

## Revealing Two New Species of the *Rhinella margaritifera* Species Group (Anura, Bufonidae): An Enigmatic Taxonomic Group of Neotropical Toads

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**ABSTRACT:** The level of species richness of amphibians found in Brazil is the greatest in the world. Despite increasing anthropic pressures on the Brazilian Cerrado and Amazon biomes, several new anuran species are described each year. In this study, two new species of *Rhinella* found in Brazil are described and assigned to the *Rhinella margaritifera* species group, based on morphological features. *Rhinella sebbeni* sp. nov. occurs in forested environments in the Cerrado biome and is characterized by the combination of the following features: snout–vent length (SVL; range = 48.5–59.7 mm in males, and 54.7–66.7 mm in females); presence of bony protrusion at the angle of jaws and rostral keel at the tip of snout; skin on the dorsum with a few granules, but more concentrated on the flanks; parotoid gland is well delineated and small and elliptical in shape showing a lateral line of tubercles. *Rhinella gildae* sp. nov. is found in southern Amazonia and is characterized by the combination of following features: SVL (range = 69.6–76.4 mm in males); cephalic crests poorly developed; parotoid gland without lateral line of tubercles; in dorsal view, supratympanic crest not exceeding the angle of the jaws; and presence of a vertebral line. We discuss data on morphological variation, natural history attributes, and distribution of the two new species.

**Key words:** Amazon; Biodiversity; Cerrado; Dry forest; Natural history; Rain forest

EVERY YEAR many new species of amphibians are described, particularly in South America (see Frost 2014), where Brazil holds the greatest richness of species worldwide (Frost 2014; Segalla et al. 2014). However, biodiversity in Brazil is under heavy anthropic pressure. Biomes such as the Cerrado are under extraordinary stress, mainly because of the rapid conversion of this landscape into farmland and pastureland (Klink and Moreira 2002; Klink and Machado 2005). Nevertheless, this biome is the source of many descriptions of new species of anurans (e.g., Pugliese et al. 2009; Vaz-Silva and Maciel 2011; Vaz-Silva et al. 2012; Brandão et al. 2013).

The toad genus *Rhinella* Fitzinger 1826 currently comprises 88 species that are distributed from the southern United States to southern South America (Frost 2014). Currently, 17 species constitute the *Rhinella margaritifera* species group (Moravec et al. 2014), occurring from Panama, throughout Amazonia to Bolivia and Paraguay, and the central and Atlantic Forest regions of Brazil (Frost 2014). Given the continual discoveries of new anuran species, there is presumably a large number of cryptic species awaiting description or inclusion among some of the previously described taxa (e.g., Hass et al. 1995; Fouquet et al. 2007b). The taxonomic history of species associated with this toad group has been problematic (Hoogmoed 1986, 1989, 1990; Lavilla et al. 2013). Duellman and Schulte (1992) recognized seven species belonging to the *R. margaritifera* species group (labeled as the *Bufo typhonius* group). Vélez-Rodríguez (2005) later moved *Rhinella sternosignata* to the *Rhinella granulosa* group, and Pereyra et al. (2015) recovered this species as sister taxon of *R. margaritifera* + *Rhinella veraguensis* groups. Pramuk (2006) recognized 10 species in the *R. margaritifera* group (as *Bufo*) by also including

*Rhinella alata*, *Rhinella castaneotica*, *Rhinella cristinae*, and *Rhinella sclerocephala*. She suggested that species presenting an expansion of the posterior ramus of the pterygoid be included in the *R. margaritifera* group. This feature is considered by her as a unique and unreversed synapomorphy of the *R. margaritifera* group. However, Pramuk (2006) did not examine specimens of *Rhinella ceratophrys*, *R. cristinae*, and *Rhinella iserni*. Fouquet et al. (2007a) suggested that *Rhinella nasica*, currently within the genus *Rhaebo* (see Frost 2014), should be removed from the *Rhinella margaritifera* group. Pramuk (2006) suggested that *R. nasica* does not belong to the *R. margaritifera* group. Fouquet et al. (2007a) also excluded *R. cristinae*, *R. sternosignata*, *R. iserni*, and *R. ceratophrys* from the *R. margaritifera* group as these species do not possess morphological synapomorphies proposed by Vélez-Rodríguez (2004, as cited in Fouquet et al. 2007a): the depressor mandibulae muscle formed by two slips, and the presence of a thickening on the ventrolateral border of the quadratojugal that can be seen as a process on the extreme forms. Accordingly, we recognize the following species in the *R. margaritifera* species group (Moravec et al. 2014): *Rhinella acutirostris* (Spix 1824), *Rhinella alata* (Thomiot 1884), *R. castaneotica* (Caldwell 1991), *Rhinella dapsilis* (Myers and Carvalho 1945), *Rhinella hoogmoedi* Caramaschi and Pombal 2006, *Rhinella lescurei* Fouquet, Gaucher, Blanc and Vélez-Rodríguez 2007a, *R. magnussoni* Lima, Menin and Araújo 2007, *R. margaritifera* (Laurenti 1768), *Rhinella martyi* Fouquet, Gaucher, Blanc and Vélez-Rodríguez 2007a, *Rhinella ocellata* (Günther 1858), *Rhinella paraguayensis* Ávila, Pansonato and Strüssmann 2010, *Rhinella proboscidea* (Spix 1824), *Rhinella roqueana* (Melin 1941), *Rhinella scitula* (Caramaschi and Niemeyer 2003), *Rhinella sclerocephala* (Mijares-Urrutia and Arends 2001), *Rhinella stanlaui* (Lötters and Köhler 2000), and *Rhinella yunga* Moravec, Lehr, Cusi, Córdova and Gvozdík 2014.

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Herein we describe two new species of *Rhinella* from two Brazilian biomes: one occupying dry forested areas of the Cerrado in central Brazil and another from Amazonian rain forest. Both species are currently allocated in the *R. margaritifera* species group based on their having an expansion of the posterior ramus of the pterygoid (see Pramuk 2006), and also by a combination of other morphological features.

#### MATERIALS AND METHODS

Specimens used for descriptions of the new species or examined for comparisons are deposited in Museu Nacional, Rio de Janeiro, Brazil (MNRJ); Coleção Herpetológica do Centro de Estudos e Pesquisas Biológicas da Pontifícia Universidade Católica de Goiás, Goiânia, Brazil (CEPB-PUCGO); Coleção Zoológica da Universidade Federal de Goiás, Goiânia, Brazil (ZUFUG); Coleção Herpetológica da Universidade de Brasília, Brasília, Brazil (CHUNB); and Coleção Célio F. B. Haddad, Universidade Estadual Paulista, Rio Claro, Brazil (CFBH; see Appendix). Sixteen external morphological characters were measured following Cei (1980), Duellman (2001), and Ávila et al. (2010): snout-vent length (SVL); head length (HL); head width (HW); internarial distance (IND); eye-nostril distance (END); eye diameter (ED); upper eyelid width (UEW); interorbital distance (IOD); postorbital crest length (POCL); horizontal tympanum diameter (HTD); vertical tympanum diameter (VTD); parotoid gland length (PGL); hand length (HAL); thigh length (THL); tibia length (TBL); foot length (tarsus + foot; FL). Measurements were obtained by using digital calipers ( $\pm 0.01$  mm). Sex was determined by examination of the presence of vocal slits (present on males), coloration on the gular region (black coloration on adult males), and/or by presence of secondary follicles in females. Head shape and digit numbers follows Heyer et al. (1990), webbing formulas follow Savage and Heyer (1967) as modified by Myers and Duellman (1982), and cephalic crest description follows Narvaes and Rodrigues (2009). Diagnoses and comparisons were provided by direct examination of the specimens of the *R. margaritifera* species group (see Appendix) and images of the holotype (*R. alata* available at the web site of the Muséum National D'Histoire Naturelle of France, <http://coldb.mnhn.fr/catalognumber/mnhn/ra/1884.285>). We dissected partially the lateral head of topotype (ZUFUG 8702) of *R. sebbeni* sp. nov. and the paratype of *R. gildae* sp. nov. (MNRJ 23837) to check the presence of the posterior ramus of pterygoid (see Pramuk 2006) in these new species in order to support their inclusion in the *R. margaritifera* group.

For comparative purposes we also included specimens of *R. cristinae* (Vélez-Rodriguez and Ruiz-Carranza 2002), *R. iserni* (Jiménez-de-la-Espada 1875), and *R. ceratophrys* (Boulenger 1882), which had been excluded from the *R. margaritifera* group by Fouquet et al. (2007a). Our purpose here is not to suggest the reallocation of *R. cristinae*, *R. iserni*, and *R. ceratophrys* to the *R. margaritifera* species group, but only to consider a broader spectrum of comparison with the species described herein. Therefore, although we used the synapomorphy (possession of an expansion of the posterior ramus of the pterygoid) suggested by Pramuk (2006) to include the two new species in the *R. margaritifera* group, we did not confine the list of species

comparisons to the species she considered as belonging to this group. We acknowledge that the *R. margaritifera* group of species still lacks a comprehensive systematic study.

#### SPECIES DESCRIPTIONS

##### *Rhinella sebbeni* sp. nov.

(Figs. 1–2)

**Holotype.**—MNRJ 53073, adult male, Parque Ecológico Altamiro de Moura Pacheco (16°34'24"S, 49°10'58"W; 790 m above sea-level [asl]; in all cases, datum = WGS84), Goiânia municipality, State of Goiás, Brazil, collected on 11 November 2006 by R.P. Bastos.

**Paratypes.**—CHUNB 56423 and 56445, adult males, collected on 27 August 2004, and CHUNB 57354, adult female, collected on 17 October 2004 by A.K. Peres, Jr., same municipality of holotype. CEPB 4724, adult male, 10 January 1997; CEPB 4711, adult female, 9 December 1996; CEPB 4723, adult female, 9 June 1997; CEPB 4725–4728, adult females, 20 December 1996; CEPB 4731, adult female, 2 December 1996; CEPB 4739, adult female, 4 December 1996, Niquelândia municipality (14°09'S, 48°22'W; 690 m asl), State of Goiás, Brazil, collected by Systema Naturae Team during the Fauna Rescue of Hydroelectric Power Plant Serra da Mesa. CFBH 11591, adult female, collected on 23 March 2006 by C.A. Brasileiro and M. Oyamaguchi; CFBH 18652, juvenile, collected on 28 January 2008 by T.G. Santos and K. Kopp, both in Fazenda Boa Vista, Ouro Verde de Goiás municipality (16°13'S, 49°11'W; 1040 m asl), State of Goiás, Brazil.

**Diagnosis.**—A species of the *R. margaritifera* group as defined by Pramuk (2006) based on the presence of an expanded posterior ramus of the pterygoid. *Rhinella sebbeni* sp. nov. is distinguished from other species by the following combination of features: (1) snout-vent length (SVL; range = 48.5–59.7 mm, in males; 54.7–66.7 mm, in females); (2) supraorbital and parietal crests evident; (3) supratympanic crest well-developed, wider than bony protrusion at the angle of jaws in dorsal view; (4) presence of dorsolateral line tubercles; (5) tympanum evident; (6) snout nearly acute in lateral view and mucronate in dorsal view; (7) presence of bony protrusion at the angle of jaws; (8) presence of rostral keel at the tip of snout; (9) dorsal skin with a small concentration of granules, more concentrated on the flanks; (10) absence of vertebral apophyses; (11) foot webbing poorly developed; and (12) parotoid gland well-delimited, with small and elliptical shape presenting a lateral line of tubercles.

**Comparisons with other species.**—*Rhinella sebbeni* sp. nov. differs from *R. margaritifera* by well-developed cephalic crests (vs. hypertrophied cephalic crests), absence of vertebral apophyses (vs. presence), and an evident bony protrusion at the angle of jaws (vs. bony protrusion slightly evident; see Lavilla et al. 2013). From *R. acutirostris* and *R. alata*, this new species differs by the presence of rostral keel at the tip of snout, cephalic crests well-developed, a bony protrusion at the angle of jaws, and larger size of males 48.5–59.7 mm (vs. absence or poorly bony protrusion at the angle of jaws, cephalic crest poorly developed, and males with 35.3 mm in *R. acutirostris* and 36.8 mm in *R. alata*; see Thomiot 1884; Lötters and Köhler 2000; Santos et al., 2015). Further, from *R. acutirostris* by the well-developed cephalic crests (vs. poorly developed) and larger size of males 48.5–59.7 mm





FIG. 1.—Dorsal and ventral views of the holotype of *Rhinella sebbei* sp. nov. (MNRJ 53073; snout-vent length = 55.9 mm).

(vs. 35.3 mm; see Lötters and Köhler 2000). *Rhinella sebbei* sp. nov. can be distinguished from *R. proboscidea* by the presence of an evident continuous line of tubercles along the lateral side body, including the posterior border of the parotoid gland (vs. less evident), cephalic crests well-developed (poorly developed in *R. proboscidea*), snout lacking a developed proboscis (vs. developed proboscis), nearly acute snout in lateral view (vs. pointed), skin on

dorsum slightly granulose (vs. smooth), and parotoid gland well-delimited (vs. indistinct). From *R. roqueana*, this new species is distinguished by having an evident tympanum (vs. barely distinct), snout in lateral view nearly acute (vs. snout nearly vertical), snout mucronate in dorsal view (vs. truncated), absence of vertebral apophyses (vs. presence), parietal crest poorly developed (vs. well-developed), and smaller size in males 48.5–59.7 mm (vs. SVL 70.0–79.0 mm; Melin 1941). *Rhinella sebbei* sp. nov. is distinguished from *R. dapsilis* by the posterior border of the parotoid gland having a line of tubercles that are continuous along the lateral side of body (vs. absence), presence of lateral line of tubercles on parotoid gland (vs. absence), snout lacking a developed proboscis (vs. developed proboscis), postorbital crest well-developed (vs. poorly developed), skin on dorsum slightly granulose (vs. smooth), and bony protrusion well-developed at the angle of the jaws (vs. poorly developed; Myers and Carvalho 1945). From *R. castaneotica*, *R. sebbei* sp. nov. differs in having a well-developed supratympanic crest (vs. poorly developed), larger size of males 48.5–59.7 mm (vs. 30.9–36.8 mm), well-developed postorbital crest (vs. poorly developed), presence of lateral line of tubercles on parotoid gland (vs. absence), tympanum evident (vs. not evident), and skin on dorsum slightly granulose (vs. smooth; see Caldwell 1991). This new species differs from *R. stanlani* by having a poorly developed supraorbital crest (vs. hypertrophied), postorbital crest well-developed (vs. poorly developed), snout in lateral view nearly acute (vs. protruding), and presence of vocal slits (vs. absence; Lötters and Köhler 2000). From *R. sclerocephala*, *R. sebbei* sp. nov. differs by the absence of vertebral apophyses (vs. presence), snout mucronate in dorsal view (vs. truncated), and foot webbing poorly developed (vs. developed; see Mijares-Urrutia and Arends 2001). From *R. scitula*, this new species is distinguished by well-developed cephalic crests (poorly developed), bony protrusion at the angle of jaws poorly

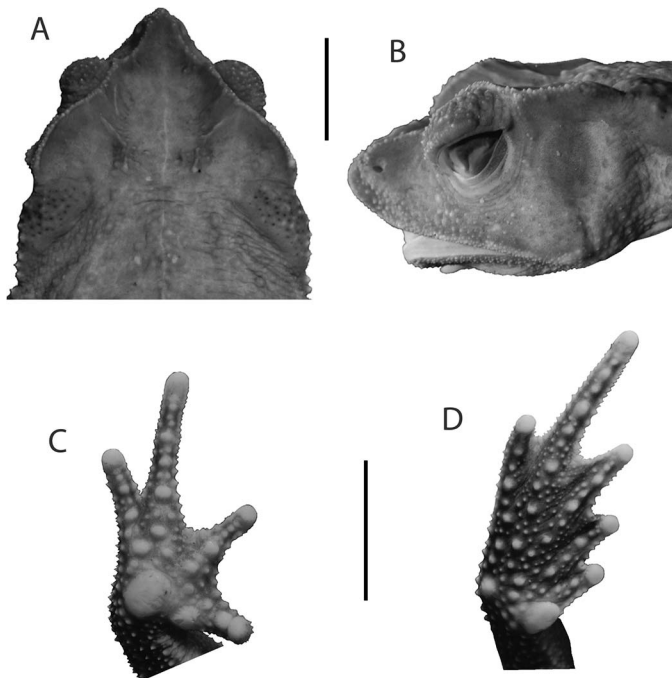


FIG. 2.—Holotype of *Rhinella sebbei* sp. nov. (MNRJ 53073). Dorsal (A) and lateral (B) views of head (scale bar = 10 mm) and palmar (C) and plantar views (D). Scale bar = 5 mm.

developed (vs. well-developed), skin on dorsum slightly granulose (vs. extremely granulose), presence of lateral line of tubercles on parotoid gland (vs. absence), small and elliptical parotoid gland (vs. globose), and larger size of males 48.5–59.7 mm (vs. 33.8–46.1 mm; see Caramaschi and Niemeyer 2003).

From *R. hoogmoedi*, this new species is distinguished by its slightly granulose dorsal skin (vs. rugose), rostral keel at the tip of snout (vs. absence), and larger parotoid gland (Caramaschi and Pombal 2006). This new species is distinguished from *R. paraguayensis* by the well-developed cephalic crests (vs. poorly developed), snout nearly acute in lateral view (vs. rounded), rostral keel at the tip of snout poorly developed (developed) and skin on dorsum slightly granulose (vs. very granulose; Ávila et al. 2010). From *R. lescurei*, this new species is distinguished by its snout nearly acute in lateral view (vs. pointed), by the well-developed postorbital crest (vs. poorly developed), larger size of males 48.5–59.7 mm (vs. 30.2–38.9 mm), and vestigial webbing in toes (vs. developed; Fouquet et al. 2007a). From *Rhinella magnussoni*, *R. sebbeni* sp. nov. can be distinguished by the well-developed supratympanic crest (vs. poorly developed), larger size of males 48.5–59.7 mm (vs. 36.0–45.3 mm), snout nearly acute in lateral view (vs. pointed), snout mucronate in dorsal view (vs. pointed), and skin on dorsum slightly granulose (vs. rugose; Lima et al. 2007). From *R. martyi*, this new species differs in the absence of vertebral apophyses (vs. presence), snout in lateral view nearly acute (vs. slightly rounded), vestigial webbing in toes (vs. developed; see Fouquet et al. 2007a). This new species is distinguished from *R. ocellata* by its well-developed cranial crests (vs. undeveloped), tubercles on dorsal skin poorly developed (vs. developed), snout mucronate in dorsal view and nearly acute in lateral view (vs. rounded in dorsal and lateral views), rostral keel at the tip of snout (vs. absent), dorsal cream uniform sometimes presenting small spots (vs. dorsal ocelli color pattern; see Leão and Cochran 1952; Caldwell and Shepard 1997), bony protrusion at the angle of jaws poorly developed (vs. well-developed). *Rhinella sebbeni* sp. nov. is distinguished from *R. yunga* by its snout being mucronate in dorsal view (vs. slightly pointed), tympanic membrane and tympanic annulus present (vs. absent), bony protrusion at the angle of jaws poorly developed (vs. undeveloped), vestigial webbing in toes (vs. developed), and by its cephalic crests being well-developed (vs. poorly developed; Moravec et al. 2014).

*Rhinella sebbeni* sp. nov. differs from *R. cristinae* by the evident tympanum (vs. absent), larger size of males 48.5–59.7 mm (vs. 30.7–34.3 mm,  $n = 9$ ), and the bony protrusion at the angle of jaws poorly developed (vs. bony protrusion undeveloped; Veléz-Rodríguez and Ruiz-Carranza 2002). From *R. iserni*, this new species differs in the absence of vertebral apophyses (vs. presence), and tympanum evident (vs. absent; Jiménez-de-la-Espada 1875; Caramaschi and Pombal 2006). From *R. ceratophrys*, this new species differs in the absence of triangular projecting dermal flaps on the eyelids and at the corners of mouth (vs. presence; Fenolio et al. 2012).

**Description of the holotype.**—Body robust; head wider than long, head length 70.8% of head width; head length 29.5% of SVL; head width 41.6% of SVL. Snout mucronate in dorsal view, with a rostral keel at the tip of snout; nearly

acute in lateral view. Tip of snout and head slightly concave; *canthus rostralis* well-defined by canthal crests, curved; loreal region slightly concave. Nostrils slightly protuberant, slightly directed dorsolaterally, nearer to the tip of snout than to eyes; internarial distance shorter than the eye–nostril distance, eye diameter, upper eyelid width, and tympanum diameter; eye–nostril distance longer than the eye diameter, tympanum diameter, and upper eyelid width; eye diameter slightly longer than the upper eyelid width and tympanum diameter; upper eyelid width 48.8% of interorbital distance. Canthal and supraorbital crests developed, parietal poorly developed and absence of preorbital crest; well-developed supratympanic crest, forming conspicuous lateral ledges; distance of the extremities of the supratympanic crests nearly larger than head width. Tympanum large, with a distinct annulus only anteriorly; vertical tympanum diameter nearly equal to the diameter of the eye. Parotoid glands, in dorsal view, small, triangular; in lateral view, elliptical, continuous to the supratympanic crest; parotoid gland length larger than the supraorbital crest length. External border of the parotoid gland with a line of pointed tubercles, which continues along the lateral side of body to the groin. Absence of salient apophyses on dorsum. Lips with small numerous tubercles; eyes visible from below. A nearly V-shaped incision in the maxilar symphysis; presence of a bony protrusion at the angle of jaws. Vocal sac not expanded externally and vocal slits present. Choanae small, ovoid, lateral, widely separated; medium tongue size, longer than wide, free and not notched posteriorly.

Forelimbs robust, forearms slightly more robust than the arms. Hand with medium-sized fingers; slender fingers with reduced webbing only at the base of fingers; fingers in ascending order of size,  $IV < II < I < III$ ; lateral fingers with a line of spinulose tubercles. Finger tips not expanded, smooth, posteriorly delimited on the dorsal and ventral faces by a groove. Palmar tubercle large, rounded, smooth; thenar tubercle small, approximately one fourth of the palmar tubercle, nearly rounded, smooth. Subarticular tubercles developed, conical, unique. Many supernumerary tubercles of varied sizes, distinct, conical, irregularly distributed on the ventral surfaces of hand and fingers. A few scattered black spinules on the inner dorsal surface of Finger I.

Hindlimbs short, robust. Tibia length slightly shorter than thigh length; tibia length 95.5% of thigh length and 42.8% of the SVL; thigh length 44.8% of SVL; sum of tibia and thigh lengths 87.0% of the SVL; tarsus–foot length larger than the tibia and thigh lengths, 58.9% of the SVL. Foot with short toes, moderately robust, in ascending order of size,  $I < II < V \approx III < IV$ ; toes webbing poorly developed; external surfaces of the first, fifth, and free part of the third toes with a line of spinulose tubercles; webbing formula  $I1 - 2III1 - 2^{1/2}III1^{1/2} - 4IV4 - 1^{1/2}V$ . Tips of toes slightly expanded, smooth. Outer metatarsal tubercle small, rounded, protruding; inner metatarsal tubercle large, approximately three times the outer, ovoid, with the distal border free. Subarticular tubercles small, conical, unique. Many supernumerary tubercles distinct, conical, unequal in size, approximately aligned on the ventral surfaces of foot and toes.

Skin on dorsum, flanks, and limbs granulose, with many small tubercles, rounded, irregularly distributed without forming a defined pattern; dorsal region poorly granulose. Ventral surfaces finely granulose.



TABLE 1.—Measurements (millimeters) of *Rhinella sebbeni* sp. nov. ( $n = 13$ ). For each sex, means are reported  $\pm 1$  SD, followed by range.

Character <sup>a</sup>	Males		Females	
	$\bar{x} \pm \text{SD}$	Range ( $n = 4$ )	$\bar{x} \pm \text{SD}$	Range ( $n = 9$ )
SVL	53.7 $\pm$ 5.0	48.5–59.7	60.6 $\pm$ 4.4	54.7–66.7
HL	15.8 $\pm$ 0.9	14.5–16.5	16.6 $\pm$ 2.1	13.9–19.7
HW	22.2 $\pm$ 2.6	18.4–24.4	24.5 $\pm$ 1.8	20.6–27.1
IND	3.1 $\pm$ 0.2	2.9–3.3	3.3 $\pm$ 0.3	3.0–4.0
END	4.9 $\pm$ 0.6	4.0–5.3	5.0 $\pm$ 0.4	4.3–5.7
ED	4.5 $\pm$ 0.9	3.8–5.8	4.9 $\pm$ 0.6	3.9–5.6
UEW	4.0 $\pm$ 0.3	3.6–4.3	4.8 $\pm$ 0.4	3.8–5.2
IOD	9.0 $\pm$ 1.7	7.7–11.5	9.5 $\pm$ 0.8	8.5–11.1
POCL	8.9 $\pm$ 0.9	7.9–10.0	10.0 $\pm$ 0.8	8.8–11.3
HTD	2.6 $\pm$ 1.7	0.2–3.9	3.9 $\pm$ 0.4	3.2–4.5
VTD	4.2 $\pm$ 0.7	3.3–4.8	4.4 $\pm$ 0.4	3.7–4.8
PGL	9.6 $\pm$ 0.3	9.4–9.9	11.0 $\pm$ 1.5	8.8–12.7
HAL	14.3 $\pm$ 1.7	12.0–16.2	15.6 $\pm$ 1.5	13.4–18.3
THL	23.7 $\pm$ 2.3	21.4–26.4	25.8 $\pm$ 2.3	22.0–29.6
TL	22.4 $\pm$ 1.8	20.0–23.8	24.2 $\pm$ 1.8	20.5–26.8
FL	30.9 $\pm$ 2.4	27.4–32.7	33.2 $\pm$ 3.4	28.8–40.4

<sup>a</sup> SVL, snout–vent length; HL, head length; HW, head width; IND, internarial distance; END, eye–nostril distance; ED, eye diameter; UEW, upper eyelid width; IOD, interorbital distance; POCL, postorbital crest length; HTD, horizontal tympanum diameter; VTD, vertical tympanum diameter; PGL, parotoid gland length; HAL, hand length; THL, thigh length; TBL, tibia length; FL, foot length (tarsus + foot).

**Measurements of the holotype (in mm).**—Snout–vent length 55.9; head length 16.5; head width 23.3; internarial distance 3.0; eye–nostril distance 5.3; eye diameter 4.7; upper eyelid width 4.3; interorbital distance 8.8; postorbital crest length 9.0; horizontal tympanum diameter 3.6; vertical tympanum diameter 4.8; parotoid gland length 9.9; hand length 16.1; thigh length 24.7; tibia length 23.6; foot length (tarsus + foot) 32.7.

**Color of the holotype in preservative.**—Dorsum and laterals of body and limbs uniformly brown; a grayish brown bar on forearm, tibia, and tarsus; ventral surfaces of feet and tarsus gray with brown tubercles. A whitish thin dorsal line from head to the posterior third of the body.

**Variation and color in life.**—Variations in measurements are summarized in Table 1. In life, dorsum uniformly brown or presenting small spots (Fig. 3). Sometimes, presence of a dark stripe on dorsal forearm and slight vertebral line (Fig. 4). CFBH 18652 is brownish with light spots throughout the body, especially on the limbs. In preservative, its dorsum is cream or brown. Specimens CEPB 4721, 4724–4726, and CEPB 4728 present dark spots on dorsum and the other specimens in type-series present uniform coloration. Blotch on frontoparietal region poorly marked in the specimen CEPB 4726. Ventral pattern well reticulated in specimen CHUNB 56445. Specimen CEPB 4711 presents malformation of left toes and CEPB 4731 and its third finger of the right hand is shorter than the left hand.

**Etymology.**—The specific name is a tribute to our friend and colleague Antonio Sebben (Universidade de Brasília) a notable morphologist, physiologist, and photographer, for his contribution to the knowledge of the Brazilian herpetofauna.

**Natural history.**—Adults or subadults of *R. sebbeni* sp. nov. can be found inside pristine forest (Ciliar and Dry Seasonal forests), in the leaf litter. On one occasion, males were observed vocalizing in ponds at the edges of a forest. In a survey conducted by RPB and C. Alves in the area flooded by the reservoir of the João Leite River, *R. sebbeni* sp. nov. was collected in pitfall traps in the rainy season (November–



FIG. 3.—*Rhinella sebbeni* sp. nov., (A) adult in life from type-locality (uncollected specimen; photograph by D.M. Silva), and (B) a juvenile specimen (CFBH 18652) from Ouro Verde de Goiás, Goiás state, Brazil (photograph by K. Kopp).

March) in periods from 2006 to 2010. A female (CEPB 4723) had 2,225 brown and cream ovarian eggs, with an average diameter of  $1.73 \pm 0.19$  mm ( $n = 10$ ; range = 1.34–1.99 mm). *Rhinella sebbeni* sp. nov. was sympatric and more abundant in areas where it occurs than *Rhinella schneideri* (Werner 1894), a species common in open areas. We captured 18 specimens of *R. sebbeni* sp. nov. in each rainy season between 2006 and 2010, compared to only four individuals of *R. schneideri*.

**Geographic distribution.**—*Rhinella sebbeni* sp. nov. has been found in Goiânia (type locality), Ouro Verde de Goiás, and Niquelândia municipalities, in the State of Goiás, Central Brazil (Fig. 5). These localities are located within Cerrado biome. This biome covers approximately 2 million km<sup>2</sup>, representing 22% of Brazil's land area (extending from the southern borders of the Amazonian forest to areas in the southern States of São Paulo and Paraná), plus small areas in eastern Bolivia and northwestern Paraguay. The distribution of Cerrado is coincident with the plateau of central Brazil (Oliveira-Filho and Ratter 2002).

*Rhinella gildae* sp. nov.  
(Figs. 6–7)

**Holotype.**—MNRJ 23838, adult male, São Pedro da Água Branca municipality (approximately 05°05'S, 48°19'W; 150

m asl), State of Maranhão, Brazil, collected on 28 October 1998 by G.V. Andrade and J.D. Lima.

**Paratype.**—MNRJ 23837, adult male, collected with the holotype.

**Diagnosis.**—A species of the *R. margaritifera* group as defined by Pramuk (2006) based on the presence of an expanded posterior ramus of the pterygoid. *Rhinella gildae* sp. nov. is distinguished from other members of this group by the following combination of characters: (1) SVL (range = 69.6–76.4 mm, in males); (2) preorbital, supraorbital, and parietal crests poorly developed; (3) in dorsal view, supratympanic crest poorly developed and not exceeding the angle of jaws; (4) presence of tubercles on the dorsolateral line; (5) tympanum evident; (6) snout mucronate in dorsal view and nearly acute in profile, with prominent nostrils; (7) absence or very small vertebral apophyses; (8) foot webbing poorly developed; and (9) parotoid gland well-delimited, with small and elliptical shape without a lateral line of tubercles.

**Comparisons with other species.**—*Rhinella gildae* sp. nov. differs from *R. sebbei* sp. nov. by its larger size with SVL ranging 69.6–76.4 mm, in males (vs. 48.5–59.7 mm), cephalic crests poorly developed (vs. well-developed), parotoid gland with lateral line of tubercles absent (vs. present), in dorsal view supratympanic crest not exceeding the angle of jaws (vs. supratympanic crest on the limit or exceeding the angle of jaws). From *R. margaritifera*, this new species is distinguished by having a poorly developed cephalic crest (vs. hypertrophied cephalic crests), absence or very small vertebral apophyses (vs. presence), and presence of bony protrusion at the angle of jaws (vs. bony protrusion slightly evident; see Lavilla et al. 2013). From *R. acutirostris* and *R. alata*, *R. gildae* sp. nov. differs by the evident bony protrusion at the angle of jaws (vs. absence), and absence of tubercles on the lateral of parotoid gland (vs. presence), and larger size of males of males 69.6–76.4 mm (vs. 35.3 mm in *R. acutirostris*, and 36.8 mm in *R. alata*; see Thomiot 1884; Lötters and Köhler 2000; Santos et al. 2015). Further, *R. gildae* sp. nov. is distinguished from *R. acutirostris* by the development of the cephalic crest (vs. undeveloped), and larger size of males 69.6–76.4 mm (35.3 mm; Lötters and Köhler 2000).

From *R. proboscidea*, *R. gildae* sp. nov. differs by the presence of a line of tubercles continuous along lateral body side (vs. poorly evident), snout lacking a developed proboscis (vs. developed proboscis), and nearly acute snout in lateral view (vs. pointed). From *R. roqueana*, *R. gildae* sp. nov. differs by the presence of a line of tubercles continuous along lateral body side (vs. absence), tympanum evident (vs. barely distinct), snout in lateral view nearly acute (vs. nearly vertical), in dorsal view mucronate (vs. truncated), and absence or very small vertebral apophyses (vs. presence; Melin 1941). This new species differs from *R. dapsilis* by the presence of a line of tubercles along the posterior border of the parotoid gland that continues along the lateral side of body (vs. absence), snout lacking a developed proboscis (vs. developed proboscis), skin on dorsum poorly granulose (vs. smooth), and a bony protrusion at the angle of the jaws (vs. poorly developed; Myers and Carvalho 1945). *Rhinella gildae* sp. nov. is distinguished from *R. castaneotica* by larger size of males 69.6–76.4 mm (vs. 30.9–36.8 mm), tympanum evident (vs. not evident), and skin on dorsum poorly

granulose (vs. smooth; Caldwell 1991). From *R. stanlaui*, *R. gildae* sp. nov. differs by poorly developed cephalic crests (vs. hypertrophied), postorbital crest well-developed (vs. poorly developed), snout in lateral view nearly acute (vs. protruding) and, size of males 69.6–76.4 mm (vs. 39.1–54.1 mm; Lötters and Köhler 2000).

From *R. sclerocephala*, *R. gildae* sp. nov. differs by the absence or very small vertebral apophyses (vs. presence), snout mucronate in dorsal view (vs. truncated), foot webbing poorly developed (vs. developed), and size of males 69.6–76.4 mm (vs. 55.4–67.3 mm; Mijares-Urrutia and Arends 2001). This new species differs from *R. scitula* by its skin on dorsum poorly granulose (vs. extremely granulose), elliptical parotoid gland (vs. globose), and size of males 69.6–76.4 mm (vs. 36.8–46.1 mm; Caramaschi and Niemeyer 2003). From *R. hoogmoedi*, *R. gildae* sp. nov. differs by having a dorsum with poorly granulose skin texture (vs. rugose), absence or very small vertebral apophyses (vs. presence in some specimens), and size of males 69.6–76.4 mm (vs. 39.4–52.1 mm; Caramaschi and Pombal 2006). From *R. paraguayensis*, *R. gildae* sp. nov. differs by its snout nearly acute in lateral view (vs. rounded), rostral keel at the tip of snout poorly developed (vs. developed), larger size of males 69.6–76.4 mm (vs. 42.3–52.6 mm), and skin on dorsum weakly granulose (vs. rugose; Ávila et al. 2010). From *R. lescurei*, this new species differs by snout being nearly acute in lateral view (vs. pointed), size of males 69.6–76.4 mm (vs. 30.2–38.9 mm), the supratympanic crest being well-developed (vs. poorly developed), and vestigial webbing in toes (vs. developed; Fouquet et al. 2007a). From *R. magnussoni*, *R. gildae* sp. nov. differs by the well-developed supratympanic crest (vs. poorly developed), larger size of males 69.6–76.4 mm (vs. 36.0–45.3 mm), line of tubercles absent along the parotoid gland (vs. present), snout nearly acute in lateral view (vs. pointed), snout mucronate in dorsal view (vs. pointed), and skin on dorsum weakly granulose (vs. rugose; Lima et al. 2007). From *R. martyi*, *R. gildae* sp. nov. is distinguished by the absence or very small vertebral apophyses (vs. presence), poorly developed cephalic crests (vs. well-developed), size of males 69.6–76.4 mm (vs. 49.5–61.1 mm), snout in lateral view nearly acute (vs. slightly rounded; Fouquet et al. 2007a), vestigial webbing in toes (vs. developed; Fouquet et al. 2007a).

From *R. ocellata*, this new species is distinguished by developed cephalic crests (vs. undeveloped), granules on dorsal skin poorly developed (vs. more developed), snout mucronate in dorsal view and nearly acute in lateral (vs. rounded in dorsal and lateral view), rostral keel present at the tip of snout (vs. absent), dorsal color pattern lacking ocelli (vs. ocelli present on dorsal pattern; Leão and Cochran 1952; Caldwell and Shepard 1997). *Rhinella gildae* sp. nov. is distinguished from *R. yunga* by larger size of males 69.6–76.4 mm (vs. 57.5–59.5 mm in males; Moravec et al. 2014), parotoid gland lacking lateral line of tubercles (vs. present), snout mucronate in dorsal view (vs. slightly pointed), bony protrusion evident at the angle of jaws (vs. not evident), vestigial webbing in toes (vs. developed), presence of tympanic membrane and tympanic annulus (vs. absent; see Moravec et al. 2014). *Rhinella gildae* sp. nov. differs from *R. cristinae* by larger size of males 69.6–76.4 mm (vs. 30.7–34.3 mm) and presence of bony protrusion at the angle of jaws (vs. absent; Veléz-Rodríguez and Ruiz-Carranza 2002).



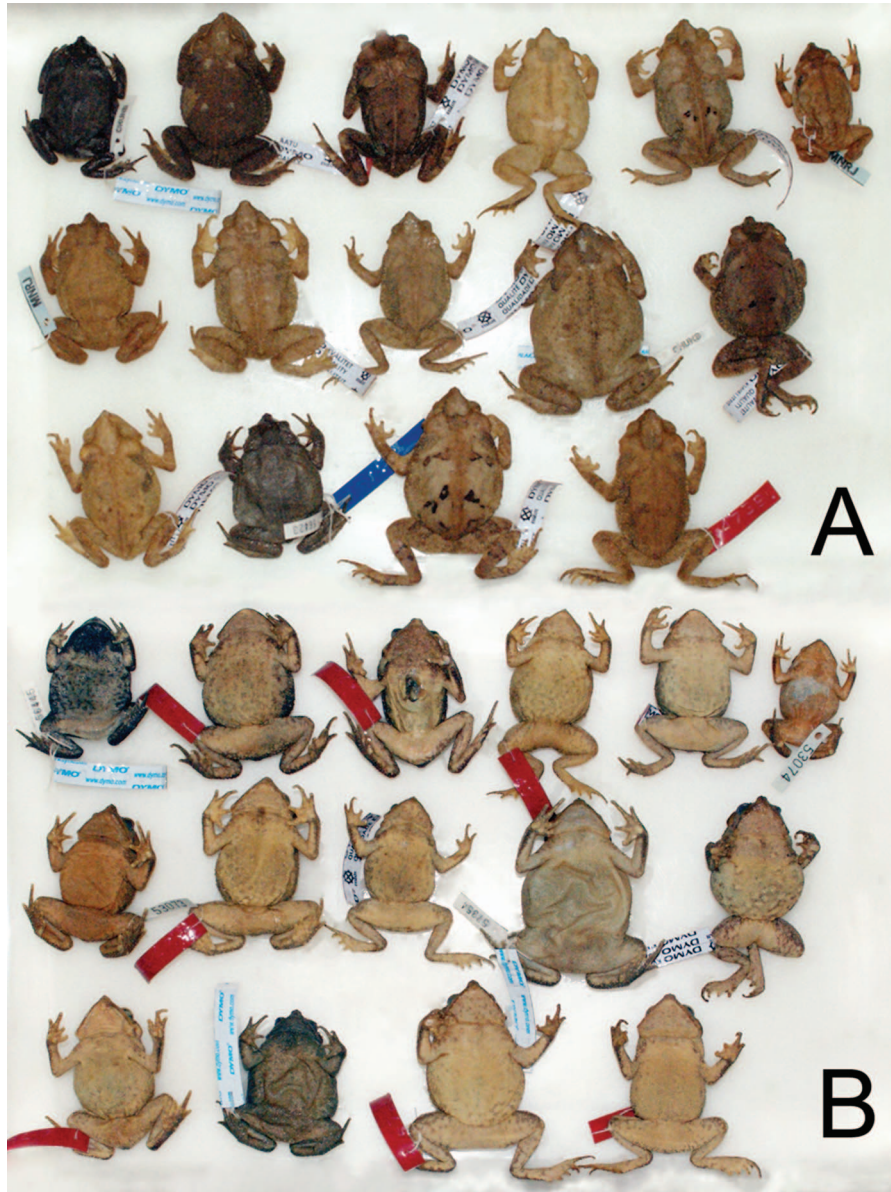


FIG. 4.—Variation in dorsal (A) and ventral (B) color pattern of *Rhinella sebbeni* sp. nov. (type series).

From *R. ceratophrys*, this new species differs by the absence of triangular projecting dermal flaps on the eyelids and at the corners of mouth (vs. presence; Fenolio et al. 2012). From *R. iserni*, *R. gildae* sp. nov. differs by the absence or very small vertebral apophyses (vs. presence), and tympanum evident (vs. absent; Jimenez-de-la-Espada 1875; Caramaschi and Pombal 2006).

**Description of the holotype.**—Body robust; head wider than long, head length 86.4% of head width; head length 31.9% of SVL; head width 37% of SVL. Snout mucronate in dorsal view, with a rostral keel at the tip of snout; in profile, nearly acute. Rostrum slightly concave, a pair of bony protrusions between the supratympanic crests; *canthus rostralis* well-defined by canthal crests, curved; loreal region weakly concave. Nostrils lateral, protuberant, slightly directed dorsally and backwards, nearer to the tip of snout than to eyes; internarial distance shorter than the eye–nostril distance, eye diameter, upper eyelid width, and tympanum

diameter; eye–nostril distance shorter than the eye diameter, vertical tympanum diameter; eye diameter shorter than the upper eyelid width and tympanum diameter; upper eyelid width 80.5% of interorbital distance. Canthal and supraorbital crests developed, parietal crest poorly developed and preorbital crest absent; supratympanic crests well-developed, forming conspicuous lateral edges. Tympanum large, longer than wide, with a distinct annulus; vertical tympanum diameter shorter than the eye diameter. In dorsal view, parotoid glands small, triangular; in lateral view, elliptical, continuous to the supratympanic crest; parotoid gland length larger than the supratympanic crest length. Continuous lines of tubercles along lateral torso, from the posterior border of parotoid gland to the groin. Absence of apophyses on dorsum. Lips with small numerous tubercles; eyes visible from below. Presence of a bony protrusion at the angle of jaws. Vocal sac not expanded externally and vocal slits present. Choanae small, ovoid, lateral, widely separated;

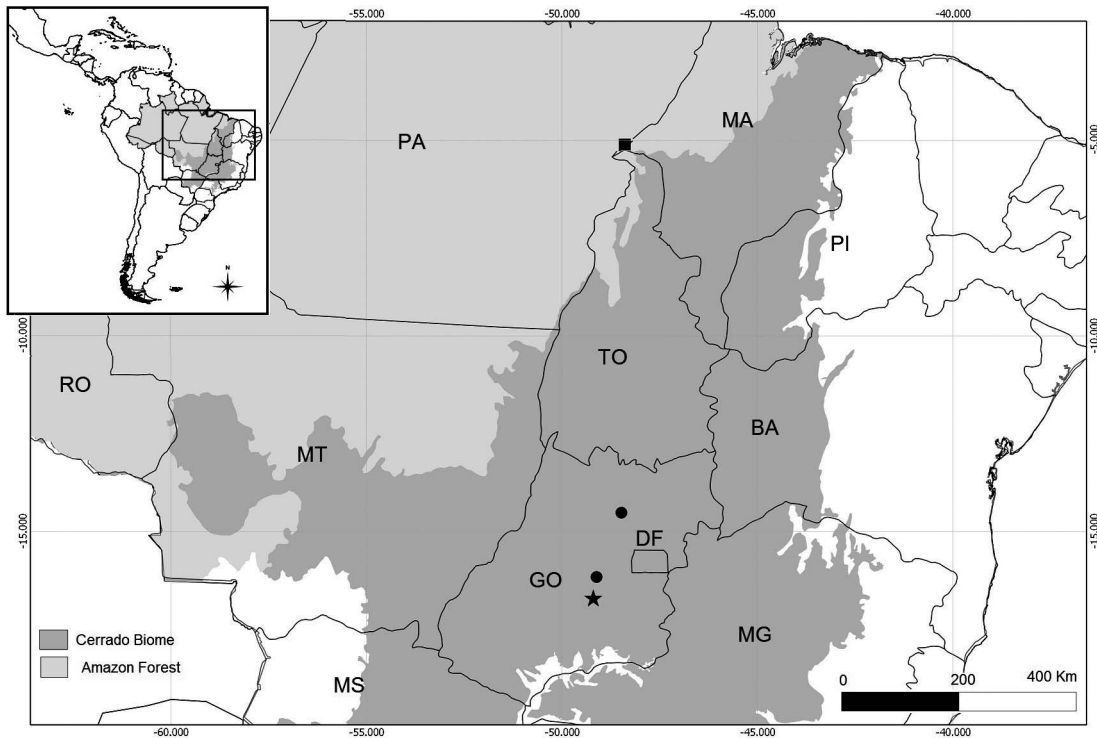


FIG. 5.—Geographic distribution of *Rhinella sebbeni* sp. nov. (circles), showing Goiânia Municipality (type locality; star), and *Rhinella gildae* sp. nov., São Pedro da Água Branca (type locality; square). GO = State of Goiás; DF = Federal District; TO = State of Tocantins; MS = State of Mato Grosso do Sul; MT = State of Mato Grosso; BA = State of Bahia; MA = State of Maranhão; PI = State of Piauí; MG = State of Minas Gerais; SP = State of São Paulo; PR = State of Paraná.

medium size tongue, longer than wide, free and not notched posteriorly.

Forelimbs robust and slightly more robust than the arms. Hand with medium-sized fingers; slender fingers without

webbing; fingers in ascending order of size,  $IV \approx II < I < III$ ; lateral fingers with a line of spinulose tubercles. Finger tips not expanded, smooth, posteriorly delimited on the dorsal and ventral faces by a groove. Palmar tubercle large, ovoid,



FIG. 6.—Dorsal and ventral views of holotype of *Rhinella gildae* sp. nov. (MNRJ 23838; snout-vent length = 69.6 mm).



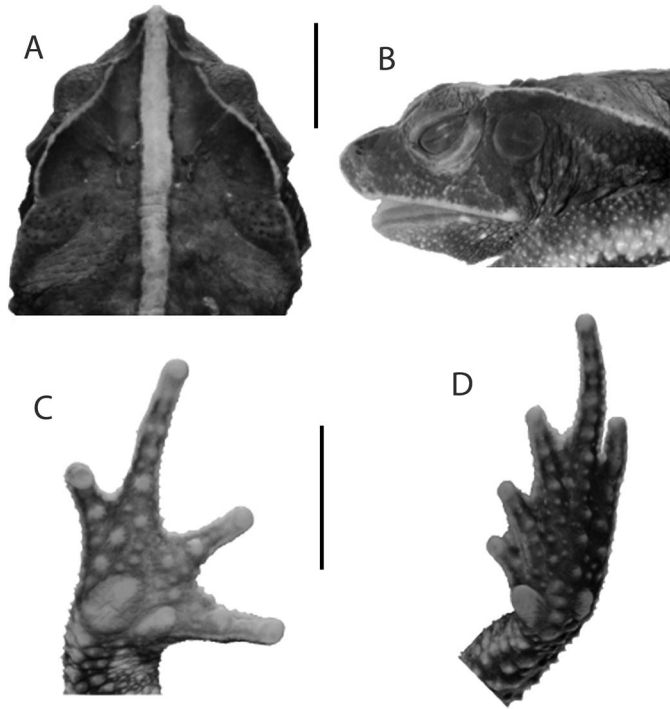


FIG. 7.—Holotype of *Rhinella gildae* sp. nov. (MNRJ 23838). Dorsal (A) and lateral (B) views of head (scale bar = 10 mm) and palmar (C) and plantar views (D; scale bar = 5 mm).

smooth; thenar tubercle small, nearly elongated, smooth. Subarticular tubercles developed, conical, unique, except by a double distal subarticular on Finger III. Many supernumerary tubercles of varied sizes, distinct, conical, irregularly distributed on the ventral surfaces of hand and fingers. Black spinulose nuptial pad on the inner dorsal surface of Finger I.

Hindlimbs short, robust. Tibia length slightly shorter than thigh length; tibia length 88.8% of thigh length and 39.0% of the SVL; thigh length 43.9% of SVL; sum of tibia and thigh lengths 83.0% of the SVL; tarsus-foot length longer than the tibia and thigh lengths, 52.2% of the SVL. Foot with short toes, moderately robust, in ascending order of size,  $I < II < V \approx III < IV$ ; toe webbing poorly developed; external surfaces of the fifth toes, and free part of the third toes with a line of spinulose tubercles; webbing formula  $II-2III-2^{1/2}III-2IV-2V$ . Tips of toes slightly expanded, smooth. Outer metatarsal tubercle small, ovoid, protruding; inner metatarsal tubercle medium-sized, approximately two times the outer, ovoid, with the distal border free. Subarticular tubercles small, conical, singular. Many supernumerary tubercles distinct, conical, unequal in size, approximately aligned on the ventral surfaces of foot and toes.

Skin on dorsum, flanks and limbs granulose, with many small tubercles, rounded, irregularly distributed without forming a defined pattern; tubercles on forelimbs smaller than hindlimbs; dorsal region poorly granulose. Ventral surfaces finely granulose.

**Measurements of the holotype (in millimeters).—**Snout-vent length 69.6; head length 22.3; head width 25.8; internarial distance 4.0; eye-nostril distance 6.2; eye diameter 6.8; upper eyelid width 7.8; interorbital distance 10.4; postorbital crest length 6.9; horizontal tympanum diameter 4.9; vertical tympanum diameter 6.3; parotoid gland length

14.7; hand length 17.4; thigh length 30.6; tibia length 27.2; foot length (tarsus + foot) 36.6.

**Color of the holotype in preservative.**—Dorsal and limbs gray brownish; a wide light gray medial dorsal band with a thin lateral line dark gray on its edge; a line gray on supraorbital and supratympanic crests; line of tubercles cream from parotoid glands to the groin; flanks below this tubercles line dark gray; superior lips and protrusion at the angle of jaw cream; a dark gray bar poorly visible on tibia, tarsus, and forearm. Mental region brown; gular region black. Venter cream with light gray blotches. Palm of hand cream; undersurfaces of foot and tarsus gray.

**Variation.**—Measurements (in mm) of the paratype are snout-vent length 76.4; head length 23.8; head width 27.3; internarial distance 5.1; eye-nostril distance 6.9; eye diameter 9.9; upper eyelid width 9.5; interorbital distance 11.8; postorbital crest length 5.7; horizontal tympanum diameter 5.3; vertical tympanum diameter 5.6; parotoid gland length 12.5; hand length 20.7; thigh length 34.2; tibia length 31.0; foot length (tarsus + foot) 42.7. General color of the paratype is more uniform. Palmar tubercle bigger than the holotype. The medial dorsal band narrower than the holotype. Presence of three very small dorsal apophysis. Coloration in life is unknown.

**Etymology.**—The specific name honors our friend and colleague Gilda V. Andrade (Universidade Federal do Maranhão) for her contributions to the knowledge of the ecology of Brazilian anurans, including the collection of the specimens used to describe this new species.

**Geographic distribution.**—*Rhinella gildae* sp. nov. is known only in São Pedro da Água Branca municipality (type locality; Fig. 5), State of Maranhão, northern Brazil.

#### DISCUSSION

In a cladistic analysis of morphological and molecular data, Pramuk (2006) found one unreversed morphological synapomorphy supporting the monophyly of the *R. margaritifer* species group: expansion of the posterior ramus of the pterygoid. *Rhinella ocellata* was considered as *incertae sedis* by Frost et al. (2006) and as a sister taxon of the *R. margaritifer* group by Pramuk (2006). Based on molecular analyses, Fouquet et al. (2007a) and Moravec et al. (2014) found *R. ocellata* to be embedded within the *R. margaritifer* group. Moravec et al. (2014) described a new species, *R. yunga*, proposing it as sister to the others species of the *R. margaritifer* group. Historically, species in the *R. margaritifer* group have been easily recognizable. The inclusion of *R. ocellata* and *R. yunga* in the group, however, makes it morphologically heterogeneous, as these species are more dissimilar than those remaining. Our analyses of the morphological characters presented here indicate that the two new species, *R. sebbeni* and *R. gildae*, should be allocated to the *R. margaritifer* species group, thus raising the total number of species in this group to 19.

Regarding its taxonomy, the *R. margaritifer* species group is considered one the most problematic Neotropical anuran groups (Hoogmoed 1986, 1989, 1990). Many candidate undescribed species have been suggested (Fouquet et al. 2007b). Only recently, *R. margaritifer*, the nominate species of the group (Laurenti 1768), was stabilized by a neotype designation (Lavilla et al. 2013).

One of the challenges of identifying undescribed species is caused by the lack of information on morphological variation that is used to delimit species. For example, juveniles frequently hold less developed apophyses at the tip snout and cephalic crest, although the development of these features seems to be related to size. Apparently several species of this clade are explosive breeders (e.g., Wells 1979; Pombal and Gordo 2004), which constrains the sampling of adults, their vocalizations, and larvae.

Neotropical toads of the *R. margaritifera* species group inhabit the forest floor of tropical humid and seasonal dry ecosystems in South America. These toads show adaptations to life in the leaf litter of primary and secondary forests. Their cryptic coloration, expanded cephalic crests, bone protrusions at angle of the jaws and, in some species, neural crests of the vertebrae combine to resemble dark, partly decomposed leaf litter. These features are effective at facilitating crypsis (Moravec et al. 2014).

*Rhinella gildae* sp. nov. is associated with the tropical rain forest (Amazonian biome), as are most species of the *R. margaritifera* group. Species of the group that occur in the Cerrado biome (*R. scitula*, *R. ocellata*, *R. paraguayensis*, and *R. sebbeni* sp. nov.; Caldwell and Shepard 1997; Caramaschi and Niemeyer 2003; Ávila et al. 2010; this study) are also associated with forested environments (Ciliar and seasonal dry forests). Among the physiognomies present in the Cerrado biome, these forests environments are increasingly fragmented, relictual, and restricted to areas of high slope on account of deforestation and conversion to agriculture. The disjunct and endemic nature of these populations (e.g., Werneck and Colli 2006; Teixeira et al. 2012; Vaz-Silva et al. 2012) highlights the uniqueness of seasonal dry forests and the urgent need to conserve them.

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## APPENDIX

### Specimens Examined

- Rhinella castaneotica*.—MNRJ 35230–35241, Serra do Navio municipality, Amapá state, Brazil.
- Rhinella dapsilis*.—MNRJ 0840 (holotype), Bom Jardim, Benjamin Constant municipality, Amazonia state, Brazil.
- Rhinella hoogmoedi*.—MNRJ 40325 (holotype), Fazenda Santa, Canavieiras municipality, Bahia state, Brazil. MNRJ 40326–40331; 40508–40510 (paratypes), Fazenda Santa, Canavieiras municipality, Bahia state, Brazil.
- Rhinella scitula*.—MNRJ 25884 (holotype), Estância Mimosa, Bonito municipality, Mato Grosso do Sul state; MNRJ 25885–25920 (paratypes). MNRJ 25921–25934 (paratypes), Margem do Rio Anhumas, Fazenda Jaraguá, Bonito municipality, Mato Grosso do Sul state, Brazil.
- Rhinella magnussoni*.—MNRJ 48259, 47880, 48140, 48253, 47900, Porto Trombetas, Oriximiná municipality, Pará state, Brazil; MNRJ 80583, Utinga, Belém do Pará municipality, Pará state, Brazil.
- Rhinella margaritifera*.—MNRJ 71538 (neotype), MNRJ 75823–75826, Humaitá municipality, Amazonas state, Brazil.
- Rhinella ocellata*.—MNRJ 28676–28677, Xavantina municipality, Mato Grosso state, Brazil; MNRJ 80000 Serra da Mesa, Goiás state, Brazil.
- Rhinella paraguayensis*.—ZUF 6447–6457, Tangará da Serra, Mato Grosso state, Brazil.
- Rhinella proboscidea*.—MNRJ 28556, Macapá municipality, Amapá state, Brazil; MNRJ 85743, Parecis municipality, Rondônia state, Brazil.
- Rhinella sclerocephala*.—MNRJ 58904, Venezuela.