includes colouring, structural and behavioural adaptations to avoid detection by predators

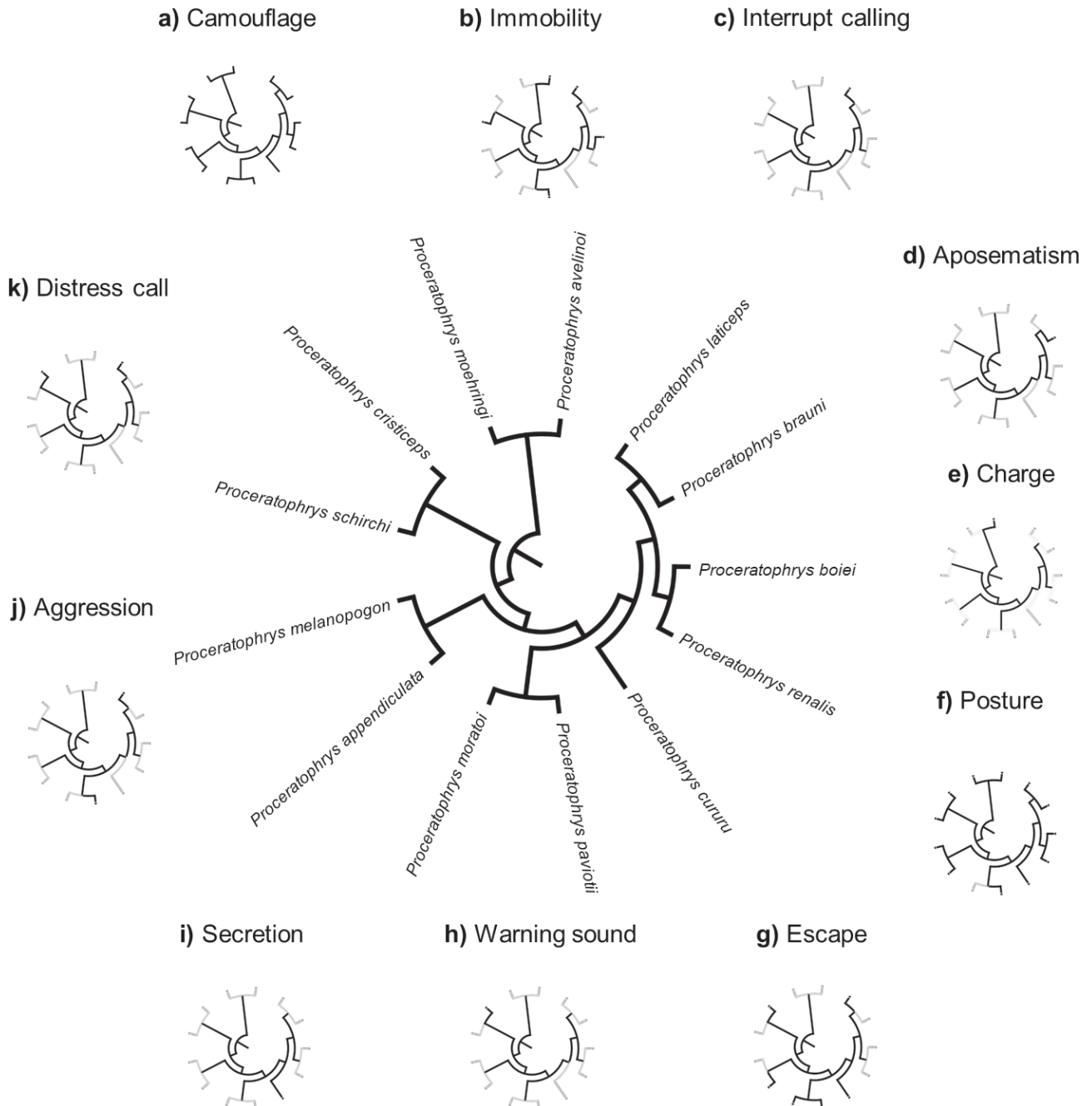


Fig. 2. Reconstruction of ancestral state of 11 antipredator mechanisms displayed by 13 species of *Proceratophrys*. A) Camouflage, B) Immobility, C) Interrupt calling, D) Aposematism, E) Charge, F) Posture, G) Escape, H) Warning sound, I) Secretion, J) Aggression, K) Distress call. Black branches = presence of the mechanism; grey branches = absence of the mechanism.

(Ferreira et al., 2019). In this context, *Proceratophrys* species have morphological adaptations such as supraciliary structures, and a variety of warts and tubercles that likely enhance camouflage (Prado and Pombal, 2008).

After being touched by a predator, *Proceratophrys* species usually display a variety of postures. Posture is

symplesiomorphic in *Proceratophrys*, showing high phylogenetic structure, being conserved in the genus and in Anura. Posture was displayed by 12 (30%) species of *Proceratophrys*, and it is the second most displayed antipredator mechanism in the genus. The eight species that displayed stretching limbs may be avoiding detection

by visually oriented predators that forage on the leaf-litter (Sazima, 1978). Body inflation can fool the predator regarding anuran body size, becoming difficult to be handled and ingested (Caro, 2014). Death feigning is displayed to resemble a dead organism and generally is displayed after the anuran has jumped away or was handled by a predator (Toledo et al., 2011; Ferreira et al., 2019). Therefore, *Proceratophrys* species likely have success in avoiding predation by displaying postures to intimidate predators, resembling a dead leaf, or making them difficult to be swallowed. We suggest that posture is an effective antipredator mechanism for species of *Proceratophrys* against predators in the leaf-litter.

Our results suggest that there are antipredator mechanisms with strong phylogenetic signal (camouflage, immobility, distress call, aggression, aposematism, and interrupt calling) in *Proceratophrys* and that their evolution is purely phylogenetic. Six antipredator mechanisms displayed by *Proceratophrys* species (i.e., camouflage, immobility, posture, escape, warning sound, and secretion) are plesiomorphic in Anura (Ferreira et al., 2019), explaining the maintenance of these mechanisms in the genus. In contrast, three antipredator mechanisms displayed by *Proceratophrys* species are homoplastic (i.e., interrupt calling, charge, and distress call), having evolved independently.

To conclude, our observations are the first records of antipredator mechanisms for *P. laticeps*, and the first report of interrupt calling in the genus *Proceratophrys*. Also, we showed that several antipredator mechanisms have high phylogenetic signal in this genus. Due to limited sample number of both records of different antipredator mechanism and examined species of the genus, our analysis should be treated as a preliminary overview of possibly more complex phylogenetic scenarios of a number of different mechanisms. We suggest also that further studies on this topic should use standardized induction methods and classification system for antipredator mechanisms (see Ferreira et al., 2019).

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