Acta Herpetologica 17(1): 59-70, 2022
DOI: 10.36253/a_h-11315

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

Brena da Silva Gonçalves[1,*], Carla D. Hendges[2], Bruno Madalozzo[2], Tiago G. Santos[2,3]

[1] *Universidade Federal do Amazonas, Departamento de Biologia. Avenida Rodrigo Otávio, 6500, Japiim, CEP 69077000 - Manaus, Amazonas, Brasil*

[2] *Programa de Pós-Graduação em Biodiversidade Animal, Universidade Federal de Santa Maria, CEP 97105-900, Santa Maria, Rio Grande do Sul, Brazil*

[3] *Universidade Federal do Pampa Campus São Gabriel, CEP 97307-020, São Gabriel, Rio Grande do Sul, Brazil*

**Corresponding author*. E-mail: brenasgoncalves@outlook.com

Submitted on: 2021, 8th June; revised on: 2021, 25th October; accepted on: 2022, 1st April
Editor: Raoul Manenti

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

ISSN 1827-9635 (print)
ISSN 1827-9643 (online)

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; Provete 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 (Cei, 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. macrogolossa* housed in the herpetological collections of the Universidade Federal de Santa Maria, Brazil (ZUFSM). 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

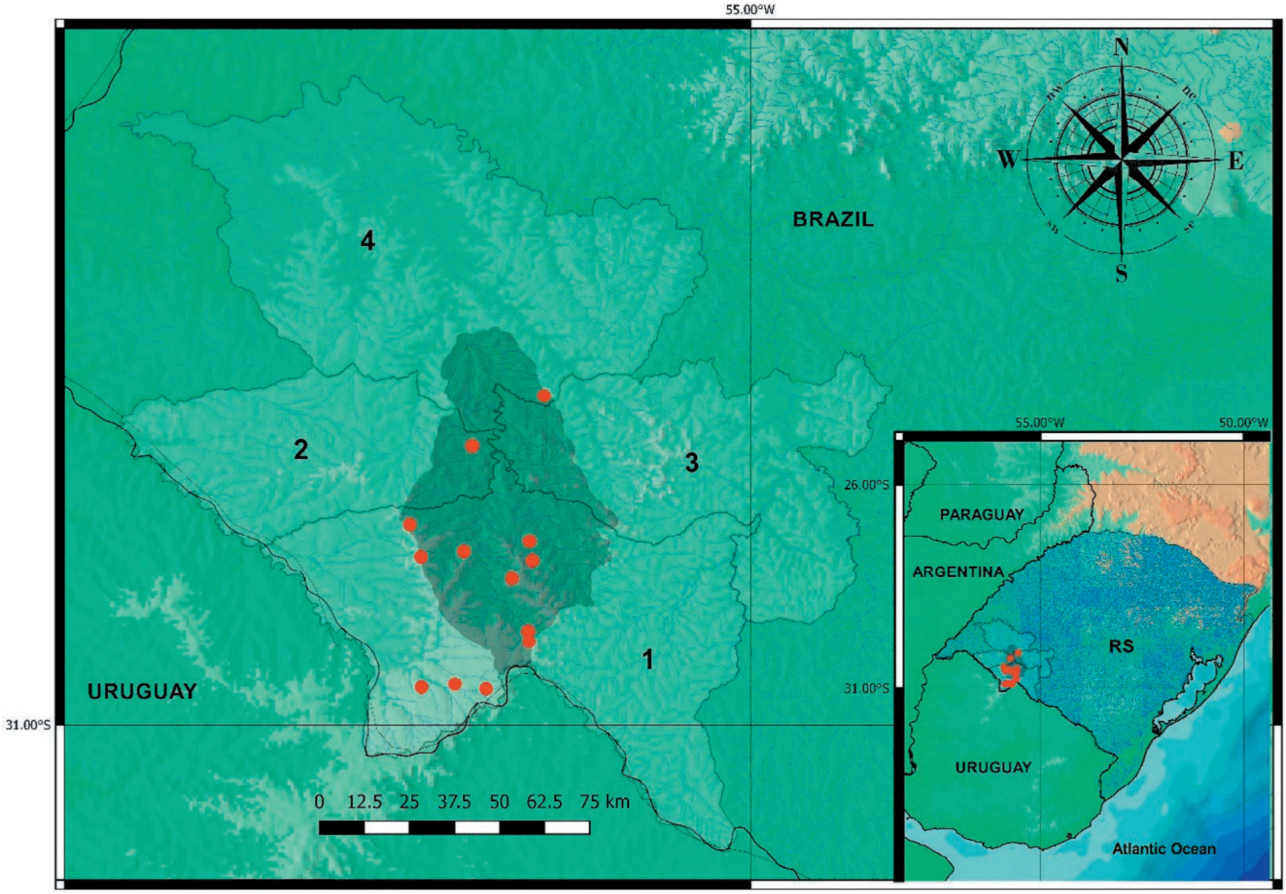

Fig. 1. Distribution of the 13 streamlets in the Environmental Protect Area of the Ibirapuitã and surroundings, where tadpoles of *Limnomedusa macroglossa* were collected. The grey area represents boundaries of the APA of the Ibirapuitã encompassing four Brazilian municipalities in the state of Rio Grande 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 semilandmarks 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

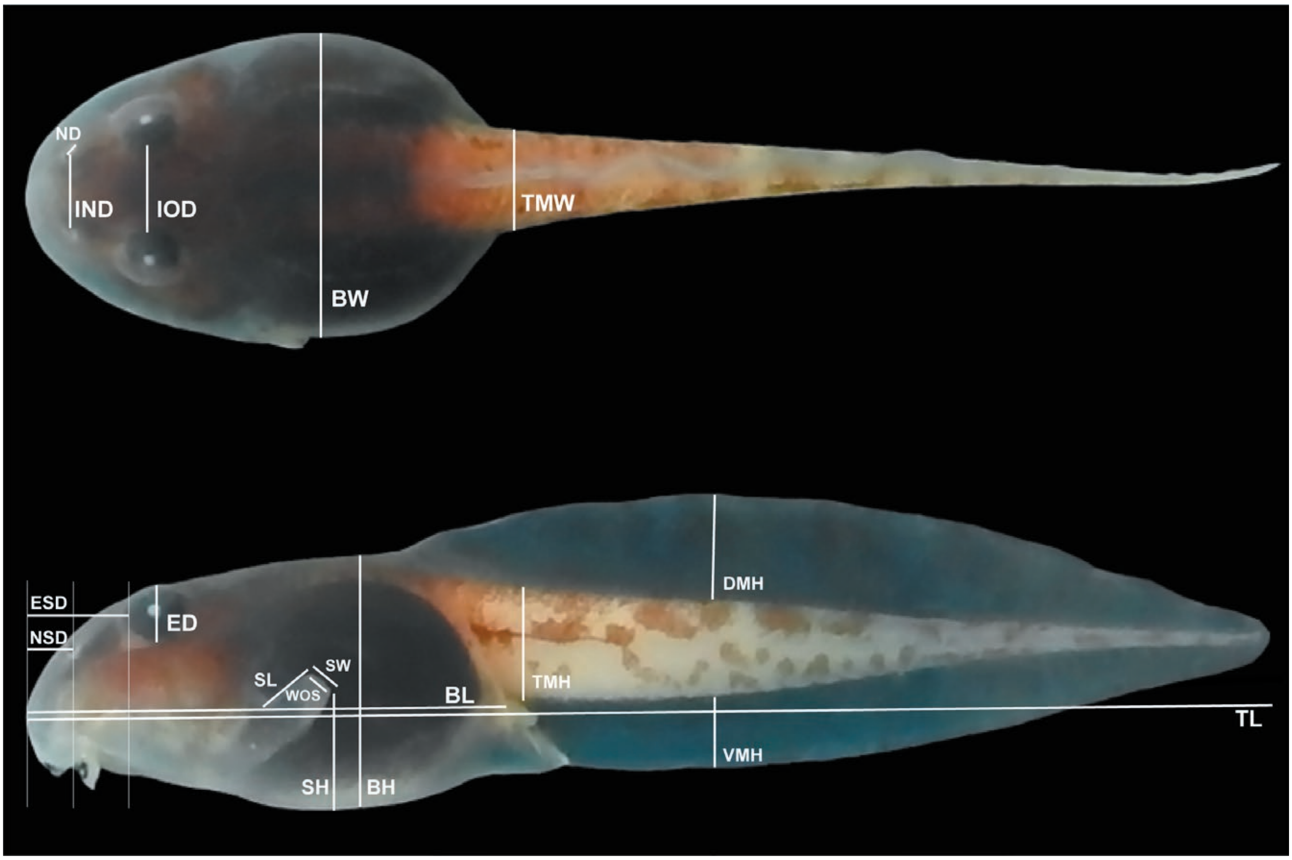

Fig. 2. Representation of the measures carried out in the larvae of *Limnomedusa macroglossa* used for description of external morphology in dorsal and lateral view (sensu Lavilla and Scrocchi (1986), McDiarmid and Altig (1999) and Altig (2007)): BH = body height, SH = spiracle height, TMH = tail musculature 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: $R2 = 0.07$, $F = 11.756$, $P < 0.05$; 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 ± 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

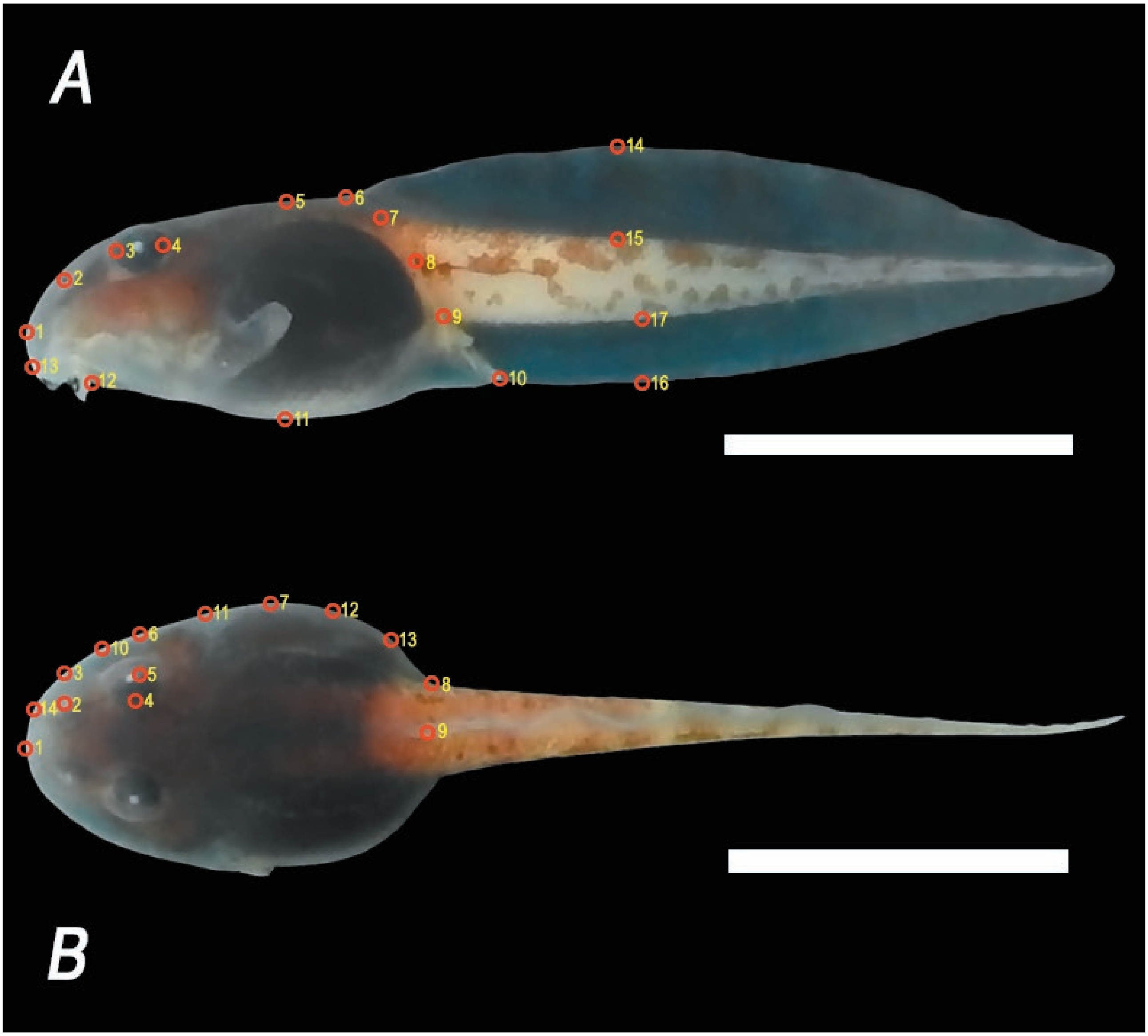

Fig. 3. Position of the landmarks and semilandmarks on the lateral (A) and dorsal view (B) of the tadpole of *Limnomedusa macroglossa*. Landmark and semilandmarks descriptions are in Table S2.

posterodorsally oriented tip) (SH: 2.89 ± 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 ± 0.94, BW: 9.11 ± 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 ± 0.54, VMH: 1.70 ± 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 lateroventral 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,

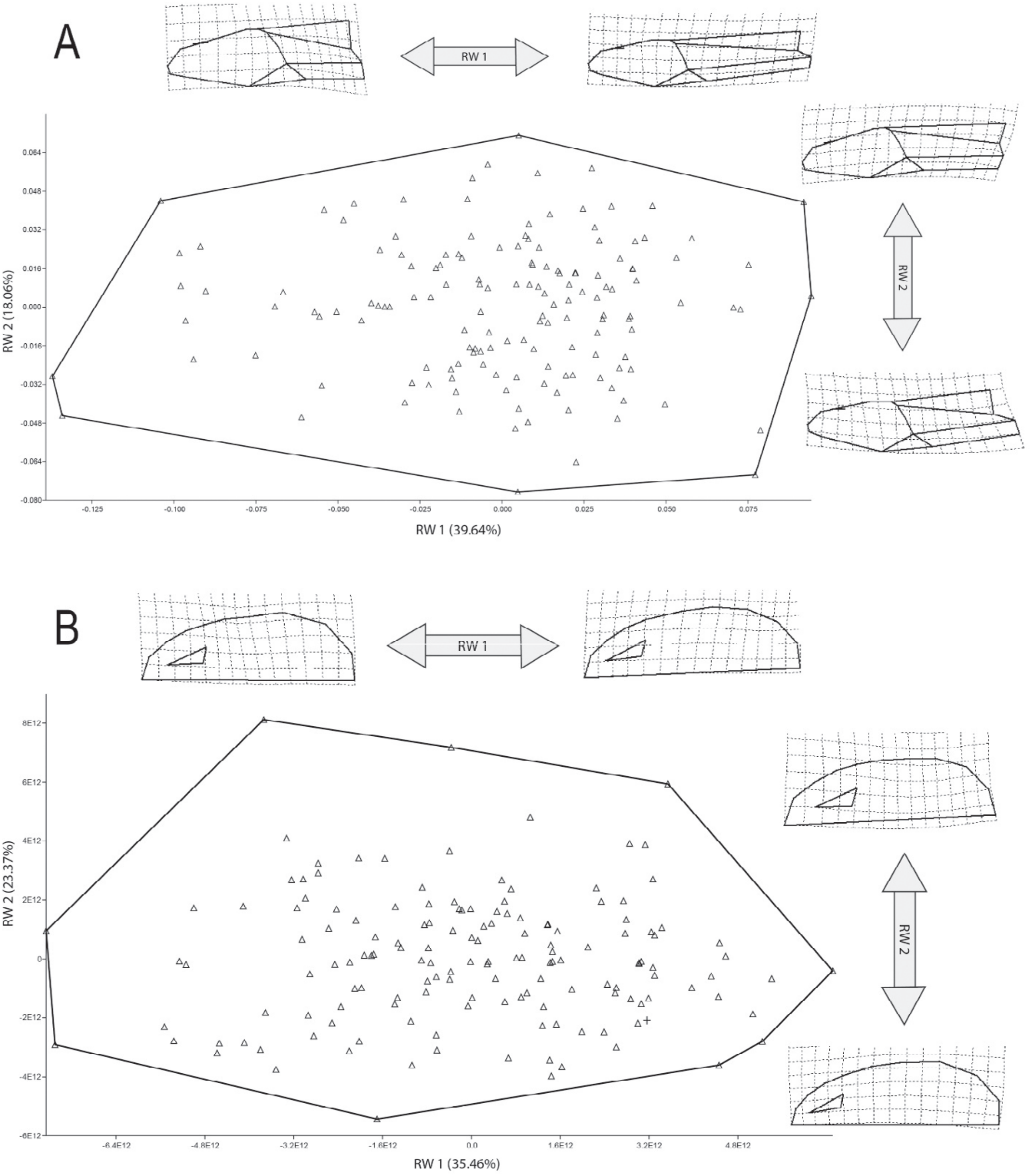

Fig. 4. Scatter plot of RW1 vs. RW2. The deformation grids demonstrate the average deformation of the shape in lateral view (A) and dorsal view (B), at the positive and negative scores of the Relative Warp axes for tadpoles of *Limnomedusa macroglossa*.

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

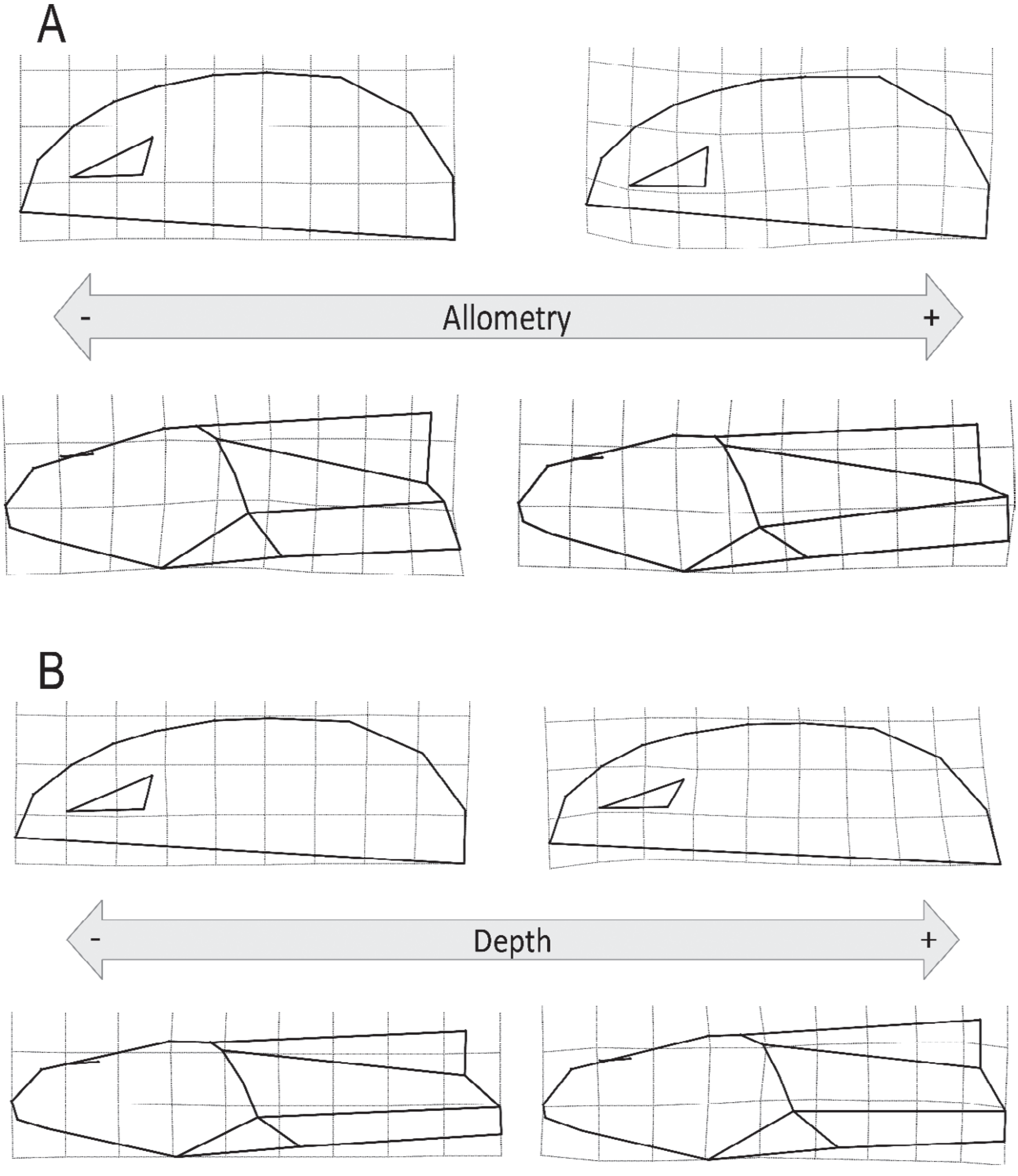

Fig. 5. Shape deformations related to the multivariate regression going from the most extreme negative score (left) to the most positive scores (right). In (A) deformations are from the effect of allometry. In (B) from the depth influence in the lateral and dorsal views of tadpoles of *Limnomedusa macroglossa.*

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.

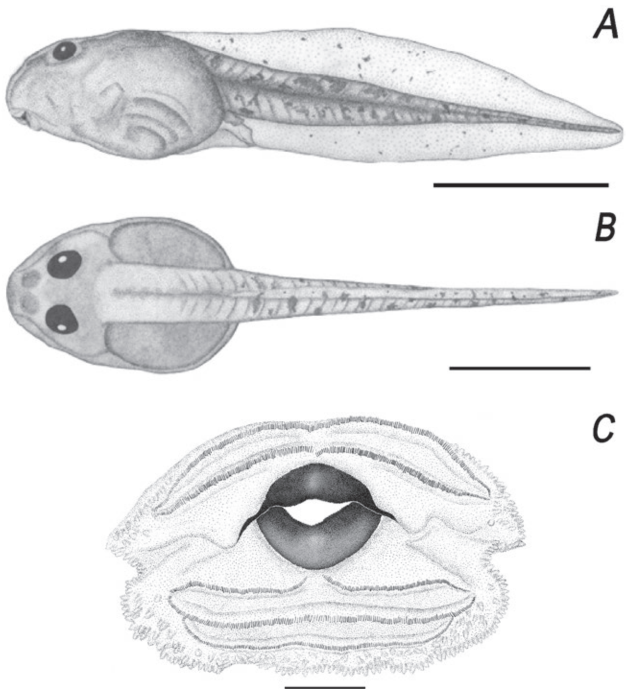

Fig. 6. Tadpole of *Limnomedusa macroglossa* of stage 33: (A) in lateral view (scale 1 cm); (B) in dorsal view (scale 1 cm); (C) Oral disc (scale 1mm). Drawings by B.S.G.

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, especial 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 Cei (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 Cei (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 Candioti 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).

ACKNOWLEDGEMENTS

We are grateful to SISBio-ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade) for providing the collection permit (License #33975/March 2012). We are also grateful to the anonymous reviewers for their comments and suggestions on the manuscript.

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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