

**Larvae of *Proceratophrys melanopogon* (Amphibia: Anura),  
with Emphasis on Internal Oral Morphology and Comparisons  
with *P. Cururu* and *P. Moratoi***

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## LARVAE OF *PROCERATOPHRYS MELANOPOGON* (AMPHIBIA: ANURA), WITH EMPHASIS ON INTERNAL ORAL MORPHOLOGY AND COMPARISONS WITH *P. CURURU* AND *P. MORATOI*

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**ABSTRACT:** We provide novel data on the biology of *Proceratophrys melanopogon* and describe its larval external morphology and internal oral features on the basis of specimens from the Serra do Mar, southeastern Brazil. We also review the larval internal oral features of the genus, including descriptions and comparisons with *P. cururu* and *P. moratoi*. The external morphology of tadpoles of *P. melanopogon* is very similar to that of closely related species. The internal oral features are very conserved in this genus, with the majority of species having lingual papillae unusually bifurcated, four infralabial papillae, and a transverse ridge on the prenarial arena. Our results also provide data for phylogenetic studies on the genus *Proceratophrys*.

**Key words:** Atlantic rain forest; Larval morphology; natural history

RECENT molecular phylogenies (e.g., Pyron and Wiens, 2011) have challenged the traditional classification within Hyloidea (Frost et al., 2006). For example, several families were not recovered as monophyletic, such as Ceratophryidae and Cycloramphidae (Pyron and Wiens, 2011). Furthermore, the genera *Macrogenioglottus*, *Odontophrynus*, and *Proceratophrys*, corresponding to the former tribe Odontophrynini (Lynch, 1971), appeared as the sister group of Ceratophryidae, which is now restricted to the former Ceratophryinae subfamily (Frost et al., 2006; Frost, 2013).

The genus *Proceratophrys* presently comprises 29 valid species (AmphibiaWeb, 2013) distributed from western, eastern, and southern Brazil, into northeastern Argentina and Paraguay (Giaretta et al., 2000; Frost, 2013). Traditionally, species within the genus have been classified into three phenetic groups (Giaretta et al., 2000; Prado and Pombal, 2008). However, these groups seem to be nonmonophyletic (Amaro et al., 2009; Pyron

and Wiens, 2011), but further phylogenetic studies with an improved taxon sampling are needed.

*Proceratophrys melanopogon* is a poorly known species occurring along the Atlantic rain forest in southeastern Brazil (Frost, 2013), to which only limited information is available for natural history (Heyer et al., 1990) and osteology (Izecksohn et al., 2005). *Proceratophrys melanopogon* is the sister species of *P. appendiculata* (Amaro et al., 2009; Pyron and Wiens, 2011). In fact, it was until recently considered a junior synonym of *P. appendiculata* (Izecksohn and Peixoto, 1981), but its status was revalidated by Heyer et al. (1990).

Several tadpoles of *Proceratophrys* species have been described (Provete et al., 2012). They are usually benthic, and occupy lotic to lentic habitats (Altig and McDiarmid, 1999b). However, little information is available on larval internal oral features of the genus *Proceratophrys* (Wassersug and Heyer, 1988; De Sá and Langone, 2002; Vieira et al., 2007; Nascimento et al., 2010). Here, we provide novel data on the biology of *P. melanopogon* and describe its larval external morphology

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and internal oral features on the basis of specimens from the highlands of the Serra da Bocaina, São Paulo, southeastern Brazil. We also provide a review of larval internal oral features of the genus, including detailed descriptions for *P. cururu* and *P. moratoi*.

#### MATERIAL AND METHODS

We carried out fieldwork in the Serra da Bocaina National Park, São José do Barreiro, São Paulo, southeastern Brazil (22°43'33.3"S, 44°37'15.6"W; 1500 m above sea level [asl]; datum = South American datum 1969) monthly from April 2008–July 2009. We collected tadpoles by dipnetting along the bottom of narrow, slowly flowing streams and ponds inside primary forests. We anesthetized tadpoles with 5% benzocaine, then fixed and preserved them in 10% formalin in the field. We provide brief but novel data on the biology of *P. melanopogon* on the basis of the fieldwork, and then describe the larval morphology in detail.

Our description of external morphology is based on 10 tadpoles between Stages 31 and 38 (Gosner, 1960) collected in the field. We followed Altig and McDiarmid (1999a) for tadpole measurements, with minor modifications, except for internarial and interocular distance, which were taken from the internal margins of those structures. We took the following measurements: total length (TOL); body length (BL); tail length (TL); body height; tail height; dorsal fin height; ventral fin height; tail musculature width; eye diameter; interorbital distance; nare diameter; internarial distance; oral disc diameter; anterior jaw sheath width (AJSW); anterior jaw sheath height (AJSH); eye–snout distance; nare–snout distance; body width (BW); tail musculature height; vent tube length; spiracle length; spiracle width; spiracle opening width. One tadpole at Stage 33 had its oral disc damaged, which prevented us from taking AJSW and AJSH measurements. We took the measurements using a stereomicroscope (Leica MZ715) coupled with an ocular grid, except for BL, TOL, and TL, which we measured with digital calipers to the nearest 0.01 mm. We assessed larval diet in three tadpoles of *P. melanopogon* following Rossa-Feres et al. (2004).

For the description of internal oral features, we dissected three tadpoles of *P. melanopogon* at Stages 36 and 37. We also describe the internal oral features of two additional species: one specimen of *P. cururu* at Stage 38 from Serra do Cipó, Minas Gerais, and three specimens of *P. moratoi* at Stages 36, 38, and 40, from Botucatu, São Paulo, Brazil; unfortunately, larvae of other species were not available for dissection. We analyzed the buccopharyngeal morphology of tadpoles using a scanning electron microscope (SEM; model Jeol JSM-6610) following Conte et al. (2007), replacing acetone with 95% ethanol. Terminology for those structures follows Wassersug (1976). Additional SEM pictures are available at MorphoBank (<http://morphobank.org/permalink/?P778>). Specimens used in descriptions are housed at the tadpole collection of the Departamento de Zoologia e Botânica (DZSJRP) of the Universidade Estadual Paulista, Brazil (see Appendix).

#### RESULTS

*Proceratophrys melanopogon* occurs in areas of Atlantic forest in southeastern Brazil at elevations ranging from 800–1480 m asl. It is an explosive breeder (sensu Wells, 1977), with calling males forming agglomerations with up to 30 individuals at night, after heavy rains. Males call from small concavities on stream banks, during the rainy season, between October and April. Calling activity begins usually at 1800 h and ends by midnight. Adults and tadpoles occur only in slow-flowing, forested streams. Tadpoles of *P. melanopogon* inhabit narrow (60–80-cm wide) temporary and permanent streams, between October and February, specifically in stream pools with clay, coarse or fine sand, and fine gravel on the bottom. Other tadpoles that commonly co-occurred with *P. melanopogon* were those of *Aplastodiscus arildae*, *A. albosignatus*, *Bokermannohyla ahenea*, *B. circumdata*, *Hylodes* sp., *Hypsiboas* sp. (aff. *polytaenius*), and *Scinax* sp. (aff. *obtriangulatus*). Potential predators that co-occurred with tadpoles of *P. melanopogon* were odonate larvae of the genera *Dythemis* sp., *Cacoides* sp., *Neocordulia* sp., *Orthemis* sp., and *Dasythemis* sp. Tadpoles of *P. melanopogon*

usually occurred at a density of approximately 0.86 individuals/m<sup>2</sup>. Common items in tadpoles' diet included diatoms, coarse particulate organic matter, and algae of the genera *Closterium*, *Microspora*, and *Trachelomonas*.

#### *External Morphology*

*Proceratophrys melanopogon* has an Orton Type IV, exotroph, lotic, and benthic tadpole (Fig. 1, Table 1). At Stage 37, the body is ovoid in dorsal view, and slightly depressed in lateral view (BL/BW = 1.47; Fig. 1A). The snout is rounded in dorsal view, slightly curved in lateral view. The eyes are dorsal and oriented dorsolaterally. The nares are oval with a small marginal rim, dorsally positioned, located closer to the eyes than the tip of the snout, directed upward. Narial opening is located on a depression on the body wall. A single sinistral spiracle, directed posterodorsally, with inner wall present as a slight ridge. The vent tube is short, dextral, with right wall displaced dorsally. Lateral line system is indistinct. The tail is short (TL/TOL = 0.53). The dorsal fin originates at the body-tail junction and is widely arched and higher than the ventral fin. The maximum height of the dorsal fin is at the middle third of tail. Ventral fin arched. The tail musculature is high, not reaching tail tip, which is rounded.

Externally, the oral disc is ventral, and laterally emarginated (Fig. 1C). The marginal papillae are conical, disposed in a single row laterally, alternated posteriorly, with a wide anterior gap, which is slightly shorter than A-1. Papillae without pigmentation, about 15 papillae per linear millimeter. The number and arrangement of submarginal papillae vary, see section below. Labial tooth row formula (LTRF) 2(2)/3(1), A-2 slightly longer than A-1; P-1 larger than P-2 and P-3, P-2 larger than P-3. The labial teeth are keratinized and arranged in single rows, one per tooth ridge. The tooth ridge is straight, slightly curved on the outer edges. There are about 48 labial teeth/mm (estimated on P-1,  $n = 3$ ), curved toward the oral opening. Anterior jaw sheath widely arched, posterior jaw sheath V-shaped and curved inward, both slightly keratinized and finely serrated, with 33 serrations/mm, serrations conical.

#### *Coloration*

In formalin, the body wall is transparent in dorsal view, with small, scattered light-gray melanophores along the body. Broad transverse stripes on the caudal musculature in dorsal view. Tail musculature is cream. Fins and caudal musculature are transparent with a few small blotches. The frequency of spots increases in the middle of tail. The iris is black and pupil is white.

#### *Intraspecific Variation*

Submarginal papillae on the lower labium vary in number from zero to eight. One specimen had a row with 14 submarginal papillae on the right side of the oral disc. Submarginal papillae also vary in number and position laterally on both anterior and posterior labia: specimens may have none or up to six papillae on upper labium and three on lower labium. Additionally, about 70% of tadpoles analyzed had some oral deformity, such as a fragmented labial tooth row, papillae unusually high, undulated labial teeth rows, teeth in nonnormal hillocks of ridge tissue, and scattered submarginal papillae. Furthermore, nares are slightly reniform at Stage 31.

#### *Internal Oral Features of P. melanopogon*

The buccal floor is triangular, wider than long (Fig. 2A). Two pairs of fingerlike infralabial papillae, one smaller anteromedial pair projecting toward the jaw sheath, one posterolateral curved to the midline, with small pustulations all along the axis of the papillae. Its length about 10% of the buccal floor width (BFW). All pairs with secondary projections. Anterior portion of buccal floor arena (BFA) bearing a large tongue anlage. Five tall lingual papillae, with blunt apices and small pustulations scattered along its length. In the majority of specimens, all papillae are bifurcated, but a few have no bifurcated papillae; papillae height about 15% of the BFW. BFA rounded, bearing about 50 pointed papillae, the largest ones anteriorly positioned, all projecting toward the BFA. Four tall prepocket papillae oriented toward the BFA. About 180 pustulations uniformly distributed, and 5–12 short papillae posteriorly, following the ventral velum. Buccal pockets perforated, twice wider than long,

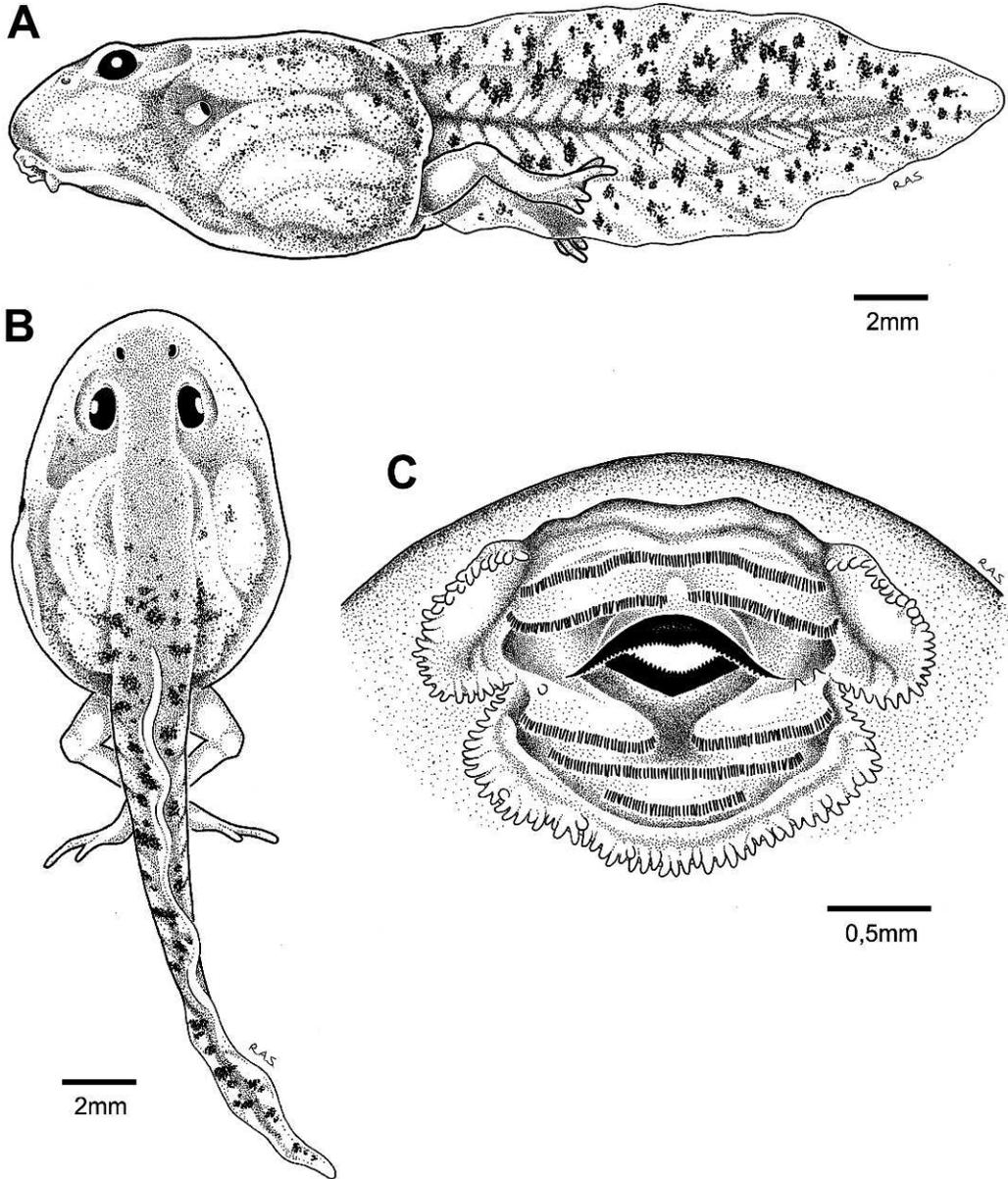


FIG. 1.—External morphology of the tadpole of *Proceratophrys melanopogon* (Departamento de Zoologia e Botânica [DZS]RP] 2398.1) at Gosner Stage 41. Changes in external morphology begin at, and especially after, Stage 41 in tadpoles of this species (D. Provete, personal observation); we illustrated this stage because the specimen had the best fixation. (A) Lateral view; (B) dorsal view; (C) oral disc.

convex, so that part of the buccal pocket papillae is transversely disposed and the remaining are longitudinally disposed. Length of the free velar surface about 60% of the BFW, its height 10% of the BFW; spicular support indistinct; posterior margin gently

scalloped, median notch with 5–10 papillae covering the glottis. Secretory pits along the edge of the free velar surface. Ventral pharynx: length of branchial baskets is 50% of the BFW, oriented about 30° from the midline. The width of the glottis is about 10%

TABLE 1.—Measurements of the tadpole of *Proceratophrys melanopogon* between Gosner Stages 31 and 41. At and especially after Stage 41, larval features begin to change. Data are in millimeters and presented as mean  $\pm$  SD. Abbreviations: total length (TOL); body length (BL); tail length (TL); body width (BW); tail musculature width (TMW); body height (BH); dorsal fin height (DFH); ventral fin height (VMH); tail musculature height (TMH); interorbital distance (IOD); internarial distance (IND); eye–snout distance (ESD); nare–snout distance (NSD); eye diameter (ED); nare diameter (ND); vent tube length (VTL); spiracle length (SL); spiracle width (SW); spiracle opening width (SOW); oral disc diameter (OD); anterior jaw sheath height (AJSH); anterior jaw sheath width (AJSW).

Larval features	Stage (n)					
	31 (2)	33 (2)	34 (2)	37 (2)	38 (2)	41 (2)
TOL	23.34 $\pm$ 2.72	27.75 $\pm$ 4.49	27.63 $\pm$ 1.51	27.79 $\pm$ 2.47	28.99 $\pm$ 0.47	29.24 $\pm$ 1.51
BL	11.48 $\pm$ 1.92	12.2 $\pm$ 3.45	12.71 $\pm$ 1.20	13.06 $\pm$ 0.41	13.37 $\pm$ 0.91	12.88 $\pm$ 0.11
TL	11.87 $\pm$ 0.8	15.55 $\pm$ 1.04	14.92 $\pm$ 0.31	14.73 $\pm$ 2.06	15.62 $\pm$ 0.44	16.36 $\pm$ 1.4
BW	7.84 $\pm$ 1.64	8.58 $\pm$ 3.37	8.91 $\pm$ 1.98	8.84 $\pm$ 0.83	10.14 $\pm$ 1.66	9.2 $\pm$ 0.11
TMW	1.95 $\pm$ 0.07	1.95 $\pm$ 0.64	2.3 $\pm$ 0.14	2.65 $\pm$ 0.07	2.55 $\pm$ 0.07	2.65 $\pm$ 0.07
BH	5.5 $\pm$ 0.57	7.15 $\pm$ 7.15	6.8 $\pm$ 1.41	7.2 $\pm$ 0.28	7.7 $\pm$ 1.13	6.64 $\pm$ 0.34
DFH	2.35 $\pm$ 0.07	3.05 $\pm$ 1.48	2.9 $\pm$ 1.13	2.5 $\pm$ 0	3.1 $\pm$ 0.71	2.85 $\pm$ 0.49
VFH	1.8 $\pm$ 0	2 $\pm$ 0.42	2.1 $\pm$ 0.57	2 $\pm$ 0.28	2.35 $\pm$ 0.64	1.8 $\pm$ 0.28
TMH	2.25 $\pm$ 0.07	2.65 $\pm$ 0.64	2.55 $\pm$ 0.35	2.85 $\pm$ 0.35	3 $\pm$ 0	3.05 $\pm$ 0.07
IOD	1.84 $\pm$ 0.04	2.09 $\pm$ 0.58	2.19 $\pm$ 0.45	1.91 $\pm$ 0.13	2.19 $\pm$ 0.26	1.93 $\pm$ 0.06
IND	1.39 $\pm$ 0.03	1.57 $\pm$ 0.16	1.66 $\pm$ 0.08	1.6 $\pm$ 0	1.66 $\pm$ 0.14	1.53 $\pm$ 0.04
ESD	2.47 $\pm$ 0.05	2.84 $\pm$ 0.13	2.93 $\pm$ 0	2.81 $\pm$ 0.08	2.90 $\pm$ 0.04	3.12 $\pm$ 0.11
NSD	1.28 $\pm$ 0.13	1.15 $\pm$ 0.04	1.34 $\pm$ 0.4	1.5 $\pm$ 0.26	1.47 $\pm$ 0.13	1.6 $\pm$ 0.23
ED	0.94 $\pm$ 0	1.06 $\pm$ 0.11	1.13 $\pm$ 0.06	1.17 $\pm$ 0	1.17 $\pm$ 0	1.31 $\pm$ 0
ND	0.35 $\pm$ 0	0.43 $\pm$ 0.11	0.45 $\pm$ 0.08	0.43 $\pm$ 0.06	0.43 $\pm$ 0	0.39 $\pm$ 0
VTL	1.57 $\pm$ 0.22	1.31 $\pm$ 0.58	1.16 $\pm$ 0.25	1.84 $\pm$ 0.06	1.69 $\pm$ 0.28	1.7 $\pm$ 0.06
SL	1.15 $\pm$ 0.52	1.06 $\pm$ 0.39	1.53 $\pm$ 0.11	1.25 $\pm$ 0.61	1.65 $\pm$ 0.33	1.91 $\pm$ 0.4
SW	0.92 $\pm$ 0.08	1.2 $\pm$ 0.3	1.14 $\pm$ 0.22	1.08 $\pm$ 0.13	1.27 $\pm$ 0.25	1.06 $\pm$ 0.09
SOW	0.43 $\pm$ 0.06	0.49 $\pm$ 0.02	0.51 $\pm$ 0.16	0.62 $\pm$ 0	0.56 $\pm$ 0.08	0.58 $\pm$ 0.04
OD	2.4 $\pm$ 0.14	2.7 $\pm$ 0	2.95 $\pm$ 0.35	2.7 $\pm$ 0.28	2.75 $\pm$ 0.07	2.78 $\pm$ 0.13
AJSH	0.24 $\pm$ 0	0.15 $\pm$ 0	0.24 $\pm$ 0.14	0.24 $\pm$ 0	0.27 $\pm$ 0.10	0.17 $\pm$ 0.01
AJW	2.32 $\pm$ 0.10	2.20 $\pm$ 0	2.51 $\pm$ 0.03	2.54 $\pm$ 0.34	2.61 $\pm$ 0.24	1.22 $\pm$ 0.03

of the BFW, lips broad, almost occluded. Esophageal tunnel broad, about 20–30% of the BFW. Lung buds small, uninflated.

The buccal roof (Fig. 2B) is trapezoidal, slightly longer than wide; nares about 20% distance from front of the mouth to esophagus, median ridge about 35% distance from front of the mouth to esophagus. Prenarial arena large with one medial large pustulation and a short lateral one. The width of the internal nares is about 30% of the buccal roof width (BRW), nearly perpendicular. Anterior wall very thick, its height 4% of the BRW, prenarial papillae absent. The height of the posterior wall varies intraspecifically, but commonly it is higher than the anterior wall, its width three times its height. About 10–12 conical postnarial papillae, with secondary projections, forming a diagonal line. Median ridge very depressed, trapezoidal, with three bifurcated papillae. Two large, handlike lateral ridge papillae, with secondary projections on their margins. Buccal roof arena (BRA)

squarelike with 14–30 papillae, none bifurcated, anterior ones are the largest. About 100–200 pustulations forming a heart-shaped arrangement. Glandular zone poorly defined. Secretory pits indistinct. The length of the dorsal velum is about 42% of the BRW, widely interrupted at the midline; dorsal velum with about 15 short papillae on each side. Pressure cushions distinct.

#### *Internal Larval Oral Features of Proceratophrys cururu*

The buccal floor is ellipsoid (Fig. 2C), slightly wider than long, broader medially. One pair of tall, multiple-branching infralabial papillae, positioned anteriorly and projecting toward the midline. Four conical lingual papillae, all bifid, at the middle of a tongue anlage; the height of the papillae represents 1% of the BFW. BFA is hexagonal with approximately 50 papillae, the largest ones medially located; one specimen had a handlike papilla medially; about 40 pustulations cover-

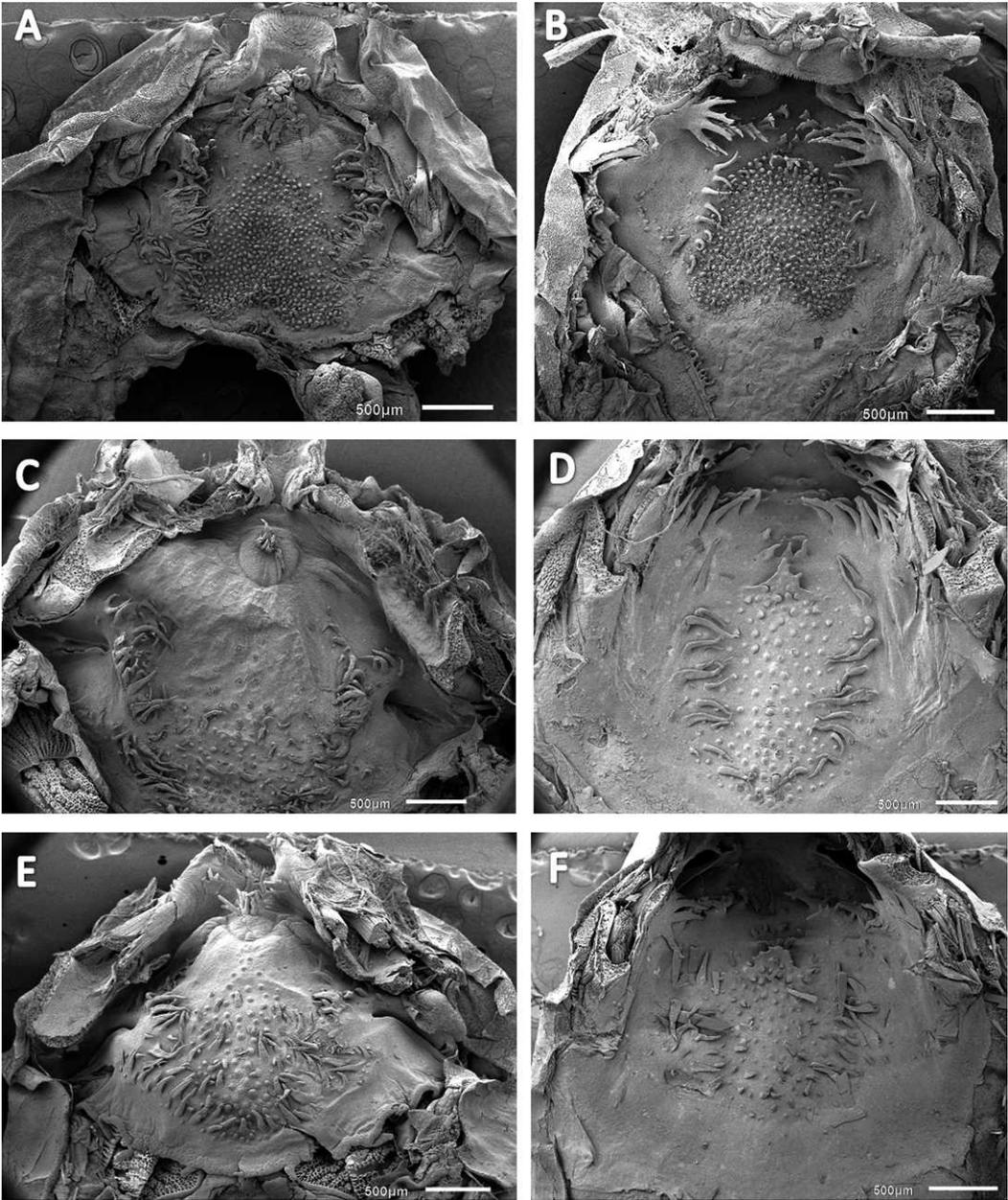


FIG. 2.—Internal oral features of *Proceratophrys* tadpoles. (A), (B) Internal oral features of tadpoles of *Proceratophrys melanopogon* at Stage 37. (C), (D) *P. cururu* at Stage 38. (E), (F) *P. moratoi* at Stage 38. Infralabial papillae, prenarial area, and medial ridge of *P. melanopogon* are best visualized from MorphoBank images.

ing the BFA, they are concentrated posteriorly; about 10 papillae lay inside the BFA; there are some small prevelar papillae around the outer margin of the BFA. There are four to five prepocket papillae; the highest are

medially located. The buccal pockets are transverse, twice wider than long, deep, perforated. The free velar surface comprises 12% of the BFW, spicular support conspicuous; posterior margin slightly curved, peaks

over the filter cavities small, without projections on the middle, median notch narrow. Secretory pits conspicuous, not very dense, covering the entire posterior margin. Ventral pharynx: branchial baskets small, triangular, 40% longer than wide, their width about 50% of the BFW, filter cavities oriented 45° from the middle plane. Glottis completely visible, 75% longer than wide, lips broad, laryngeal disc indistinct, glottis occluded; esophageal funnel narrow, 25% of the BFW. Lung buds small, uninflated.

The buccal roof is hexagonal (Fig. 2D), longer than wide, nares 20% distance from front of the mouth to esophagus, median ridge 65% distance from front of the mouth to esophagus. Prenarial arena very long and curved; three tiny papillae on a medial transverse flap, other four papillae posterolateral, two on each side. Nares wider than long, oriented 45° from the transverse plane; anterior wall very thick and long, with sharply indented margin. The narial valve is tall, twice wider than long; internarial distance 25% of the BRW. There are 12 tall and conical postnarial papillae, with pustulations along their margins, projecting toward the center of the postnarial arena. Postnarial arena bears approximately five short and conical papillae. The median ridge is triangular, with two rows of papillae anterior to the median ridge, each one with three papillae; those of anterior row are the smallest. There is one pair of fingerlike lateral ridge papillae. The BRA is elliptical and also bears about 28 papillae, arranged in a U-shape, the largest ones are anterior, without bifurcations. There are 80–90 pustulations homogeneously distributed along the BRA, beginning on the median ridge. There are 8–12 small papillae anterior to the glandular zone. Secretory pits indistinct, anterior margin of the glandular zone not well defined, its height about 17% of the BRW. Dorsal velum very long, broadly interrupted at the midline, papillated, with tall, bifurcated papillae. Pressure cushions poorly defined.

#### *Internal Larval Oral Features of Proceratophrys moratoi*

The buccal floor is triangular (Fig. 2E), slightly wider than long, oral opening about 25% of BFW. Two tall, fingerlike infralabial

papillae and two multiple-branching papillae posterolateral, the height of the most anterior ones about 33% of the oral opening width. Four conical lingual papillae, all bifid. BFA diamond shaped, surrounded by about 40–50 conical papillae of different in size. About 10 tall prepocket papillae projecting toward the BFA; those in front of the buccal pocket are bifurcated or handlike; additionally there are about 50 pustulations homogeneously distributed along the BFA, in some specimens 10–15 papillae of different size lay inside the BFA. Buccal pockets are four times longer than wide, shallow, oriented 45° to the transverse plane, perforated. Free velar surface comprising about 8% of the BFW, its posterior margin gently curved and bearing long marginal projections over filter cavities, median notch absent. Small spiculae measuring about 5% of the BFW. Secretory pits along the posterior margin of the free velar surface. Ventral pharynx: branchial baskets triangular, shallow, its width 50% of the BFW, 1.5 times longer than wide, oriented about 30° from the transverse plane, filter cavities small, with three projections over the filter plates. Ceratobranchial 3 with five filter cavities and ceratobranchial 4 with four. Glottis fully visible and occluded, longer than wide, lips uniformly broad, laryngeal disc and esophageal funnel broad.

The buccal roof is rectangular (Fig. 2F), 1.5 times longer than wide, median ridge 40% distance from front of the mouth to esophagus, nares 20% distance from front of the mouth to esophagus. Prenarial arena small, strongly curved, and with three pustulations transversely. Nares length about 25% of the BFW, internarial distance about 30% of the BFW, oriented about 30° from the transverse plane, anterior wall thin with irregular margin, with several projections; posterior wall thin, about five times wider than high. There are six tall and conical postnarial papillae, with several secondary projections. Postnarial arena with about seven tall papillae, three on each side, with pustulated margins curved toward the middle of the postnarial arena and arranged almost transversally, the shortest ones are medial. Median ridge triangular, with serrated margins; two rows of papillae anterior to the median ridge, the anterior one

TABLE 2.—Main internal oral features of *Proceratophrys* larvae. Abbreviations: buccal floor arena (BFA); buccal roof arena (BRA); infralabial papillae (IP); lingual papillae (LP); papillae/pustulation number (P/P); pre-pocket papillae number (PRP); velar margin (VM); secretory pits (SP); papillae number/features (P/F); papillae on anterior wall of nares (AWN); median ridge (MR); lateral ridge papillae (LRP); glandular zone (GZ).

	BFA				Prenatal arena				Postnatal arena				BRA			References
	IP	LP	P/P	PRP	VM	SP	P/F	AWN	P/P	MR	LRP	P/P	SP	GZ		
<i>P. boiei</i>	2	4	50	?	Papillated	?	1/medial	-	-/several	Trapezoidal/?	At least 1 hand-like	30/20-30	Large	Well defined	Wassersug and Heyer, 1988	
<i>P. melanogon</i>	4	5	27-50/130-180	4	Papillated	+	2	-	4-5/-	Trapezoidal/papillated	2 hand-like	7-15/100-200	?	Poorly defined	Present study	
<i>P. appendiculata</i>	4	4	45-60/50	16-40	Over gnotis papillated	Nar-row	2/verse ridge	3 tall and several smalls	10-11/?	Trapezoidal/papillated	2 hand-like	60-70/130	Few isolated	Poorly defined	Wassersug and Heyer, 1988	
<i>P. cristiceps</i>	4	3	28-34/?	12	Serrated	?	0	Only pustulations	4/5-8	Semicircular/papillated	2 hand-like	18-20/?	?	Well defined	Vieira et al., 2007	
<i>P. cururu</i>	2	4	40/70	4-5	Papillated	+	4/trans-verse ridge	2	5/-	Triangular/papillated	2 hand-like	34/80-90	+	Well defined	Present study	
<i>P. moratoi</i>	2	4-6	10-15/50	10	Papillated	-	3	2-3	7/-	Triangular/serrated	2 handlike	15/40-50	+	Well defined	Present study	
<i>P. avelinoi</i>	4	4	40-50/130	?	Papillated	-	-	4-5	8/-	Trapezoid/papillated	2 handlike	30/140-150	?	Poorly defined	De Sá and Langone, 2002	
<i>P. renalis</i>	2	6	26-28/>200	8-10	Papillated	+	4/trans-verse ridge	+	12/-	Trapezoidal/papillated	2 bifurcated*	24/170		Well defined	Nascimento et al., 2010	

+, present; -, absent; ?, not mentioned in the original papers; \*, handlike from the image.

TABLE 3.—Main external morphological characteristics that distinguish known tadpoles of *Proceratophrys*.

	Stage	Total length (mm)	Nostril shape	Spiracle orientation	Spiracle's inner wall	Tail tip	Posterior labium emargination	LTRF	References
<i>P. appendiculata</i>	36	33	Reniform	Posterodorsal	?	Rounded	Two folds	2/3(1)	Peixoto and Cruz, 1980
<i>P. avelinoi</i>	36	32.6	Rounded	Posterodorsal	?	Rounded	Two folds	2(2)/3(1)	De Sá and Langone, 2002
<i>P. boiei</i>	34	31	Reniform	Posterodorsal	Free	Rounded	None	2(2)/3(1)	Izecksohn et al., 1979
<i>P. concavitympanum</i>	36	50.1	Rounded	Posterodorsal	Small and free	Pointed	None	2(2)/3(1)	Giaretta et al., 2000
<i>P. cristiceps</i>	39	30.31	Rounded	Dorsal	Fused	Pointed	None	2(2)/3(1)	Vieira et al., 2007
<i>P. cururu</i>	38	39.5	Rounded	Dorsal	?	Pointed	Two folds	2(2)/3(1)	Eterovick and Szazma, 1998
<i>P. laticeps</i>	36	31	Reniform	Posterodorsal	?	Pointed	Two folds	2(2)/3(1)	Peixoto et al., 1981
<i>P. melanopogon</i>	37	27.76	Oval with internal rim	Posterodorsal	Small and free	Rounded	None	2(2)/3(1)	Present study
<i>P. moehringi</i>	29	23.6	Rounded	Posterodorsal	?	Rounded	Two folds	2(2)/3(1)	Weygoldt and Peixoto, 1985
<i>P. morato</i>	37	32	rounded	posterodorsal	fused	rounded	None	2(2)/3(1)	Rossa-Feres and Jim, 1996
<i>P. palustris</i>	37	36	rounded	posterodorsal	fused	pointed	Two folds	2(2)/3(1)	Giaretta and Szazma, 1993
<i>P. renalts</i>	36	33.8	oval with internal rim	posterodorsal	free	pointed	Two folds	2(2)/3(1)	Nascimento et al., 2010
<i>P. schirechi</i>	34	31	reniform	posterodorsal	fused	pointed	Two folds	2(2)/3(1)	Peixoto et al., 1984
<i>P. tupinamba</i>	28	22.7	rounded	posterodorsal	small and free	pointed	Two folds	2/3(1)	Fatorelli et al., 2010

with about five small papillae and the posterior row with three papillae with wide and serrated apices; short handlike lateral ridge papillae. BRA rounded and bearing about 15 papillae arranged in U-shape, the largest ones anterior; additionally there are about 40–50 pustulations homogeneously distributed along the BRA, beginning on the median ridge; about 10 small papillae lay inside the BRA. Secretory pits distinct (Table 2).

DISCUSSION

Currently, 14 species (48%) of the genus *Proceratophrys* have known tadpoles (Provette et al., 2012). All known tadpoles of *Proceratophrys* have a very conserved body shape (Rossa-Feres and Jim, 1996; Altig and McDiarmid, 1999b; Nascimento et al., 2010; this study): an oval body, low fins, a sinistral spiracle with midlateral opening, dextral vent tube, ventral and emarginated oral disc, a single row of marginal papillae with a wide dorsal gap (except for *P. concavitympanum* with double row posterolaterally and posteriorly; Giaretta et al., 2000), serrated and keratinized jaw sheaths, LTRF 2(2)/3(1) or 2/3(1), and large nares (Altig and McDiarmid, 1999a; De Sá and Langone, 2002). However, some external morphological features vary within the genus, such as nostril shape, tail tip, folds on the lower labium, and spiracle tube orientation (Table 3).

Nares are rounded in 57% of tadpoles of *Proceratophrys*, 29% have reniform nares, and 14% have elliptical nares with internal marginal rim. The spiracle is oriented posterodorsally in the majority of species (86%), and dorsally in the remaining species. Most larvae (57%) bear a rounded tail tip, and 47% have a pointed tail tip. Most species (64%) have two folds in the lower labium, and 36% do not have emarginations. The LTRF in the majority of species (86%) is 2(2)/3(1), including *P. melanopogon*, with *P. appendiculata* and *P. tupinamba* differing from all other known tadpoles by having a LTRF 2/3(1) (Peixoto and Cruz, 1980; Fatorelli et al., 2010; Table 3).

*Proceratophrys melanopogon* is the sister species of *P. appendiculata* (Pyron and Wiens, 2011). However, the larvae of these

two species are easily differentiated: *P. melanopogon* differs from *P. appendiculata* by having LTRF 2(2)/3(1), lacking emarginations on the lower labium, oval nares with marginal rim. *P. melanopogon* differ from *P. boiei* by having oval nares, inner wall of the spiracle present as a slight ridge, nares and eyes smaller than *P. boiei*. The tadpole of *P. melanopogon* can be distinguished from *P. renalis* by the tail tip rounded (pointed in *P. renalis*), and shorter spiracle's inner wall.

Other external morphological traits are useful to differentiate tadpoles of *Proceratophrys*. The spiracle is completely fused to the body wall in *P. moratoi* (Rossa-Feres and Jim, 1996), *P. palustris* (Giaretta and Sazima, 1993), and the two sister species *P. schirchi* (Peixoto et al., 1984) and *P. cristiceps* (Vieira et al., 2007); the inner wall is free in *P. melanopogon*, *P. boiei* (Izecksohn et al., 1979), *P. concavitympanum* (Giaretta et al., 2000), *P. renalis* (Nascimento et al., 2010), and *P. tupinamba* (Fatorelli et al., 2010). We examined the tadpoles used in the description of *P. cururu* (Eterovick and Sazima, 1998); its oral disc is laterally emarginated and also has two folds in the lower labium. The arrangement of submarginal papillae greatly varies interspecifically, and species of *Proceratophrys* can be divided into two groups (Vieira et al., 2007): one bearing submarginal papillae on only one labium (*P. avelinoi*, *P. concavitympanum*, *P. boiei*, *P. renalis*, and *P. schirchi*) and another with submarginal papillae on both labia (*P. cururu* [D. Provete, personal observation] and *P. cristiceps*). However, detailed information about intraspecific variation on this character may be necessary, since the number and arrangement of submarginal papillae in *P. melanopogon* vary greatly, and it cannot be assigned to any group.

The internal oral features of *Proceratophrys* larvae are similar to stream-dwelling species (Wassersug and Heyer, 1988), namely: numerous BFA/BRA papillae and pustulations, handlike lateral ridge papillae, and a papillated ventral velum. Additional common features of larvae of the genus include lingual papillae unusually bifurcated (except for *P. avelinoi*), and a transverse ridge on the prenarial arena (Wassersug and Heyer, 1988; De Sá and Langone, 2002; Vieira et al., 2007;

Nascimento et al., 2010; this study). Most species have small papillae inside the BRA (except for *P. avelinoi*); small papillae inside BFA occur in *P. cristiceps*, *P. renalis*, and *P. moratoi*. The majority of species has four infralabial papillae, except for *P. renalis* and *P. boiei*, which have two infralabial papillae (Wassersug and Heyer, 1988; Vieira et al., 2007). However, the number of lingual papillae is extremely variable: ranging from three to six papillae, but usually four (Table 2); only *P. renalis* has three pairs of bifid papillae (Vieira et al., 2007). *Proceratophrys cristiceps* is distinct from other larvae by having rows of papillae anterior to the median ridge (Vieira et al., 2007).

The tadpole of *P. melanopogon* can be diagnosed by following combination of external morphological traits (Table 3): (1) small size, (2) oval nares with marginal rim, (3) rounded tail tip, (4) inner wall of the spiracle as a slight ridge, (5) absence of emarginations in the lower labium, and (6) LTRF 2(2)/3(1). Larval characters have always played a pivotal role in anuran classification (e.g., Orton, 1953; Starrett, 1973). However, its inclusion in large-scale phylogenetic studies (e.g., Haas, 2003) is still rare. There is much variation in the external morphological characters of *Proceratophrys* tadpoles, apparently without a strong phylogenetic signal. This variation is likely due to the high plasticity of tadpoles in general. However, a comprehensive study of the phylogenetic patterns of anuran larval morphology is lacking. Therefore, our results provide valuable data for future phylogenetic and morphological studies of the genus *Proceratophrys*.

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#### LITERATURE CITED

- Altig, R., and R.W. McDiarmid. 1999a. Body plan: Development and morphology. Pp. 24–51 in R.W. McDiarmid, and R. Altig (Eds.), *Tadpoles: The Biology of Anuran Larvae*. University of Chicago Press, USA.
- Altig, R., and R.W. McDiarmid. 1999b. Diversity: Familial and generic characterizations. Pp. 295–337 in R.W. McDiarmid, and R. Altig (Eds.), *Tadpoles: The Biology of Anuran Larvae*. University of Chicago Press, USA.
- Amaro, R.C., D. Pavan, and M.T. Rodrigues. 2009. On the generic identity of *Odontophrynus moratoi* Jim and Caramaschi, 1980 (Anura, Cycloramphidae). *Zootaxa* 2071:61–68.
- AmphibiaWeb. 2013. Information on amphibian biology and conservation. University of California, Berkeley, USA. Available at <http://amphibiaweb.org>.
- Conte, C.E., F. Nomura, D.C. Rossa-Feres, A. D'Heursel, and C.F.B. Haddad. 2007. The tadpole of *Scinax catharinae* (Anura: Hylidae) with description of the internal oral morphology, and a review of the tadpoles from the *Scinax catharinae* group. *Ambibia–Reptilia* 28:177–192.
- De Sá, R.O., and J.A. Langone. 2002. The tadpole of *Proceratophrys avelinoi* (Anura: Leptodactylidae). *Journal of Herpetology* 36:490–494.
- Eterovick, P.C., and I. Sazima. 1998. A new *Proceratophrys* (Anura: Leptodactylidae) from southeastern Brazil. *Copeia* 1998:159–164.
- Fatorelli, P., P.N. Costa, R.C. Laia, and M. Almeida-Santos. 2010. Description, microhabitat and temporal distribution of the tadpole of *Proceratophrys tupinamba* Prado and Pombal, 2008. *Zootaxa* 2684:57–62.
- Frost, D.R. 2013. Amphibian species of the world: an online reference. Version 5.6 (9 January 2013). Available at [http://research.amnh.org/herpetology/amphibia/American\\_Museum\\_of\\_Natural\\_History\\_USA](http://research.amnh.org/herpetology/amphibia/American_Museum_of_Natural_History_USA).
- Frost, D.R., T. Grant, J. Faivovich, R.H. Bain, A. Haas, C.F.B. Haddad, R.O. De Sá, A. Channing, M. Wilkinson, S.C. Donnellan, C.J. Raxworthy, J.A. Campbell, B.L. Blotto, P. Moler, R.C. Drewes, R.A. Nussbaum, J.D. Lynch, D.M. Green, and W.C. Wheeler. 2006. The amphibian tree of life. *Bulletin of the American Museum of Natural History* 297:1–370.
- Giaretta, A.A., and I. Sazima. 1993. Nova espécie de *Proceratophrys* Mir. Rib. do sul de Minas Gerais, Brasil (Amphibia, Anura, Leptodactylidae). *Brazilian Journal of Biology* 53:13–19.
- Giaretta, A.A., P.S. Bernarde, and M.N.C. Kokubum. 2000. A new species of *Proceratophrys* (Anura: Leptodactylidae) from the Amazon Rain Forest. *Journal of Herpetology* 34:173–178.
- Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183–190.
- Haas, A. 2003. Phylogeny of frogs as inferred from primarily larval characters (Amphibia: Anura). *Cladistics* 19:23–89.
- Heyer, W.R., A.S. Rand, C.A.G. Cruz, O.L. Peixoto, and C.E. Nelson. 1990. Frogs of Boracéia. *Arquivos de Zoologia* 31:231–410.
- Izecksohn, E., and O.L. Peixoto. 1981. Nova espécie de *Proceratophrys* da hiléia Bahiana, Brasil (Amphibia, Anura, Leptodactylidae). *Brazilian Journal of Biology* 41:19–24.
- Izecksohn, E., C.A.G. Cruz, and O.L. Peixoto. 1979. Notas sobre o girino de *Proceratophrys boiei* (Weid) (Amphibia, Anura, Leptodactylidae). *Brazilian Journal of Biology* 39:233–236.
- Izecksohn, E., S.P. Carvalho-E-Silva, and I. Deiss. 2005. O osteocrânio de *Proceratophrys boiei* (Wied-Neuwied), *P. appendiculata* (Günther), *P. melanopogon* (Miranda-Ribeiro) e *P. laticeps* Izecksohn and Peixoto (Anura, Leptodactylidae). *Revista Brasileira de Zoologia* 22:225–229.
- Lynch, J.D. 1971. Evolutionary relationships, osteology, and zoogeography of Leptodactyloid frogs. University of Kansas Publications, Museum of Natural History 53:1–238.
- Nascimento, F.A.C., B.S. Lisboa, G.O. Skuk, and R.O. De Sá. 2010. Description of the tadpole of *Proceratophrys renalis* (Miranda-Ribeiro, 1920) (Anura: Cycloramphidae). *South American Journal of Herpetology*. 5:241–248.
- Orton, G.L. 1953. The systematics of vertebrate larvae. *Systematics Zoology* 2:63–75.
- Peixoto, O.L., and C.A.G. Cruz. 1980. Observações sobre a larva de *Proceratophrys appendiculata* (Günther, 1873) (Amphibia, Anura, Leptodactylidae). *Brazilian Journal of Biology* 40:491–493.
- Peixoto, O.L., E. Izecksohn, and C.A.G. Cruz. 1981. Notas sobre o girino de *Proceratophrys laticeps* Izecksohn and Peixoto (Amphibia, Anura, Leptodactylidae). *Brazilian Journal of Biology* 41:553–555.
- Peixoto, O.L., C.A.G. Cruz, E. Izecksohn, and S.P. Carvalho-e-Silva. 1984. Notas sobre o girino de *Proceratophrys precrenulata* (Amphibia, Anura, Leptodactylidae). *Arquivos da Universidade Federal Rural do Rio de Janeiro* 7:83–86.
- Prado, G.M., and J.P. Pombal. 2008. Espécies de *Proceratophrys* Miranda-Ribeiro, 1920 com apêndices palpebrais (Anura; Cycloramphidae). *Arquivos de Zoologia* 39:1–85.
- Provete, D.B., M.V. Garey, F.R. Silva, and M.X. Jordani. 2012. Knowledge gaps and bibliographical revision about descriptions of free-swimming anuran larvae from Brazil. *North-Western Journal of Zoology* 8:283–286.
- Pyron, R.A., and J.J. Wiens. 2011. A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. *Molecular Phylogenetics and Evolution* 61:543–583.
- Rossa-Feres, D.C., and J. Jim. 1996. Tadpole of *Odontophrynus moratoi* (Anura, Leptodactylidae). *Journal of Herpetology* 30:536–539.
- Rossa-Feres, D.C., J. Jim, and M.G. Fonseca. 2004. Diets of tadpoles from a temporary pond in southeastern

- Brazil (Amphibia, Anura). *Revista Brasileira de Zoologia* 21:745–754.
- Starrett, P.H. 1973. Evolutionary patterns in larval morphology. Pp. 251–271 in J.L. Vial (Ed.), *Evolutionary Biology of the Anurans: Contemporary Research on Major Problems*. University of Missouri Press, USA.
- Vieira, W.L.S., K.S. Vieira, and G.G. Santana. 2007. Description of the tadpole of *Proceratophrys cristiceps* (Anura: Cycloramphidae: Odontophrynini). *Zootaxa* 1397:17–24.
- Wassersug, R.J. 1976. Oral morphology of anuran larvae: terminology and general description. *Occasional Papers of the Natural History Museum of the University of Kansas* 48:1–23.
- Wassersug, R., and W.R. Heyer. 1988. A survey of internal oral features of Leptodactyloid larvae (Amphibia: Anura). *Smithsonian Contributions to Zoology* 457:1–99.
- Wells, K.D. 1977. The social behaviour of anuran amphibians. *Animal Behaviour* 25:666–693.
- Weygoldt, P., and O.L. Peixoto. 1985. A new species of horned toad (*Proceratophrys*) from Espírito Santo, Brazil (Amphibia: Salientia: Leptodactylidae). *Senckenbergiana Biologica* 66:1–8.

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#### APPENDIX

##### *Specimens Examined*

*Proceratophrys melanopogon* BRAZIL: São Paulo: São José do Barreiro: Parque Nacional da Serra da Bocaina DZSJRP 1986.1; 1909.1; 2131.3; 2386.1; 2387.1; 2388.1. *P. moratoi* BRAZIL: São Paulo: Botucatu JJ 30. *P. cururu* BRAZIL: Minas Gerais: Serra do Cipó ZUEC 9575. *P. boiei* BRAZIL: Paraná: Fazenda Rio Grande: Fazenda Galha Azul DZSJRP 819.3.