

## THE TADPOLE OF *PHYLLOMEDUSA ITACOLOMI* (ANURA, HYLIDAE), WITH A DESCRIPTION OF THE INTERNAL ORAL MORPHOLOGY

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**ABSTRACT.** We describe the external morphology and the internal oral features of the tadpole of *Phyllomedusa itacolomi* (Anura, Hylidae) from Serra de Ouro Branco, Municipality of Ouro Branco, State of Minas Gerais, Brazil. Tadpoles were found at mid water in a temporary pool, in an open montane meadow. *Phyllomedusa itacolomi* (Gosner's stage 37) is characterized by a tooth row formula 2(2)/3(1), labial tooth row P3 smaller than P1 and P2, marginal papillae of oral disc with wide dorsal and narrow ventral gaps. External morphological features were compared with other tadpoles of the *Phyllomedusa hypochondrialis* species group. Additionally, we provide information on egg clutches of *P. itacolomi* and *P. megacephala*.

**KEYWORDS.** Anura; Phyllomedusinae; *Phyllomedusa itacolomi*; Taxonomy; Tadpoles; Southeastern Brazil.

### INTRODUCTION

The neotropical frog genus *Phyllomedusa* Wagler, comprises 32 valid species (Frost, 2007; Giaretta *et al.*, 2007) arranged in four species groups, with some species not currently assigned to any group (Faivovich, 2005). The *Phyllomedusa hypochondrialis* species group (*sensu* Caramaschi, 2006) occurs in Cis-Andean South America in Venezuela, Guyanas, Peru, Ecuador, Brazil, Bolivia, Paraguay, and northern Argentina (Caramaschi, 2006) and is the most diversified one. Eleven species are included in the group: *P. araguari*, *P. ayeaye*, *P. azurea*, *P. centralis*, *P. hypochondrialis*, *P. itacolomi*, *P. megacephala*, *P. nordestina*, *P. oreades*, *P. palliata*, and *P. rohdei*. However, molecular data suggest that *P. palliata* is not related to any species group (Faivovich *et al.*, 2005). Brandão and Álvares (2009) suggested that *P. araguari* and *P. oreades* could be synonymized based on morphometrics, coloration pattern, and call similarities.

Herein, we describe the external morphology and internal oral features of the tadpole of *Phyllomedusa itacolomi*, from Serra de Ouro Branco, Municipality of Ouro Branco, State of Minas Gerais, Brazil. Information on egg clutches of *P. itacolomi* and *P. megacephala* is also provided.

### MATERIAL AND METHODS

*Phyllomedusa itacolomi* tadpoles at stages 25-37 (Gosner, 1960) were collected from pools formed by temporary streams, surrounded by sparse vegetation

and bushes, located in open montane meadows ("campos rupestres"), at the Serra do Ouro Branco, Municipality of Ouro Branco, State of Minas Gerais, Brazil, on February 07, 2006. Some tadpoles were reared until the end of metamorphosis to confirm species identity. Tadpoles were anesthetized with 0.1% lidocaine and then fixed in 10% formalin. Specimens used in the description are deposited at the DZSJRP Collection, Department of Zoology and Botany, Universidade Estadual Paulista, São Paulo State, Brazil (Lot DZSJRP 1260.1). External morphology descriptions were based on fourteen tadpoles at stage 37. Measurements and terminology followed Altig and McDiarmid (1999). Measurements were taken with calipers (*i.e.*, total, body and tail lengths) or under a stereomicroscope with an ocular micrometer (other measures) to the nearest 0.1 mm. We present quantitative data as mean ( $\pm$  SD; range; sample size). Three tadpoles at stages 37-38 were dissected and the internal oral structures were stained with 1% methylene blue solution for descriptions. All observations were made under a Zeiss stereomicroscope at a magnification of 1.25X. Buccopharyngeal terminology follows Wassersug (1976). Tadpole coloration is described for life and in preserved specimens. Morphological characteristics of tadpoles of the *P. hypochondrialis* group (*sensu* Caramaschi, 2006) were obtained from Lutz and Lutz (1939), Lutz (1966), Pyburn and Glidewell (1971), Cei (1980), Cruz (1982), Lescure *et al.* (1995), Brandão (2002), Giaretta *et al.* (2007), and also from comparison with material deposited at the DZSJRP Collection, Department of Zoology and Botany, Universidade Estadual Paulista, São Paulo State, Brazil (*P. megacephala* Lot DZSJRP 1345.1).

## RESULTS

Description of the tadpole of *Phyllomedusa itacolomi*

*External morphology* – Measurements for all available developmental stages are given in Table 1. Body oval in dorsal and lateral views (Fig. 1A and 1B). Total length 2.5-3.0X body length; body 1.5-2.0X longer than wide and 1.5-2.0X longer than high. Snout sloped in lateral view and rounded in dorsal view. Nostrils oval and positioned closer to tip of snout than to eyes. Eyes laterally positioned and directed; interorbital distance 0.8-0.9X of maximum body width and 3.6-4.0X of eye diameter. Oral disc anteroventral (Fig. 1C), its width about 0.4X the maximum body width; a single row of alternate marginal papillae on upper lip with a wide dorsal gap; two rows of marginal papillae on lower lip and lateral portions, with a narrow ventral gap. A few scattered submarginal papillae are present on the ventrolateral portions. Tooth

row formula (TRF) 2(2)/3(1); A1 and A2 of the same length; P3 smaller than P1 and P2. Jaw sheaths darkly pigmented and finely serrated on the margins; upper jaw sheath “M” shaped and lower sheath “V” shaped.

Spiracle ventral, sinistral, short and wide; directed posteriorly and opening on the middle third of the body; inner wall absent. Vent tube dextral, associated with the ventral fin, short and narrow, with opening directed ventrally. Tail length about 0.5-0.7X the total length; tail musculature well developed; dorsal fin emerging on the posterior third of the body, originating anteriorly to the body-tail junction, at a median slope; ventral fin beginning anterior to the vent tube; ventral fin depth approximately 1.2-1.5X the depth of the dorsal fin.

*Color in life* – Body and tail grayish-yellow with darker gray dots scattered on dorsal and lateral surfaces of body and on tail musculature; in lateral view

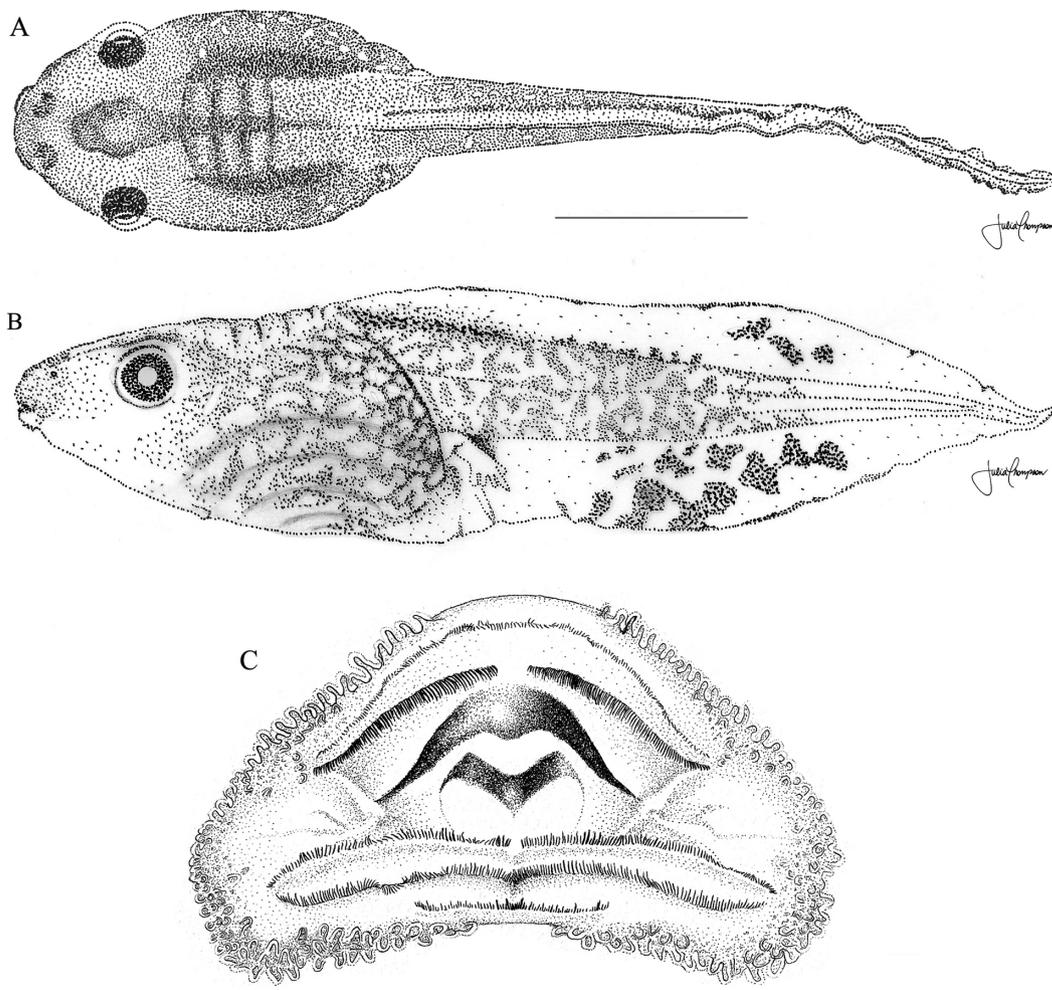


FIGURE 1. Tadpole of *Phyllomedusa itacolomi* (DZSJRP 1260.1-A) at stage 37 of Gosner (1960): (A) Dorsal view; (B) lateral view (scale = 10 mm); (C) oral disc (scale = 1 mm).



pustulations extensively distributed throughout the buccal roof arena; glandular zone conspicuous; dorsal velum longer laterally than medially, with pustulated margins, and interrupted at midline.

*Natural history notes* – In the Quadrilátero Ferrífero region (southernmost portion of Espinhaço Range Mountain Chain) of Minas Gerais State, Brazil, *P. itacolomi* tadpoles were observed exclusively in rain-filled pools created by temporary streams, surrounded by sparse vegetation and bushes, in open montane meadows known as “campo rupestre” or “campo limpo” (Rizzini, 1979). *Phyllomedusa itacolomi* tadpoles were found from December to April. A deep filled pond was found in July (middle of the dry-cold season) holding many tadpoles at various development stages, from stage 25 to newly metamorphosed froglets. This suggests that, although the reproductive activity takes place mostly during the rainy months, it may also occur in dryer months as long as pools are available.

*Phyllomedusa itacolomi* tadpoles were observed at mid water, floating with quick and short movements of their tail tips. They remained with their bodies inclined upwards, at an angle of approximately 45° with the water surface. When disturbed they promptly fled to deeper regions. They did not aggregate or school. In deeper (ca. 4-5 m<sup>3</sup>) temporary stream pools they were found in great abundance (more than a hundred).

*Egg clutches* – Adults of *Phyllomedusa itacolomi* (Fig. 3A) deposit egg clutches within folded, single, leaves on the adaxial surface of shrubs at about

50-150 cm above water on temporary streams. Arbooreal clutches of *P. itacolomi* (Fig. 3D) collected at the municipality of Congonhas, State of Minas Gerais, from January to March had clear, non pigmented eggs and eggless capsules. The mean number of eggs per clutch was 31.3 ( $\pm$  7.4; 21-45; n = 15). Egg clutches of *P. megacephala* collected on December 2004, at the type locality, (municipality of Jabuticatubas, actually municipality of Santana do Riacho, State of Minas Gerais) were also found on folded leaves of shrubs above the water level of temporary streams, and showed a similar aspect to those of *P. itacolomi*. The mean number of *P. megacephala* eggs per clutch was 22.43 ( $\pm$  4.65; 18-30; n = 7).

#### DISCUSSION

*Species comparisons* – The tadpole of *Phyllomedusa itacolomi* differs from those of *P. ayeaye* (Lutz, 1966; Cruz, 1982), *P. azurea* (Ceil, 1980), *P. nordestina* (described as *P. hypochondrialis* Cruz, 1982), and *P. oreades* (Brandão, 2002), by the presence of a narrow gap on the posterior row of marginal papillae (Table 2). Giaretta *et al.* (2007) stated that this character could vary ontogenetically in *P. araguari* and *P. ayeaye* tadpoles. Thus, the presence/absence of a narrow gap on the posterior row of marginal papillae seems not to be a reliable diagnostic character to separate *P. ayeaye* from *P. itacolomi*, which are quite similar in external larval morphology. The tadpole of *Phyllomedusa itacolomi* also differs from those of *P. oreades* by the absence of darker dots scattered on the muscular portion of the tail, forming discrete

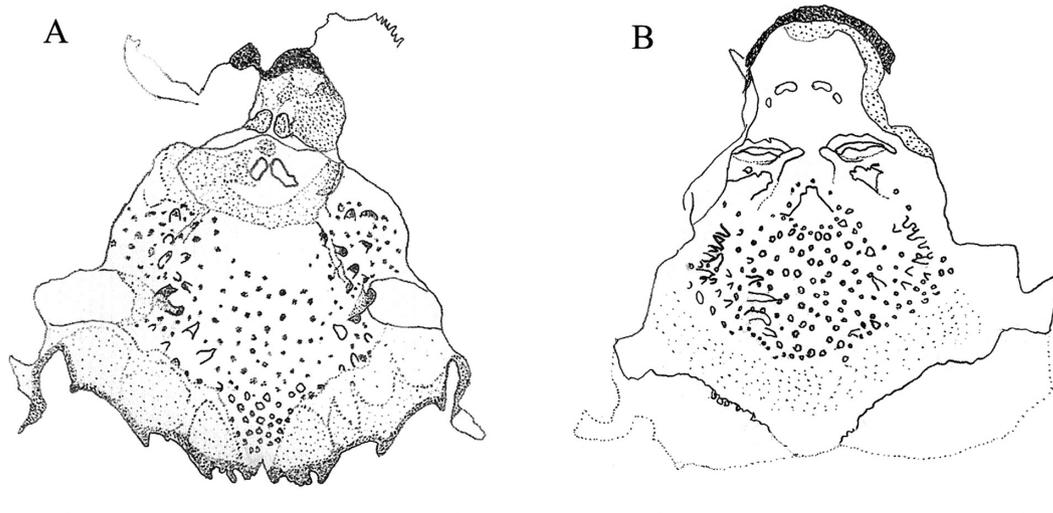


FIGURE 2. Tadpole of *Phyllomedusa itacolomi*: (A) floor and (B) roof of buccopharyngeal cavity (scale = 1 mm).

transverse bars. It differs from those of *P. azurea*, *P. nordestina*, and *P. rodhei* by the shape of the upper jaw sheath (“arc-shaped” in *P. azurea*, *P. nordestina*, and *P. rodhei* and “M-shaped” in *P. itacolomi*) and

from those of *P. azurea* by the TRF (Table 2). The tadpole of *P. itacolomi* differs from those of *P. rohdei* and from those of *P. araguari* by the origin of the dorsal fin, which originates at the body-tail junction

TABLE 2. Morphological characters and habitat occurrence of the tadpoles of *Phyllomedusa hypochondrialis* species group.

	TL mm (gosner's stage)	Tooth row formula	Gap on the posterior row of marginal papillae	Shape of the upper jaw sheath	Occurrence (Temporary stream or pound)
<i>P. araguari</i>	45,7(33)	2(2)/3(1)	Absent/present <sup>c</sup>	M <sup>d</sup>	Temporary stream/Pound
<i>P. ayeaye</i>	33,0(31)	2(2)/3 <sup>a</sup> ; 2(2)/3(1) <sup>b</sup>	Absent/present <sup>c</sup>	M	Temporary stream/Pound
<i>P. azurea</i>	—	2(2)/2(1)	Absent	Arch	Pound
<i>P. hypochondrialis</i>	—	2(2)/3(1)	Present	M	Pound
<i>P. itacolomi</i>	44,3(37)	2(2)/3(1)	Present	M	Temporary stream
<i>P. megacephala</i>	36,0(30)	2(2)/3(1)	Present	M	Temporary stream
<i>P. nordestina</i>	46,0(37)	2(2)/3(1)	Absent	Arch	Pound
<i>P. oreades</i>	46,3(37)	2(2)/3 <sup>c</sup> ; 2(2)/3(1) <sup>f</sup>	Absent	M	Temporary stream
<i>P. rohdei</i>	43,0(36)	2(2)/3(1)	Present	Arch	Pound

a = TRF presented by Lutz (1966); b = TRF presented by Cruz (1982); c = Ontogenetic variation (Giaretta *et al.*, 2007); d = Giaretta *et al.* (2007) presented the upper jaw sheath as arc-shaped with a medial point; e = Brandão (2002); f = R. A. Brandão and G. Álvares (pers. comm.).

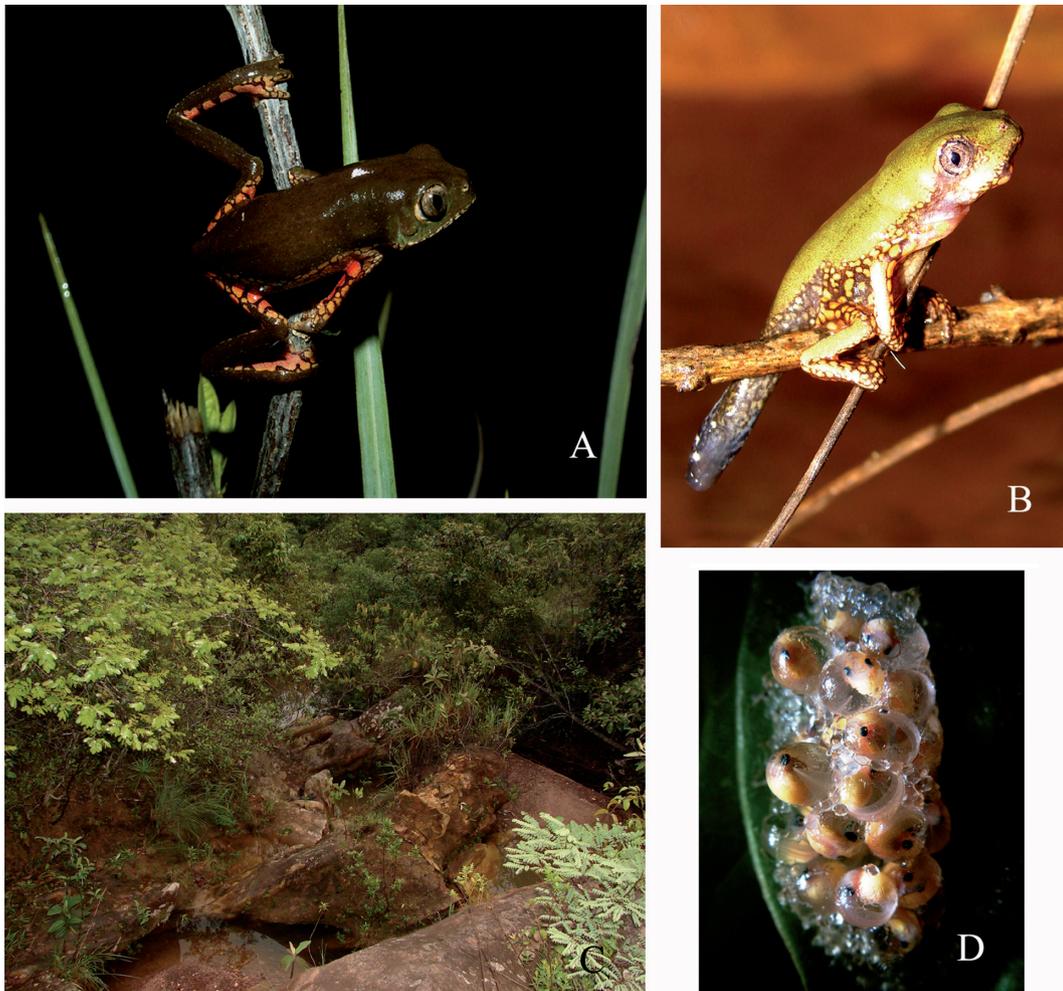


FIGURE 3. (A) *Phyllomedusa itacolomi* in life, male adult specimen photographed in calling activity (unvouchered photo); (B) froglet of *P. itacolomi* (unvouchered photo); (C) reproductive habitat of *P. itacolomi*; (D) open clutch of *P. itacolomi* (unvouchered photo). All photos are from the Municipality of Congonhas, State of Minas Gerais, Brazil.

in *P. rohdei* and in *P. araguari* and anteriorly to the body-tail junction in *P. itacolomi* (Lutz and Lutz, 1939; Cruz, 1982; Giarretta *et al.*, 2007). It also differs from those of *P. azurea*, *P. nordestina*, and *P. rohdei* by the dorsal fin height (lower in *P. azurea*, *P. nordestina*, and *P. rohdei* and higher in *P. itacolomi*). *Phyllomedusa itacolomi* and *P. megacephala* (as *P. centralis*, Cruz, 1982), are quite similar in larval external morphology characters, but differ by the origin of the dorsal fin, (at the body-tail junction in *P. itacolomi*, and posterior to the body-tail junction in *P. megacephala*), and by the life coloration of the dorsal peritoneum (dark-blue in *P. itacolomi* and black in *P. megacephala*). The tadpole of *P. itacolomi* could not be distinguished from those of *P. hypochondrialis*. Pyburn and Glidewell (1971), and Lescure *et al.* (1995) described the oral disc and dorsal pattern of coloration of *P. hypochondrialis* tadpoles. These characteristics *per se* are not enough to distinguish the larvae of these two species.

*Internal oral features* – The description of the internal oral morphology was available for only three other *Phyllomedusa* species: *Phyllomedusa* aff. *rohdei* (Dias, 2007), from Mogi das Cruzes, Brazil, *Phyllomedusa azurea* (treated as *P. hypochondrialis*), and *Phyllomedusa sauvagii* (Candioti, 2007), from Formosa and Salta, Argentina, respectively. The oral internal features of these tadpoles differ mainly in the distribution and quantity of papillae. For example, the buccal roof arena is delimited by papillae only in *P. itacolomi* and *P. sauvagii*, whereas in *P. azurea* and *P. aff. rohdei* the papillae are distributed only on the central region of the buccal roof arena (Candioti, 2007; Dias, 2007; this work). In *P. sauvagii* six papillae delimited the buccal roof arena (Candioti, 2007), whereas we found up to eight papillae in *P. itacolomi*. The pre-narial arena of the buccal roof in *Phyllomedusa* species has a medially interrupted ridge, which could be low (as in *P. rohdei* Dias, 2007) or well developed (as in *P. azurea* and *P. sauvagii* Candioti, 2007). In *P. itacolomi*, this character is variable as an uninterrupted low semicircular ridge or consisting of four pustulations rather than a ridge. Also the median ridge of *P. itacolomi* is distinct from those of other phyllomedusines: in *P. itacolomi*, it is triangular-shaped, whereas for *P. azurea* and *P. aff. rohdei* it is semicircular (Candioti, 2007; Dias, 2007). It is also triangular in *P. sauvagii* (Candioti, 2007), however it is lower than that of *P. itacolomi*. On the buccal floor, only *P. itacolomi* has infralabial papillae with no projections, whereas they are bifurcated in *P. azurea* and

are more segmented in *P. aff. rohdei* and *P. sauvagii* (Candioti, 2007; Dias, 2007). Two lingual papillae are present in phyllomedusine tadpoles, but they can be conical and tall (as in *P. aff. rohdei*), conical and short (*P. azurea*), bifurcated and tall (*P. sauvagii*), or pustulate and tall (*P. itacolomi* Candioti, 2007; Dias, 2007; this work). *Phyllomedusa itacolomi* also has the highest number of papillae on the buccal floor arena, up to 15 papillae on each side, when compared with other phyllomedusine species: two pairs of papillae in *P. azurea*, six in *P. sauvagii*, and six to seven in *P. aff. rohdei* (Candioti, 2007; Dias, 2007).

*Natural history* – The tadpoles of *P. itacolomi* also differ from those of *P. azurea*, *P. hypochondrialis*, *P. nordestina*, and *P. rohdei* by their occurrence in temporary streams (tadpoles of *P. azurea*, *P. hypochondrialis*, *P. nordestina*, and *P. rohdei* develop in lentic ponds) (Table 2). *Phyllomedusa itacolomi* egg clutches differ from those of *P. araguari* and *P. megacephala* by the larger number of eggs [31.3 ( $\pm$  7.4; 21-45; n = 15) in *P. itacolomi*; 24 in *P. araguari* (Giarretta *et al.*, 2007); 22.43 ( $\pm$  4.65; 18-30; n = 7) in *P. megacephala*]. Otherwise, *P. itacolomi* egg clutches have less eggs than those of *P. hypochondrialis* and *P. rohdei* that have been reported to range between 44-110 and 44-80 eggs, respectively (Pyburn and Glidewell, 1971; Lutz and Lutz, 1939). Despite the limited available information, it is possible that the species in the *P. hypochondrialis* group which are characterized by a reticulated pattern on the hidden parts of the flanks and limbs, a characteristic distribution on mountain ranges, and reproduction occurring in streams and rivulets (*i.e.*, *P. ayeaye*, *P. araguari*, *P. centralis*, *P. itacolomi*, *P. megacephala*, and *P. oreades*) lay smaller clutches of eggs than lowland species with striped pattern on the hidden parts of the flanks and limbs (*i.e.*, *P. azurea*, *P. hypochondrialis*, *P. nordestina*, *P. palliata*, and *P. rohdei*).

Caramaschi (2006) redefined the *P. hypochondrialis* species group based on osteology, adult and larval external characters. The larval characters common for this group were: anterior non-umbelliform oral disc, surrounded by a row of marginal papillae interrupted by a wide dorsal gap and sometimes by a narrow ventral gap; vent tube short, and fused with the ventral fin; TRF 2(2)/3(1), with P3 smaller than P1 and P2. However, *P. azurea* presents TRF 2(2)/2(1) (Cei 1980), which increases the variation of TRF in this species group. Lutz (1966) described the tadpoles of *P. ayeaye* with a TRF 2(2)/3, but Cruz

(1982), based on the same specimens, found a TRF 2(2)/3(1) (Table 2). Also, the tadpoles of *P. oreades* were described as having a TRF 2(2)/3 (Brandão, 2002), but topotypes have a distinct interruption on P1 (R. A. Brandão and G. Álvares, pers. comm.). These differences could represent ontogenic or inter-population variation.

Adults of *P. itacolomi* and *P. ayeaye* are not easy to diagnose (Caramaschi *et al.*, 2007) and their tadpoles show quite similar external larval morphology. Brandão and Álvares (2009) noted the importance of assessing population variation of described species to avoid describing such variation as new species. These authors recommend an integrative taxonomic study, including statistical comparisons of morphometry, bioacoustics, and molecular differentiation, to clarify the taxonomic status of similar species in the *P. hypochondrialis* species group.

#### RESUMO

No presente artigo são descritos a morfologia externa e as características orais internas do girino de *Phyllomedusa itacolomi* (Anura, Hylidae) da Serra de Ouro Branco, Município de Ouro Branco, Estado de Minas Gerais, Brasil. Os girinos foram encontrados à meia-água de uma poça de riacho temporário, em área de campo rupestre. *Phyllomedusa itacolomi* (Estágio 37 de Gosner) é caracterizada por apresentar fórmula oral 2(2)/3(1), fileira de denticulos P3 menor que P1 e P2, papilas marginais do disco oral com ampla interrupção dorsal e pequena interrupção ventral. As características morfológicas externas de *P. itacolomi* foram comparadas às dos girinos pertencentes ao grupo de espécies de *Phyllomedusa hypochondrialis*. Adicionalmente, são disponibilizadas informações sobre as desovas de *P. itacolomi* e *P. megacephala*.

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