

Re-description of the external morphology of *Phyllomedusa iheringii* Boulenger, 1885 larvae (Anura: Hylidae), with comments on the external morphology of tadpoles of the *P. burmeisteri* group

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Abstract. *Phyllomedusa iheringii* is a leaf frog endemic to the Uruguayan Savanna, which reproduces in water bodies in open areas. Here, based on the lack of some informative characteristics observed on the first description of this species, we re-describe the larval external morphology of *P. iheringii* from localities in Brazil and Uruguay, and compared them with other species from the *P. burmeisteri* group. The tadpoles of *P. iheringii* belong to the suspension-rasper guild. The body length corresponds to approximately one-third of the total length. The body is pyriform in dorsal view and laterally triangular. The snout is rounded in a dorsolateral view. The spiracle is single and almost ventral, not forming a free tube, and sinistral. The opening of vent tube is dextral. The oral disc is anteroventral and anteriorly directed, with single ventral emargination. Marginal papillae uniseriate, interrupted in a wide dorsal gap, and with pointed tips. The labial tooth row formula is 2(2)/3(1). The third lower row is four times shorter than the others. Furthermore, the tadpoles of *P. iheringii* showed morphological differences in relation to other species of the *P. burmeisteri* group, this species seems to be smaller in average and have fewer marginal papillae in the oral discs.

Keywords. Tadpole morphology, Phyllomedusinae, Uruguayan Savanna ecoregion.

The genus *Phyllomedusa* currently has 30 species and is widely distributed in the Neotropics, occurring from Panama, the Pacific slopes of Colombia, South America east of the Andes, and southwards to northern Argentina and Uruguay (Frost, 2014). Species of this genus are distinguished from other Neotropical hylids by their contrasting colour patterns, vertical pupils, and an arboreal reproductive mode (Caramaschi, 2006). In Brazil, 23 species of *Phyllomedusa* are recorded and all have external larval morphology known (Segalla et al., 2012). Nevertheless, larval internal oral features and chondrocranium are poorly known for most species. Similarly, the larval morphology still requires to be described or re-described,

because some previous descriptions raise doubts concerning the identification of some structures, as is the case of *P. iheringii*.

The group of *Phyllomedusa burmeisteri* comprises five species (*P. bahiana*, *P. burmeisteri*, *P. distincta*, *P. iheringii*, and *P. tetraploidea*) that share similarities in external morphology, vocalization, and cytogenetic features, and possess a complex pattern of strips on the thighs as a diagnostic characteristic (Pombal Jr. and Haddad, 1992; Silva-Filho and Juncá, 2006; Faivovich et al., 2010). Species of the *P. burmeisteri* group show a parapatric or allopatric distribution throughout the Atlantic Rainforest and replace each other throughout the geographic distri-

bution of the group (Brunes et al., 2010). *Phyllomedusa iheringii* is the only species of the group that occurs in open areas and is endemic to the Uruguayan Savanna, occurring in Uruguay and southern of the Brazilian state of Rio Grande do Sul (Olson et al., 2001; Brunes et al., 2010; Frost, 2014).

Phyllomedusa iheringii reproduces in both lentic or lotic water bodies (but in backwater zones), and uses marginal vegetation for both vocalization and spawning (Maneyro and Carreira, 2012). Males call whilst perched on vegetation and the eggs are laid in leaf nests. Tadpoles are exotrophic and drop into lentic water to complete their development (mode 24; Haddad and Prado, 2005). The first description of the *P. iheringii* tadpole was provided by de Sá and Gerhau (1983) was based on a single individual hatched from on single egg mass, from one single locality. This first effort to describe the species, lack of information concerning the sizes, length, and positions of some body structures treated as diagnostic characteristics. The dataset provided may not represent with precision the morphological intraspecific variation of the species within its distributional range and may have limited the understanding of intraspecific variation as well as inter-specific comparisons with other species of the group. Here, we re-describe the external morphology of the tadpole of *P. iheringii*, accounting for intraspecific variation and providing comparisons with other species of the *P. burmeisteri* group. In addition, we present data on natural history.

The tadpoles of *Phyllomedusa iheringii* were collected at four sites in the Uruguayan Savanna: Encruzilhada do Sul (30°33'50.7" S, 52°34'13.1" W, 414 m a.s.l.; about 100 km from the type locality; ZUFMS 8166), Santa Maria (29°49'25.3" S, 53°37'19.3" W, 229 m a.s.l.; ZUFMS8831), and São Sepé (30°15'27.5" S, 53°34'52.4" W, 346 m a.s.l.; ZUFMS 8832-3) in Rio Grande do Sul, Brazil; and in Cerro Chapeu, Rivera, Uruguay (30°57'11" S, 55°28'22" W, 257 m a.s.l.; RML 2448). The larvae were collected and immediately fixed in a formalin 10% solution in field.

The re-description was based on eight specimens of stage 37 (Gosner, 1960) (seven individuals from São Sepé and one from Rivera), from which 21 morphological measurements were taken which according to McDiarmid and Altig (1999), and Altig (2007) and Lavilla and Scrocchi (1986). To allow comparison within *P. iheringii* and with other species from *P. burmeisteri* group, we also took the same measures of other 32 individuals (stages 26-31, 34-36; Gosner, 1960) (Table 1). Tadpole measurements were made using a stereomicroscope (0.01 mm precision), except for TL, which was measured with a digital caliper (0.01 mm precision) (Table 2).

External morphology

The tadpoles of *Phyllomedusa iheringii* belong to the suspension-rasper guild (McDiarmid and Altig 1999; Both et al., 2011), and have an elongated body (BH/BW: 0.92; Tab. 1). The body length corresponds to approximately one-third of the total length (BL/TL: 0.34). The body is piriform in dorsal view and triangular in lateral view (Fig. 1A, B). The snout is rounded in a dorsolateral view. The nostrils are rounded and dorsolateral (ND: 0.25 ± 0.04). The internostril distance is larger than the distance between the nostrils and the snout (IND: 3.60 ± 0.22 ; NSD: 1.44 ± 0.27). Eyes are lateral (ED: 2.15 ± 0.19). The interorbital distance is greater than the distance between the eyes and the snout (IOD: 7.75 ± 0.41 ; ESD: 6.16 ± 0.35). The spiracle is sinistral, single and almost ventral, not forming a free tube. The opening of vent tube is dextral. The tail is pointed, with flagellum, and longer and taller than the body (TAL/TL: 0.66; MTH/BH: 1.33). The tail musculature is higher than wide (TMH/TMW: 1.16). The dorsal fin originates anterior of dorsal tail-body junction, the ventral fin is higher than the dorsal fin (VMH: 5.93 ± 1.01 ; DMH: 1.74 ± 0.24). The oral disc is anteroventral and anteriorly directed, not emarginate. Marginal papillae uniseriate, interrupted in a wide dorsal gap, and with pointed tips. Submarginal papillae laterally aggregate in the oral disc, not forming rows. The upper jaw sheath is curved, but flat in the middle, not serrated and its width is approximately seven times greater than high (UJSW/UJSH: 7.86). The lower jaw has a 'v' shape, not serrated and approximately six times longer than high (LLJ/HLJ: 6.06). The labial tooth row formula is 2(2)/3(1) and the third lower row is four times shorter than the others (Fig. 1C).

Coloration

Usually, the dorsum of the body and tail varies from yellowish to greenish. The lateral portion of the body is grey-violet, with brown pigmented areas in the middle of fin. The fins are translucent and have small, uniformly distributed melanophores. The intestine region is bluish in lateral and ventral views. There are two darkened blotches on the head, between the eyes and the nostrils. When preserved in formalin (10%), the tadpoles are uniformly yellowish or greyish.

Variation

The tadpoles of *Phyllomedusa iheringii* do not have ontogenetic variation, though geographic variation

Table 1. Morphological measurements of 40 tadpoles of *Phyllomedusa iheringii* from different localities of the Uruguayan Savana. Mean, standard deviation and range (mm) are shown. The stage and sample size are in parentheses. BH – body height, BL – body length, BW – body width, DMH – dorsal fin height, ED – eye diameter, ESD – eye–snout distance, HLJ –lower jaw sheath height, IND – inter-narial distance, IOD – interorbital distance, LLJ –lower jaw sheath length, MTH – maximum tail height, ND – nostril diameter, NSD – nostril–snout distance, OD – oral disc diameter, TAL – tail length, TL – total length, TMH – tail musculature height, TMW – tail musculature width, UJSH – upper jaw sheath height, UJSW – upper jaw sheath width, VMH – ventral fin height.

	Encruzilhada do Sul (stage 27, n = 1)	Santa Maria (stage 27, n = 3)	São Sepé (stage 26-31, n = 18)	São Sepé (stage 34-36, n = 7)	Rivera (stage 36, n = 3)	São Sepé and Rivera (stage 37, n = 8)
BH	5.60	4.80 ± 0.12 (4.67 – 5.60)	6.45 ± 0.71 (4.74 – 7.41)	7.07 ± 0.94 (8.52 – 6.04)	9.77 ± 0.93 (9.16 – 10.84)	7.74 ± 0.64 (8.35 – 6.41)
BL	8.60	6.85 ± 0.14 (6.72 – 8.60)	11.48 ± 1.54 (8.85 – 13.86)	14.78 ± 1.26 (16.79 – 13.43)	16.05 ± 1.24 (14.77 – 17.25)	15.02 ± 1.86 (16.51 – 10.95)
BW	5.08	4.45 ± 0.10 (4.39 – 5.08)	6.87 ± 0.69 (5.30 – 7.83)	8.25 ± 0.81 (9.23 – 6.94)	8.69 ± 0.33 (8.31 – 8.89)	8.42 ± 0.76 (9.39 – 7.32)
DMH	1.15	0.95 ± 0.07 (0.87 – 1.15)	1.27 ± 0.14 (1.00 – 1.28)	1.59 ± 0.14 (1.76 – 1.34)	1.66 ± 0.12 (1.56 – 1.80)	1.74 ± 0.24 (2.06 – 1.4)
ED	1.60	1.37 ± 0.03 (1.29 – 1.60)	2.03 ± 0.35 (1.29 – 2.42)	2.55 ± 0.27 (2.74 – 2.09)	2.10 ± 0.14 (2.02 – 2.26)	2.50 ± 0.19 (2.77 – 2.18)
ESD	3.47	3.00 ± 0.16 (2.87 – 3.47)	4.73 ± 0.65 (3.54 – 5.85)	5.97 ± 0.51 (6.58 – 5.25)	5.52 ± 0.37 (5.26 – 5.94)	6.16 ± 0.35 (6.9 – 5.75)
HLJ	0.13	0.10 ± 0.01 (0.09 – 0.13)	0.14 ± 0.05 (0.10 – 0.29)	0.16 ± 0.06 (0.3 – 0.11)	0.15 ± 0.03 (0.13 – 0.19)	0.15 ± 0.04 (0.19 – 0.1)
IND	2.00	1.80 ± 0.02 (1.78 – 2.00)	2.95 ± 0.29 (2.32 – 3.44)	3.40 ± 0.22 (3.08 – 3.18)	3.61 ± 0.11 (3.52 – 3.73)	3.60 ± 0.22 (3.84 – 3.22)
IOD	3.88	3.69 ± 0.03 (3.65 – 3.88)	5.73 ± 0.7 (4.42 – 6.63)	7.36 ± 0.79 (8.61 – 6.02)	7.09 ± 0.53 (6.48 – 7.46)	7.75 ± 0.41 (8.32 – 7.2)
LLJ	0.58	0.58 ± 0.08 (0.54 – 0.67)	0.73 ± 0.18 (0.54 – 1.28)	0.90 ± 0.14 (1.2 – 0.81)	1.21 ± 0.25 (0.93 – 1.42)	0.91 ± 0.09 (1.05 – 0.77)
MTH	6.92	5.50 ± 0.13 (3.72 – 6.92)	7.78 ± 1.29 (3.72 – 9.15)	9.88 ± 0.70 (10.48 – 8.6)	9.06 ± 1.30 (8.06 – 10.53)	10.33 ± 1.26 (12.22 – 8.38)
ND	0.13	0.14 ± 0.03 (0.11 – 0.16)	0.20 ± 0.06 (0.13 – 0.33)	0.23 ± 0.04 (0.23 – 0.18)	0.21 ± 0.02 (0.20 – 0.23)	0.25 ± 0.04 (0.31 – 0.21)
NSD	0.92	0.56 ± 0.24 (0.40 – 0.92)	1.09 ± 0.25 (0.75 – 1.54)	1.49 ± 0.24 (1.74 – 1.5)	1.47 ± 0.07 (1.41 – 1.55)	1.44 ± 0.27 (1.85 – 1.1)
OD	1.28	1.49 ± 0.21 (1.25 – 1.62)	2.58 ± 0.50 (1.77 – 4.15)	3.21 ± 0.62 (4.57 – 2.79)	3.41 ± 0.68 (2.89 – 4.18)	3.12 ± 0.18 (3.32 – 2.82)
TAL	15.70	15.58 ± 1.02 (14.41 – 16.26)	23 ± 3.12 (17.02 – 29.73)	28.21 ± 3.35 (32.78 – 23.64)	23.53 ± 0.86 (22.72 – 24.44)	29.61 ± 2.06 (32.28 – 26.98)
TL	24.30	22.43 ± 1.13 (21.13 – 24.30)	34.48 ± 4.01 (26.11 – 40.2)	42.99 ± 4.41 (48.93 – 37.07)	39.58 ± 2.10 (37.49 – 41.69)	44.63 ± 2.55 (47.73 – 40.88)
TMH	2.85	2.21 ± 0.18 (2.09 – 2.85)	3.63 ± 0.73 (2.32 – 4.5)	4.70 ± 0.56 (5.02 – 3.74)	3.11 ± 0.14 (3.00 – 3.27)	4.52 ± 0.50 (4.86 – 3.3)
TMW	1.99	1.52 ± 0.24 (1.30 – 1.99)	3.02 ± 0.56 (2.02 – 3.82)	3.99 ± 0.58 (4.5 – 3.03)	2.43 ± 0.24 (2.20 – 2.67)	3.88 ± 0.59 (4.79 – 2.85)
UJSH	0.16	0.12 ± 0.03 (0.10 – 0.16)	0.23 ± 0.07 (0.14 – 0.46)	0.44 ± 0.53 (0.36 – 0.210)	0.31 ± 0.06 (0.24 – 0.36)	0.22 ± 0.04 (0.29 – 0.18)
UJSW	0.99	0.84 ± 0.01 (0.22 – 0.99)	1.39 ± 0.31 (0.97 – 2.38)	1.54 ± 0.68 (2.53 – 1.58)	1.95 ± 0.36 (1.68 – 2.36)	1.73 ± 0.07 (1.82 – 1.63)
VMH	3.33	2.63 ± 0.14 (2.30 – 3.33)	4.02 ± 0.56 (2.80 – 4.88)	5.05 ± 0.42 (5.26 – 4.92)	4.87 ± 1.17 (3.75 – 6.09)	5.93 ± 1.01 (7.55 – 4.08)

occurs. Such variation may be the result of different environmental conditions (temperature, pH), food availability or presence of predators among water bodies. Although

tadpoles from the three localities are morphologically similar, some of them have small variations (Table 1). Indeed, some individuals in different developmental stag-

Table 2. Comparison of external morphological characteristics between tadpoles from the *Phyllomedusa burmeisteri* species group. *The original description uses the stage VIII of Rugh (1951). Stages were assessed according to Gosner (1960).

Species	Stage	Oral formula	Ventral gap	Marginal papillae	Reference
<i>Phyllomedusa bahiana</i> Lutz, 1925	34–36	2(2)/3(1)	Present	Mostly uniserial; biserial at emargination	Silva – Filho and Juncá, 2006
<i>Phyllomedusa burmeisteri</i> Boulenger, 1882	35	2(2)/3(1)	Absent	Two series, with four or more series on the labial emargination	Cruz, 1982
<i>Phyllomedusa distincta</i> A. Lutz in B. Lutz, 1950	37	2(2)/3(1)	Absent	Two series, with a few scattered papillae on the labial emargination	Cruz, 1982
<i>Phyllomedusa iheringii</i> Boulenger, 1885	28*	2(2)/3	Absent	Uniserial	de Sá and Gerdau, 1983
<i>Phyllomedusa iheringii</i> Boulenger, 1885	37	2(2)/3(1) 2(2)/3	Absent	Uniserial, with submarginal papillae laterally aggregate in the oral disc, not forming rows	Present study
<i>Phyllomedusa tetraploidea</i> Pombal Jr. and Haddad, 1992	37	2(2)/3(1)	Absent	Uniserial, with numerous series on the emargination	Pombal Jr. and Haddad, 1992

*The original description uses the stage VIII of Rugh (1951). Stages were assessed according to Gosner (1960).

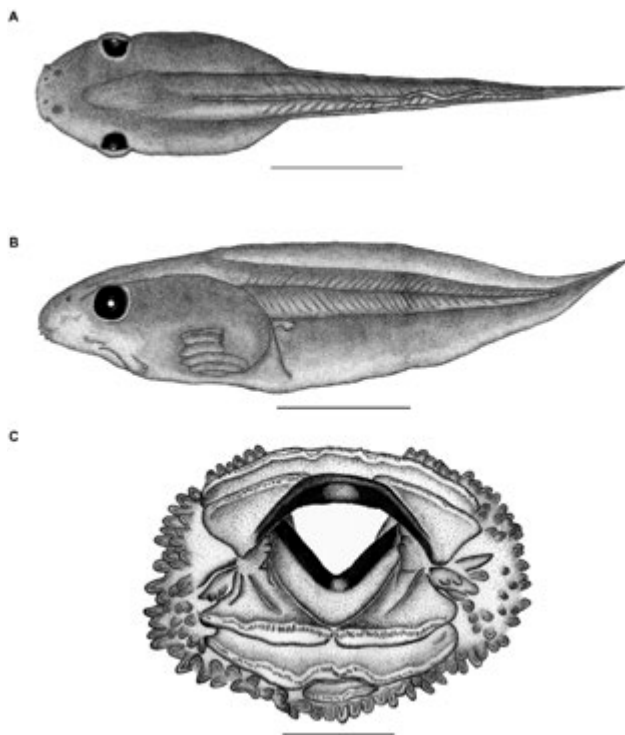


Fig. 1. Tadpole of *Phyllomedusa iheringii* (museum catalog ZUFMSM 10015) at Gosner stage 36: (A) dorsal view (scale = 10 mm); (B) lateral view (scale = 10 mm); (C) oral disc (scale = 1 mm). Drawings by B.M. and T.G.S

es (25, 26, 31, 36 and 37) have an oral formula of 2(2)/3, instead of the usual formula of 2(2)/3(1), and the third lower row in some cases, not linked to a specific devel-

opmental stage, is much smaller in comparison with the other ones. The marginal papillae can be light white or have several melanophores. Most individuals have a few blotches on the body and tail, but many others had fewer of these markings, which were less dark. Some individuals from São Sepé had melanophores on the ventral fins and blueish intestines. However, specimens from Encruzilhada do Sul, Santa Maria, and Cerro Chapeu had no melanophores on the ventral fins, and the intestine ranged from brown to black.

Natural history

Tadpoles of *Phyllomedusa iheringii* occurred throughout the spring and summer of the austral region (from September to March), and inhabit deeper and non-vegetated areas of ponds, pools of streamlets, and small dams used in cattle raising. At least, larvae of other 13 anuran species occurred syntopically with larvae of *Phyllomedusa iheringii* at the studied waterbodies: *Dendropsophus minutus*, *Elachistocleis bicolor*, *Hypsiboas pulchellus*, *Leptodactylus gracilis*, *L. latrans*, *Odontophrynus americanus*, *Physalaemus cuvieri*, *P. gracilis*, *P. henselii*, *Pseudopaludicola falcipes*, *Scinax fuscovarius*, *S. granulatus*, and *S. uruguayus*. Larvae usually forage near the water surface (head up, tilted upwards at 45°), but dive quickly when disturbed (without forming schools). On two occasions, we observed predation on tadpoles of *P. iheringii* in a temporary pond at São Sepé: in the first incident (December 2003), a tadpole was devoured by a beetle larva (Dytiscidae) early in the night, near the water sur-

face; the second occasion (November 2011) occurred in the afternoon, when a Great Kiskadee bird (*Pitangus sulphuratus*, Tyrannidae) repeatedly plunged into the water to catch and eat several tadpoles.

The morphological characteristics presented here for *Phyllomedusa iheringii* are similar to those reported by de Sá and Gerhau (1983). However, we found variation in the labial tooth row formula (Table 2), the color of papillae, body, tail, fins, and the intestine (which ranges from black to blue). The variation in coloration is probably related to the structural characteristics of the pond and to avoid predation and competitors (Thibaudeau and Altig, 2012). Indeed, experiments among tadpoles of several species in the presence of predators exhibit differences in coloration and morphology (McCullum and Leimberger, 1997; Van Buskirk and McCollum, 2000; Touchon and Warkentin, 2008). Tadpoles assessed here were collected in ponds with distinct structural variables and subject to different predation pressures, and thus we suggest that future studies could test the adaptive value of tadpole coloring on different pressures of predators and competitors.

Several characteristics of the *Phyllomedusa iheringii* tadpole are shared with other species of the *P. burmeisteri* group, such as the triangular shape of the body in dorsal view, ventral fin higher than the dorsal fin, and the oral formula 2(2)/3(1), with a reduced third row of lower teeth and dorsal gap (Cruz, 1982; de Sá and Gerhau, 1983; Pombal Jr. and Haddad, 1992; Silva-Filho and Juncá, 2006). However, the tadpole of *P. iheringii* is distinguished from other species of the *P. burmeisteri* group by fewer marginal papillae in the oral disc (only one series), whereas *P. burmeisteri* has two rows of papillae in the anterior and posterior portion of the oral disc, and four series on the labial emargination (Cruz, 1982); *P. bahiana* has one row in the anterior and posterior region and a double row on the labial emargination (Silva-Filho and Juncá, 2006); *P. distincta* has double series (Cruz, 1982); *P. tetraploidea* has one row, but with numerous rows on the labial emargination (Pombal Jr. and Haddad, 1992), and *P. bahiana* is the only species in the group that has a ventral gap of marginal papillae (Silva-Filho and Juncá, 2006). In summary, the tadpole of *P. iheringii* is distinct from other species of the *P. burmeisteri* group by the oral disc, mainly by the number of marginal papillae and the labial emargination.

The species belonging to the genus *Phyllomedusa* possess complex polypeptides on the skin that can be toxic and cause irritation, which might potentially protect the species against predation (Duellman and Trueb, 1986; Caramaschi and Cruz, 2002). However, several species of vertebrates and invertebrates prey on eggs, larvae, and adults of *Phyllomedusa* (Castanho, 1996; Feltrim and

Cechin, 2000; Toledo et al., 2005; Dias et al., 2012). Here, we add two predators of larvae of this genus (a Great Kiskadee bird and a beetle larva). Until now, odonate naiads, belostomatid bugs, spiders and fishes were identified as predators of tadpoles of *Phyllomedusa* (Azevedo-Ramos et al., 1992; Gascon, 1992; Schmidt and Amézquita, 2001; Santos-Silva et al., 2013)

Tadpoles of *Phyllomedusa iheringii* are similar to those of other species of *P. burmeisteri* group, but smaller in average and with fewer marginal papillae in the oral disc (not emarginated). Variation (between populations and individuals) on larvae of *P. iheringii* was primarily related to size, the oral disc formula (and the size of the third lower row) and the color pattern of the intestines and fins. In this sense, we believe our results add important information about the external morphology and natural history of *P. iheringii*, an endemic species of the Uruguayan Savanna ecoregion. We suggest future studies to focus on frog species that are relatively common in the Neotropics, such as *P. iheringii*, but that lack basic information as aspects of behavior, reproductive biology, and morphological descriptions of tadpoles.

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