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Two new species of the *Boana semiguttata* clade (Anura: Hylidae: Hylinae) from the Atlantic Forest of southern Brazil

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Abstract. We describe two new species of the *Boana pulchella* group, nested within the *B. semiguttata* clade and previously referred to as *Boana* sp. 1 and *Boana* sp. 2. Both species inhabit the Atlantic Forest of Santa Catarina state, southern Brazil. *Boana* sp. 1 is known from Parque Nacional da Serra do Itajaí. It resembles *B. joaquini* and *B. stellae* in external morphology but is distinguishable by its snout–vent length, coloration pattern, and advertisement call. Although its call structure differs from all other *B. semiguttata* clade species, it is strikingly similar to that of *B. marginata,* a distantly related species of the *B. pulchella* group. *Boana* sp. 2 is known only from a single locality in Serra do Quiriri (municipality of Garuva). Morphologically and acoustically, it is highly similar to *B. semiguttata,* differing only in color pattern and DNA sequences. Like all other species of the *B. semiguttata* clade, both new species breed in in montane streams and exhibit hypertrophied forearms in adult males. While *Boana* sp. 1 occurs in a protected area within a national park, the only known locality of *Boana* sp. 2 is under threat of habitat loss and other anthropogenic pressures.

Keywords. Advertisement call; Cophomantini; Santa Catarina; Taxonomy; Treefrogs.

INTRODUCTION

The Neotropical *Boana pulchella* group is the most speciose of the eight groups that comprise the genus *Boana* (Lyra et al., 2020; see Table 1 for taxonomic authorities of taxa mentioned throughout the text). The group includes 34 formally recognized species, in addition to four candidate species (Faivovich et al., 2021; Marinho et al., 2022). Its monophyly was supported in a recent molecular phylogenetic analysis that included all known species except *B. cymbalum* (Faivovich et al., 2021)—considered potentially extinct by Haddad et al. (2018) and formally declared extinct by the International Union of Conservation of Nature (IUCN; IUCN SSC Amphibian Specialist Group, Instituto Biotatá de Etnobiologia e Conservação da Fauna, 2023).

The hypothesis of Faivovich et al. (2021) presents five main clades. One of them, the *Boana semiguttata* clade, includes six named species—*B. caipora, B. curupi, B. joaquini, B. poaju, B. semiguttata,* and *B. stellae*—and two candidate species—*Boana* sp. 1 and *Boana* sp. 2. Similarly, Vasconcellos et al. (2021) found *Boana* sp. 1 (as *Boana* sp.) to be is nested within the *B. semiguttata* clade (*sensu* Faivovich et al., 2021). The *B. semiguttata* clade is restricted to an area from southern Brazil (eastern state of Rio Grande do Sul to southeastern state of São Paulo) to eastern Paraguay and northeastern Argentina (Garcia et al., 2003, 2007, 2008; Brusquetti and Lavilla, 2006; Antunes et al., 2008; Kwet, 2008; Faivovich et al., 2021 fig. 2 and Supplementary file 5).

Both *Boana* sp. 1 and *Boana* sp. 2 were found to be highly supported clades using different optimality criteria (Fig. 1; Faivovich et al., 2021). Additionally, the branch lengths of these two lineages are similar to those of other species within the *B. semiguttata* clade, and their uncorrected pairwise genetic distances (for the mitochondrial 16S gene) are similar to the values found between other pairs of species in the group. *Boana* sp. 1 differs 2.14–4.98% from other species of the *B. semiguttata* clade, *Boana* sp. 2 differs 2.14–4.99% from species of the same clade, while the other species differ between 1.66–5.16 from each other (Table 2; see also Faivovich et al., 2021: table S4.4, supplementary file 4). Herein, we describe these two previously reported candidate species and their advertisement calls.

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Table 1. List of taxa included in the main text with the respective taxonomic authorities.

BUFONIDAE Gray, 1825
Melanophryniscus biancae Bornschein et al., 2015
BRACHYCEPHALIDAE Günther, 1858a
Brachycephalus auroguttatus Ribeiro et al., 2015
B. quiririensis Pie and Ribeiro, 2015
***Ischnocnema* Reinhardt and Lütken, 1862**
CENTROLENIDAE Taylor, 1951
Vitreorana parvula (Boulenger, 1895)
HYLIDAE Rafinesque, 1815
***Aplastodiscus* Lutz, 1950**
Aplastodiscus ehrhardti (Müller, 1924)
***Boana* Gray, 1825**
B. aguilari (Lehr et al., 2010)
B. balzani (Boulenger, 1898)
B. bischoffi (Boulenger, 1887)
B. botumirim (Caramaschi et al., 2009)
B. buriti (Caramaschi and Cruz, 1999)
B. caingua (Carrizo, 1991)
B. caipora (Antunes et al., 2008)
B. callipleura (Boulenger, 1902)
B. cambui (Pinheiro et al., 2016)
B. cipoensis (Lutz, 1968b)
B. cordobae (Barrio, 1965)
B. curupi (Garcia et al., 2007)
B. cymbalum (Bokermann, 1963)
B. ericae (Caramaschi and Cruz, 2000)
B. freicanecae (Carnaval and Peixoto, 2004)
B. gladiator (Köhler et al., 2010)
B. goiana (Lutz, 1968b)
B. guarinimirim Marinho et al., 2022
B. guentheri (Boulenger, 1886)
B. jaguariaivensis (Caramaschi, et al., 2010)
B. joaquini (Lutz, 1968a)
B. leptolineata (Braun and Braun, 1977)
B. marginata (Boulenger, 1887)
B. marianitae (Carrizo, 1992)
B. melanopleura (Boulenger, 1912)
B. palaestes (Duellman et al., 1997)
B. poaju (Garcia et al., 2008)
B. polytaenia (Cope, 1870)
B. prasina (Burmeister, 1856)
B. pulchella (Duméril and Bibron, 1841)
B. riojana (Koslowsky, 1895)
B. semiguttata (Lutz, 1925)
B. stellae (Kwet, 2008)
B. stenocephala (Caramaschi and Cruz, 1999)
Bokermannohyla hylax (Heyer, 1985)
***Phyllomedusa* Wagler, 1830**
HYLODIDAE Günther, 1858b
Hylodes perplicatus (Miranda-Ribeiro, 1926)
CALLIPHORIDAE Brauer and von Bergenstamm, 1889
SARCOPHAGIDAE Macquart, 1834

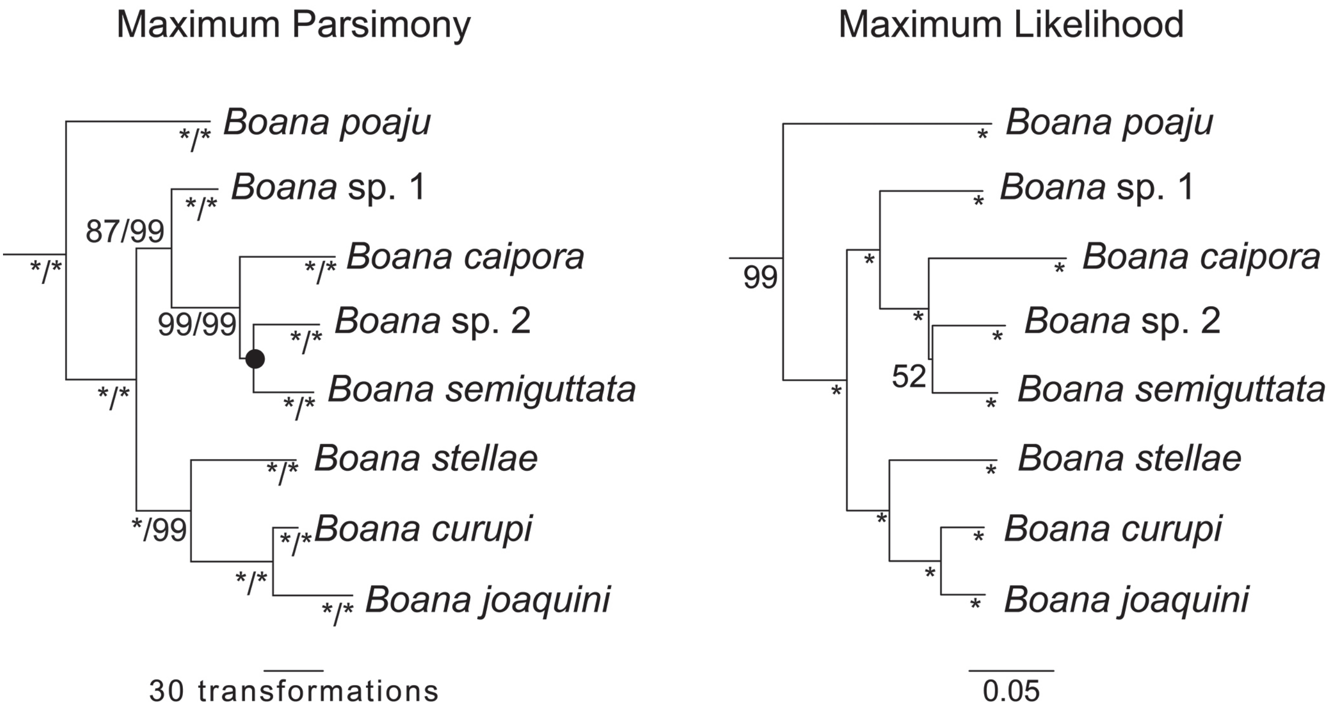


Figure 1. Pruned trees from the topologies recovered by Faivovich et al. (2021), showing the relationships between species of the *Boana semiguttata* clade; multiple terminals from each species were omitted. On the left, one of the most parsimonious trees. Values on nodes are Absolute Jackknife Frequencies for analyses considering gaps as fifth state/as missing data; the black dot point a node that collapses under strict consensus. On the right, the maximum likelihood tree; values on nodes are bootstrap values. Asterisks on nodes indicate values of 100% while absence of numbers mean values < 50% for both topologies.

Table 2. Summary of uncorrected *p*-distances (in percentage) for the final portion of the mitochondrial 16S gene of species of the *Boana semiguttata* clade reported by Faivovich et al. (2021). The candidate species proposed by the authors, *Boana* sp. 1 and *Boana* sp. 2, are highlighted in bold. See Figure 1 for phylogenetic relationship.

		1	2	3	4	5	6	7	8
1	*Boana poaju* (*n* = 2)	0							
2	***Boana* sp. 1 (*n* = 2)**	**3.56–3.74**	**0.18**						
3	*Boana caipora* (*n* = 2)	3.92–4.09	**3.20–3.56**	0.18					
4	***Boana* sp. 2 (*n* = 2)**	**3.74**	**2.14–2.31**	**2.14–2.31**	**0**				
5	*Boana semiguttata* (*n* = 10)	3.20–3.74	**2.49–3.38**	2.67–3.20	**2.31–2.49**	0–1.42			
6	*Boana stellae* (*n* = 11)	2.67–3.38	**3.21–3.91**	3.56–4.27	**3.03–3.91**	2.67–4.09	0–0.89		
9	*Boana curupi* (*n* = 6)	3.74–3.92	**2.49–3.03**	3.38–3.74	**2.85–3.03**	3.03–4.45	3.03–3.92	0–0.89	
8	*Boana joaquini* (*n* = 9)	4.27–4.98	**3.89–4.98**	3.92–4.81	**4.08–4.99**	3.92–5.16	3.56–4.45	1.66–2.67	0–1.29

MATERIALS AND METHODS

Adult morphology and species description

Adults were euthanized in 5% lidocaine, fixed in 10% formalin, and stored in 70% ethanol. Voucher specimens are housed in the Coleção de Anfíbios do Centro de Coleções Taxonômicas da Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil (UFMG); Coleção de Anfíbios da Universidade Regional de Blumenau, Blumenau, Santa Catarina (FURB); Coleção Herpetológica da Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina (CHUFSC); and Museu de Zoologia of Universidade de São Paulo, São Paulo, São Paulo (MZUSP). Additional specimens (from UFMG and Coleção de Anfíbios Célio F.B. Haddad, Rio Claro, São Paulo—CFBH) used for comparisons are listed in the Appendix. Institutional abbreviations follow Sabaj (2020).

Specimens were measured to the nearest 0.1 mm using digital calipers under a Motic K 700L stereomicroscope by PDPP. Measurements (in mm) follow Duellman (1970): snout–vent length (SVL), head length (HL), head width (HW), eye diameter (ED), eye–nostril distance (END), internarial distance (IND), eyelid width (EW), interorbital distance (IOD), tympanum diameter (TD), tibia length (TL), tarsal length (TAL), and foot length (FL). We also measured forearm length (FAL; Duellman et al., 1997); hand length (HAL) and thigh length (THL; Heyer et al., 1990); nostril to tip of snout distance (NSD), finger IV disc diameter (4FD—originally named finger III diameter, but see below), and toe IV disc diameter (4TD; Napoli and Caramaschi, 1999); anterior margins of eyes distance (AMD; Garcia et al., 2003). Terminology for external morphology follows Duellman (1970), except for the dorsal outline of the snout, which follows Heyer et al. (1990). Finger numbering follows Fabrezi and Alberch (1996). Webbing formulae follow Savage and Heyer (1967) as modified by Myers and Duellman (1982). Sex was identified on the basis of the development of the spine-shaped prepollex, vocalization, and/or presence of vocal slits in males, prepollex poorly developed and/or absence of vocalization and vocal slits in females.

Bioacoustics

Calls were recorded using a Marantz PMD 660 or a Tascam DR-40 digital recorders coupled to a Sennheiser K6/ME66 microphone at 44.1 kHz with a 16-bit sampling size. Air temperature was measured with an alcohol thermometer in degrees Celsius (°C). The data were analyzed using software Raven Pro 64 v1.5 Cornell Lab of Ornithology (Bioacoustics Research Program, 2017). Spectrograms and power spectra were produced with a window size of 256 samples, 75% overlap, hop size of 64 samples, and a Hamming window type. Brightness and contrast were set to 50% and 72%, respectively, to enhance the clarity of the spectrograms. Call voucher specimens are housed at both CHUFSC and UFMG, and recordings are deposited in the UFMG Bioacoustics collection (recordings CBUFMG 1079–1085). The following acoustic parameters were considered, following Köhler et al. (2017) definitions, employing the note centered approach: number of notes, note duration, interval between notes, call duration, interval between calls, relative amplitude of the notes (proportional to sound intensity), dominant frequency range (i.e., range between minimum and maximum frequency of most energetic band), and peak frequency (as provided by Raven Pro). Although only one type of call was identified for *Boana* sp. 1, we identify it as Call A to facilitate comparison with the call of *Boana* sp. 2.

RESULTS

Species accounts

***Boana itajahy* sp. nov.**
Figures 2–5; Table 3

Hypsiboas sp. 2—Kwet, 2008: 9, 14, fig. 5E, F.
Boana sp. 1—Faivovich et al., 2021: 2, 12.
Boana sp.—Vasconcellos et al., 2021: figs. 2, 3.

Holotype. CHUFSC 5052, adult male, Parque Nacional da Serra do Itajaí, Indaial, Santa Catarina, Brazil,

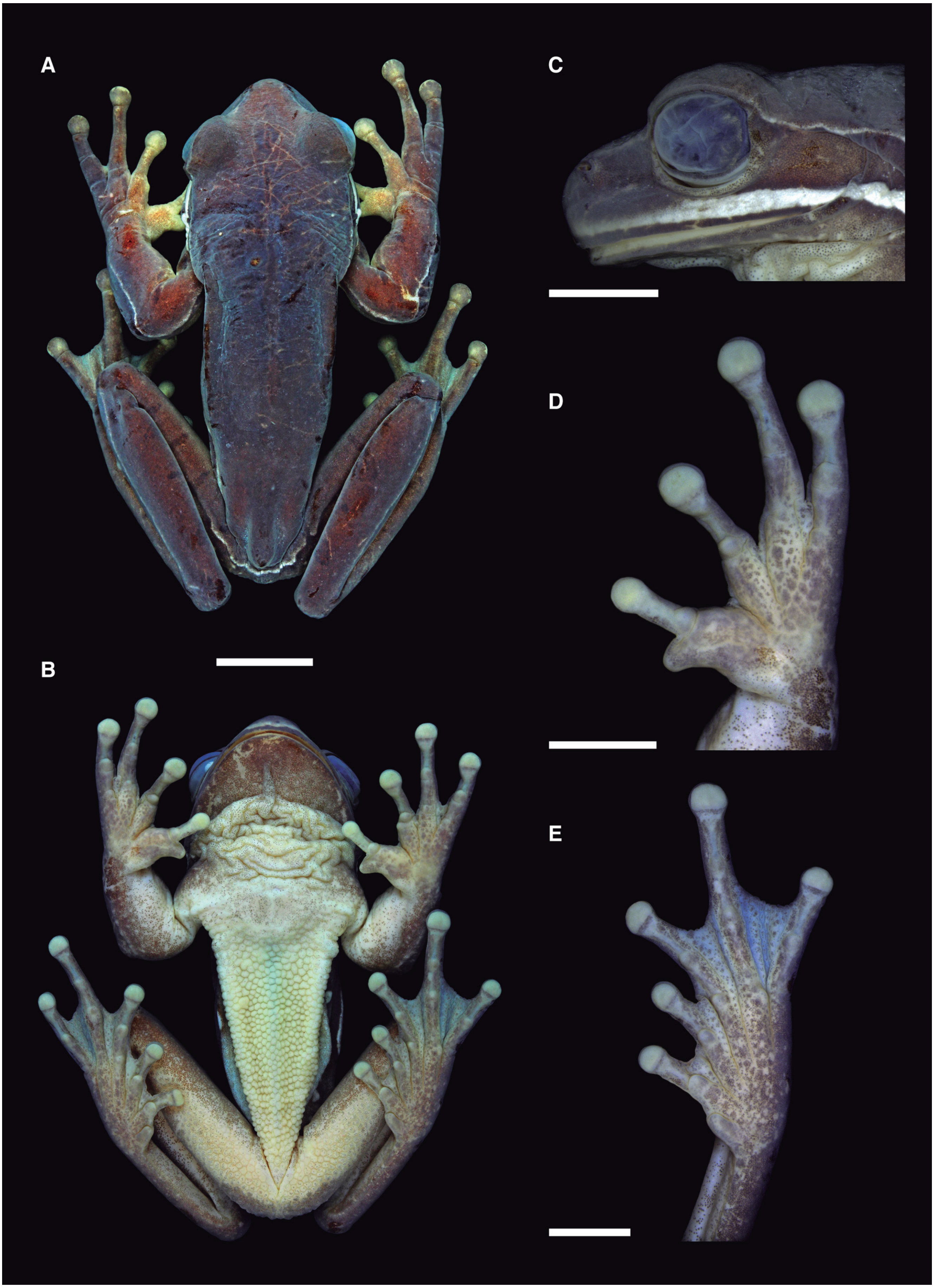


Figure 2. Holotype of *Boana itajahy* **sp. nov.** (CHUFSC 5052). **(A)** dorsal and **(B)** ventral views; **(C)** lateral profile of head; **(D)** palmar surface of left hand; (E) plantar surface of tarsal region of left foot. Scale bars: (A, B) 10 mm; (C–E) 5 mm.

27°06′26.5″S, 49°09′31.8″W, datum SAD 69, 753 m elevation, P.C.A. Garcia and F. Dallacorte, 24 October 2009.

Paratypes. Twenty specimens, all collected in Santa Catarina, Brazil. CHUFSC 5050, 5051, UFMG 4562, 4566, four adult males, UFMG 4565, adult female, same collection data as for holotype. UFMG 4564, adult male, same locality and collectors as for holotype, 25 October 2009. UFMG 5735, adult male, Lajeado Alto, Parque Nacional da Serra do Itajaí, Guabiruba, F. Dallacorte, 17 June 2007. MZUSP 138801, adult male, Parque Nacional da Serra do Itajaí, Indaial, 27°06′21.9″S, 49°09′25.3″W, datum SAD 69, 751 m elevation, F. Dallacorte, 7–13 September 2008. FURB 3301, 3307, 3308, three adult males, Parque Nacional da Serra do Itajaí, Blumenau, 27°03′29″S, 49°05′05″W, datum SAD 69, 319 m elevation, F. Dallacorte, 16–21 August 2001. FURB 3362, 3363, two adult males, same locality as for preceding, F. Dallacorte, 21 October 2001. FURB 3374, adult male, same locality as for preceding, F. Dallacorte, 16 December 2001. FURB 3467, adult male, same locality as for preceding, F. Dallacorte, 24 November 2002. FURB 3613, 3617, two adult males, Parque Nacional da Serra do Itajaí, Blumenau, 27°03′37″S, 49°06′43″W, datum SAD 69, 375 m elevation, F.O. Dias, 13 December 2004. FURB 3622, 3625, two adult males, same locality as for preceding, F.O. Dias, 09–10 February 2005. FURB 3645, adult male, same locality as for preceding, F.O. Dias, 04 March 2005. CHUFSC 5051 (previously UFMG 4561) is DNA voucher specimen (GenBank: MT823969, MT804912). UFMG 4562 is DNA voucher specimen (GenBank: MT823970, MT824109, MT824481, MT824521).

Figure 3. Coloration in life of *Boana itajahy* **sp. nov. (A)** Female paratype UFMG 4565; **(B)** male paratype FURB 3467 (note scars on male dorsum and hind limbs). **(C)** Chorus of four males (pointed by white arrows) at the margin of a creek in the Parque Nacional da Serra do Itajaí (PNSI), Blumenau, state of Santa Catarina, Brazil.

Referred material. FURB 14100, adult female, Parque Nacional da Serra do Itajaí, Indaial, Santa Catarina, Brazil, R. Pasold, 26 October 2008. DNA voucher specimen (GenBank: MW039714, MW039758, MW039803).

Diagnosis. A species of the *Boana pulchella* group, nested within the *B. semiguttata* clade (Faivovich et al., 2021). The new species shares the absence of a scapular origin of the *m. depressor mandibulae* (FURB 3645) with species of the group (Pinheiro et al., 2018). *Boana itajahy* **sp. nov.** can be diagnosed by the following combination of characters: (1) advertisement call composed of two types of pulsed notes, composed of (2) one A note followed by a series of 2–16 B notes; (3) note A with lower relative amplitude than note B; (4) note A with 43–79 pulses and note B with 4–81 pulses; (5) interval between note A and first note B 195–251 ms and between two consecutive notes B 183–321 ms; (6) no clear pattern of modulation in relative amplitude among notes (the amplitude fluctuates irregularly); (7) male SVL 42.3–54.6 mm; (8) posterior surface of the thigh with uniform coloration, lacking spots, stripes or bars; (9) dorsal surface of hindlimb varying from presenting almost indistinct blotches to presenting dark-brown blotches of variable size and shape; (10) males with hypertrophied forearm; (11) in life, dorsum uniform dark-brown to beige, or dark olive-green, with or without dark brown blotches of different sizes and shapes; (12) white dorsolateral line from behind the eyes to inguinal region present; (13) supralabial stripe present, extending posteriad past tympanum; (14) flanks with series of rounded white blotches arranged longitudinally.

Comparisons with species of the* Boana pulchella *group. The advertisement call is structured in two types of notes, a single A note followed by a series of B notes, distinguishing *Boana itajahy* **sp. nov.** from all other species of the *B. semiguttata* clade, as follows: *B. poaju* and *B. stellae* emit a single type of note in series of 5–16 and as many as 20 notes, respectively (Garcia et al., 2008; Kwet, 2008); *B. semiguttata* emits series of a single type of note (Garcia et al., 2007). The regular interval of 195–251 ms between note A and the first note B and regular intervals of 183–321 ms between consecutive notes B differentiates the advertisement call of *B. itajahy* **sp. nov.** from the call of *B. semiguttata* (advertisement call composed of irregularly spaced notes, previously described as irregularly spaced pulses in "note A" by Garcia et al., 2007). The amplitude modulation of the advertisement call of *B. itajahy* **sp. nov.** remains consistent throughout the entire call (i.e., the amplitude neither increases nor decreases), whereas the relative amplitude of the notes progressively increases as the call progresses in the advertisement call of *B. semiguttata* (possible to see in Garcia et al., 2007, fig. 5). *Boana caipora, B. curupi,* and *B. joaquini* emit calls composed of 1–2 notes (Garcia et al., 2003, 2007; Antunes et al., 2008). The larger male size (SVL 42.3–54.6 mm) of *Boana itajahy* **sp. nov.** clearly differentiates it from *B. caipora* (29.5–38.9 mm in our measurements; reported as 29.7–37.5 mm by Antunes et al., 2008) and *Boana* sp. 2 (36.7–41.5 mm; see below). *Boana itajahy* **sp. nov.** male SVL slightly overlaps with those of *B. curupi* (33–44.3 mm in our measurements; reported as 29–43.4 mm by Garcia et al., 2007), *B. poaju* (33.8–42.6 mm in our measurements; reported as 33.5–42.7 mm by Garcia et al., 2008), and *B. semiguttata* (31.2–43.9 mm in our measurements; reported as 36.1–45.2 mm by Garcia et al., 2007). The posterior surface of the thigh lacks markings or possesses white flecks near the cloaca in fixed specimens of *B. itajahy* **sp. nov.**, whereas it bears white flecks, white spots, or white reticulation in *B. stellae* (personal observations; Kwet, 2008). *Boana itajahy* **sp. nov.** can be distinguished from *B. joaquini* by the absence of white or pale markings on the dorsal surfaces of the hindlimbs (present in *B. joaquini;* Garcia et al., 2003).

The advertisement call (i.e., Call A) composed of two types of notes, in which one A note is followed by several B notes, promptly differentiates *Boana itajahy*

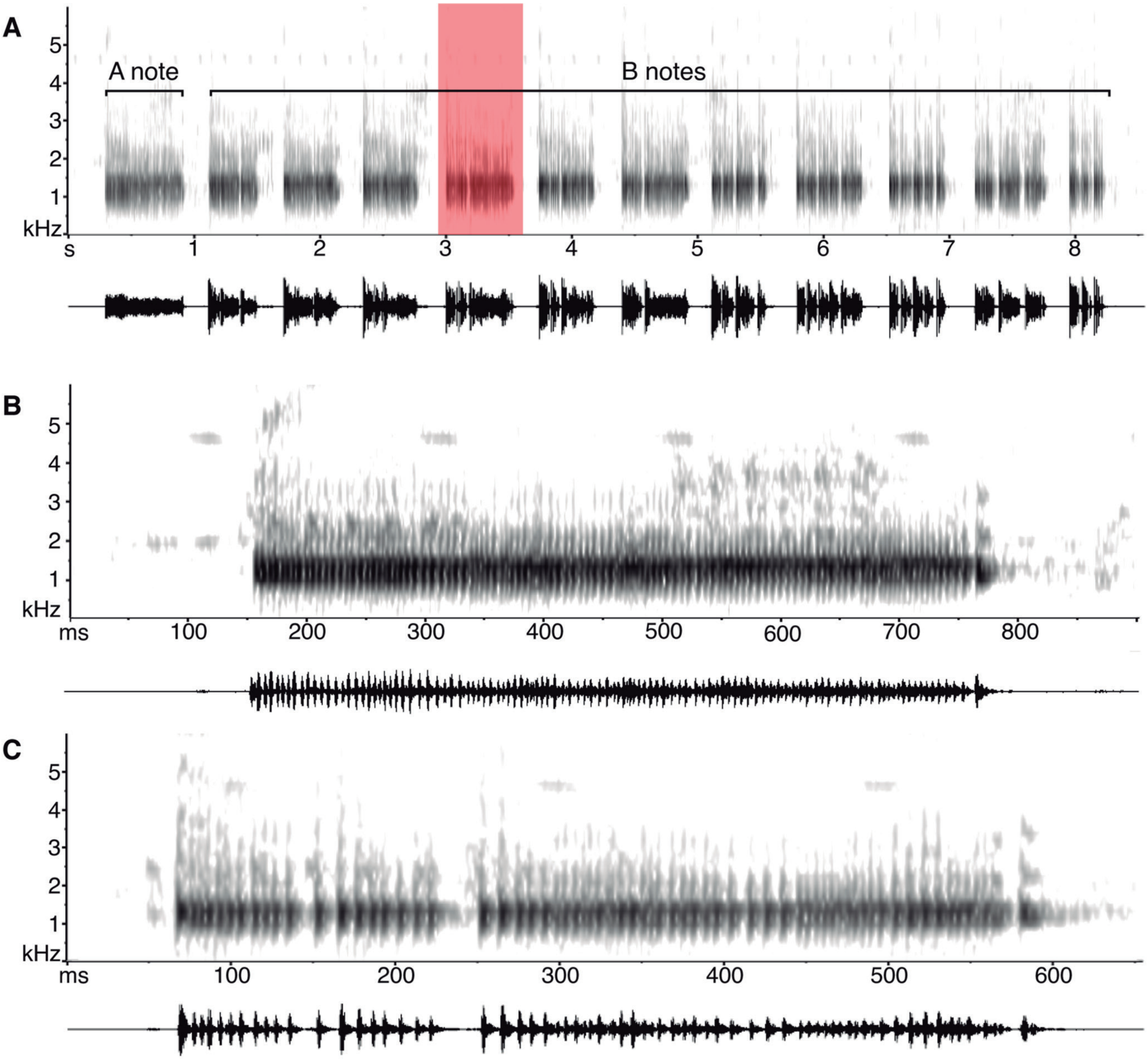
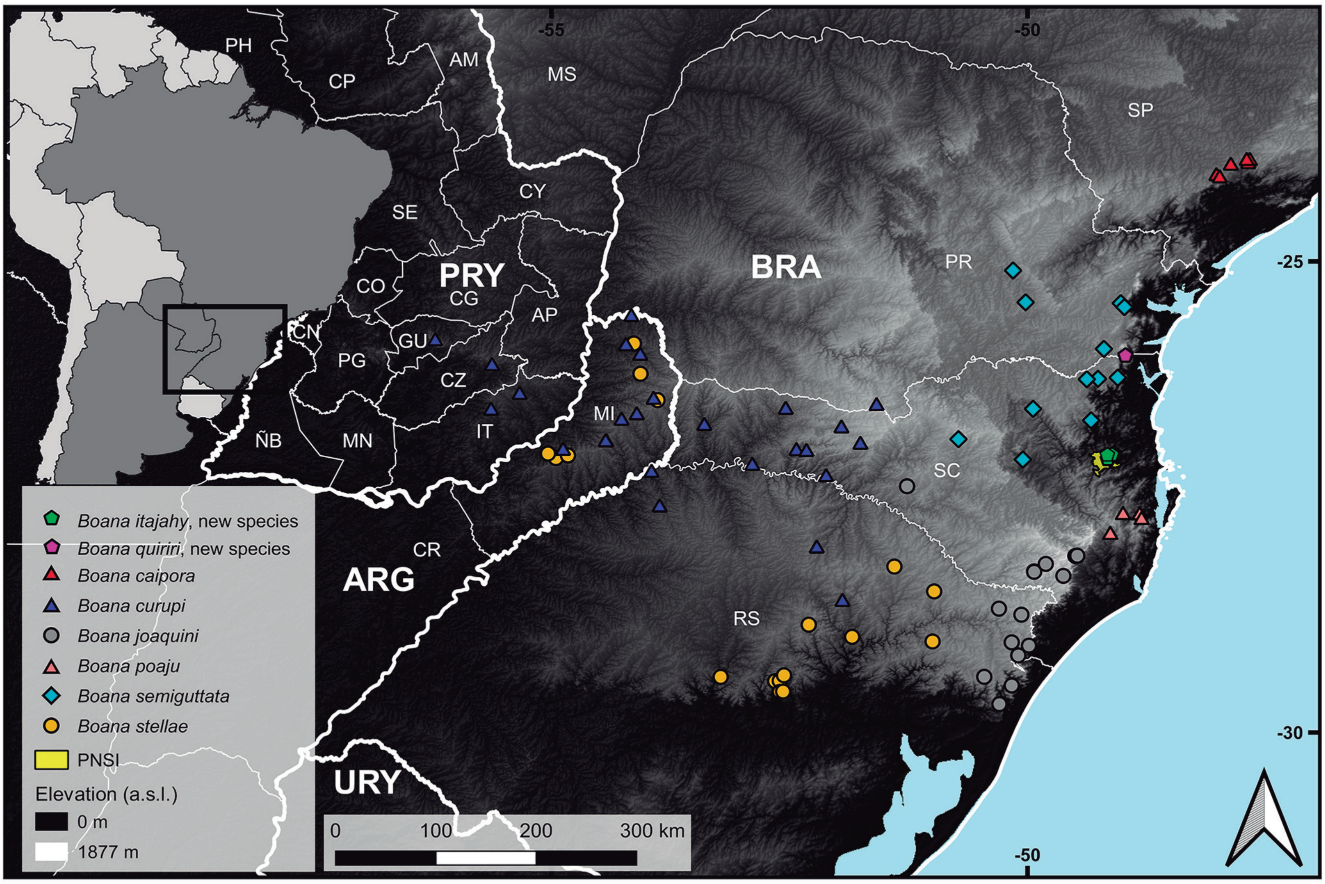


Figure 4. Advertisement call (Call A) of Boana itajahy sp. nov. from recording CBUFMG 1081 (voucher UFMG 4564), Parque Nacional da Serra do Itajaí (PNSI), Blumenau, Santa Catarina, Brazil; 25 October 2009, air temperature 16.3°C, 21:25 Brazilian daylight-saving time. **(A)** Entire advertisement call, composed of one A note followed by a series of B notes. **(B)** Expansion of A note from (A). **(C)** Expansion of B note marked in red in (A).

sp. nov. from all other species in the *B. pulchella* group except *B. marginata.* However, in *B. itajahy* **sp. nov.** note A has lower relative sound amplitude than notes B, whereas in *B. marginata* note A has higher relative amplitude than note B. Also, note A of *B. itajahy* **sp. nov.** comprises 43−79 (75 ± 19.8) pulses (vs. 10−18; 13.2 ± 4.4 pulses in *B. marginata*), followed by 2−16 notes B with 4−81 (44.3 ± 14) pulses each (note A followed by until 11 notes B with 22−48; 33.8 ± 7.5 pulses each in *B. marginata;* Garcia et al., 2001a).

The larger size of *Boana itajahy* **sp. nov.** (SVL 42.3−54.6 mm in males) differentiates it from *B. botumirim, B. buriti, B. caingua, B. cambui, B. cipoensis, B. ericae, B. goiana, B. guarinimirim, B. guentheri, B. jaguariaivensis, B. leptolineata, B. polytaenia,* and *B. stenocephala* (SVL in males 23.9−40.6 mm in those species combined; Lutz, 1968b; Braun and Braun 1977; Carrizo, 1991; Langone, 1997; Cruz and Caramaschi 1998; Caramaschi and Cruz, 1999, 2000, 2004; Caramaschi et al., 2009, 2010; Pinheiro et al., 2016; Faivovich et al., 2021; Marinho et al., 2022). The hypertrophied forearms in males of *B. itajahy* **sp. nov.** differentiates it from *B. aguilari, B. bischoffi, B. botumirim, B. buriti, B. caingua, B. cambui, B. cipoensis, B. ericae, B. goiana, B. guarinimirim, B. guentheri, B. leptolineata, B. melanopleura, B. polytaenia, B. prasina,* and *B. pulchella* (forearms not hypertrophied in those species; Braun and Braun 1977; Caramaschi and Cruz, 1999, 2000; Lehr and von May, 2004; Toledo et al., 2007; Garcia and Haddad, 2008; Lehr et al., 2010; Pinheiro et al., 2016; Marinho et al., 2022; PDPP and PCAG pers. obs.). The dorsal pattern varying from being uniform to presenting blotches of various shapes and sizes differentiates *B. itajahy* **sp. nov.** from *B. botumirim, B. buriti, B. cipoensis, B. guarinimirim, B. jaguariaivensis, B. leptolineata, B. polytaenia, B. stenocephala, B. bischoffi* (populations of the state of São Paulo; see Marcelino et al., 2009), *B. caingua,* and *B. goiana* (dorsal color pattern formed by well-marked longitudinal stripes of different brown tones and/or white in those species; Cope, 1870; Lutz, 1968b; Braun and Braun, 1977; Heyer et al., 1990; Carrizo, 1991; Cruz and Caramaschi, 1998; Caramaschi and Cruz, 1999; Caramaschi et al., 2009, 2010; Marinho et al., 2022); from *B. cambui* and *B. freicanecae* (dorsal color pattern consisting of two dorsolateral stripes connected



Elaborated with QGIS 3.10.5

Figure 5. Map of distribution of *Boana itajahy* **sp. nov.** (green pentagons), *B. quiriri* **sp. nov.** (magenta pentagon), and other species of the *Boana semiguttata* clade (for geographic coordinates used to elaborate the map see Online Supporting Information). The inset up left shows partial view of South America in light grey, with Argentina, Brazil, and Paraguay highlighted in dark grey; the black square delimits the area showed in the main map. On the main map, broad white lines delimit countries boundaries; thin white lines delimit the equivalent geopolitical subdivisions: provinces (Argentina), states (Brazil), and departments (Paraguay). The area highlighted in yellow delimits the Parque Nacional da Serra do Itajaí (PNSI). Abbreviations in larger and bold font are: ARG: Argentina; BRA: Brazil; PRY: Paraguay; URY: Uruguay. Abbreviations in smaller and regular font are: AM: Amambay; AP: Alto Paraná; CG: Caaguazú; CN: Central; CO: Cordillera; CP: Concepción; CR: Corrientes; CY: Canindeyú; CZ: Caazapá; GU: Guairá; IT: Itapúa; MI: Misiones (Argentina); MN: Misiones (Paraguay); MS: Mato Grosso do Sul; ÑB: Ñeembucú; PG: Paraguarí; PH: Presidente Hayes; PR: Paraná; RS: Rio Grande do Sul; SC: Santa Catarina; SE: San Pedro; SP: São Paulo.

Table 3. Some measurements and proportions of the type series of *Boana itajahy* **sp. nov.** Values (in mm) are reported as minimum–maximum ($\bar{x}$ ± SD). See text for measurement abbreviations.

	Measurements	
	Males (*n* = 20)	**Female (*n* = 1)**
SVL	42.3–54.6 (48.4 ± 3.7)	52.8
HL	14.6–17.9 (16.5 ± 1.1)	18.2
HW	13.9–18.3 (16.7 ± 1.1)	17.6
ED	4.4–5.7 (4.9 ± 0.4)	5.6
TD	1.8–2.5 (2.2 ± 0.2)	2.4
EW	3.2–5.0 (4.2 ± 0.5)	4.9
END	3.3–4.5 (4.1 ± 0.4)	4.5
IOD	4.1–5.6 (4.7 ± 0.4)	5.0
NSD	1.8–3.1 (2.6 ± 0.3)	3.0
IND	2.8–3.5 (3.2 ± 0.2)	3.2
AMD	8.3–10.1 (9.2 ± 0.5)	9.7
FAL	8.2–12.0 (10.2 ± 1.0)	10.4
HAL	13.5–18.5 (16.5 ± 1.3)	17.0
4FD	1.7–2.7 (2.2 ± 0.2)	2.3
THL	20.2–28.0 (25.3 ± 2.3)	28.4
TL	21.0–28.6 (26.3 ± 2.3)	29.1
TAL	12.3–16.4 (15.1 ± 1.2)	16.3
FL	17.7–25.4 (22.2 ± 2.0)	25.3
4TD	1.6–2.6 (2.1 ± 0.2)	2.0

	Proportions	
	Males (*n* = 20)	**Female (*n* = 1)**
HW/HL	0.92–1.08 (1.01 ± 0.04)	0.96
END/ED	0.72–0.99 (0.83 ± 0.08)	0.81
ED/HL	0.25–0.34 (0.30 ± 0.03)	0.31
ED/HW	0.25–0.35 (0.30 ± 0.02)	0.32
IOD/ED	0.79–1.24 (0.96 ± 0.10)	0.88
IOD/HW	0.26–0.33 (0.28 ± 0.02)	0.28
TD/ED	0.34–0.52 (0.45 ± 0.05)	0.42
TD/4FD	0.79–1.25 (1.01 ± 0.13)	1.05
FAL/SVL	0.19–0.25 (0.21 ± 0.01)	0.20
HAL/SVL	0.29–0.37 (0.34 ± 0.02)	0.32
THL/SVL	0.46–0.58 (0.52 ± 0.03)	0.54
TL/SVL	0.48–0.57 (0.54 ± 0.02)	0.55
4TD/4FD	0.84–1.02 (0.96 ± 0.05)	0.88
TL/THL	0.99–1.08 (1.04 ± 0.03)	1.02
FL/SVL	0.40–0.51 (0.46 ± 0.03)	0.47

to a dorsal triangle anterior to the eyes forming a dorsal frame in those species; Carnaval and Peixoto, 2004; Pinheiro et al., 2016); and from *B. marginata* (overall green coloration; Garcia et al., 2001a). The absence of spots, stripes, or bars on the posterior surfaces of the thighs promptly distinguishes *B. itajahy* **sp. nov.** from *B. balzani, B. bischoffi, B. caingua, B. callipleura, B. cordobae, B. cymbalum, B. gladiator, B. guentheri, B. marianitae, B. prasina, B. pulchella,* and *B. riojana* (posterior surfaces of thighs presenting heterogenous pattern consisting of vertical bars, blotches, spots, or reticulations, of variable colors in those species; Bokermann, 1963; Barrio, 1965; Heyer et al., 1990, Carrizo, 1991, 1992; Langone, 1997; Köhler et al., 2010). The presence of a white dorsolateral line in *B. itajahy* **sp. nov.** differentiates it from *B. aguilari, B. balzani, B. cordobae, B. cymbalum, B. gladiator, B. marianitae, B. melanopleura,* and *B. palaestes* (dorsolateral line absent in those species; Bokermann, 1963; Barrio, 1965; Duellman et al., 1997; Lehr and von May, 2004; Köhler et al., 2010; Lehr et al., 2010). The presence of a distinct supralabial stripe extending beyond the tympanum posteriorly differentiates *B. itajahy* **sp. nov.** from *B. balzani, B. cordobae, B. gladiator,* and *B. palaestes* (supralabial stripe absent in those species; Barrio, 1965; Duellman et al., 1997; Köhler et al., 2010). The flanks with a longitudinal series of rounded white blotches differentiates *B. itajahy* **sp. nov.** from *B. aguilari, B. balzani, B. gladiator, B. marianitae, B. melanopleura,* and *B. palaestes* (flanks with white flecks and/or irregular blotches scattered in those species; Köhler et al., 2010; Lehr et al., 2010).

Description of the holotype. Adult male, SVL 50.0 mm; body robust, head as wide as body at arm level, nearly as wide as long (HW/HL = 0.92); snout rounded in dorsal view, slightly rounded in lateral view (Fig. 2), shorter than eye diameter (END/ED = 0.75); *canthus rostralis* distinct, slightly curved in dorsal view, rounded in cross-section; loreal region concave; lips thin, not flared; internarial region not depressed; nostrils weakly protuberant, oval, directed anterolaterad; posterior margin of nasal fossa forming thin aperture partially contouring the nostril. Interorbital area flat, slightly shorter than eye diameter (IOD/ED = 0.95), three times shorter than head width (IOD/HW = 0.33). Eyes moderate in size (ED/HL = 0.32; ED/HW = 0.34), lateral, directed slightly anteriad; pupil horizontal; palpebral membrane translucent, without reticulations. Supratympanic fold present, prominent, covering posterodorsal margin of tympanic ring, starting behind upper eyelid, almost reaching arm insertion. Tympanum small (TD/ED = 0.40), distinct, directed laterad, distance to eye less than tympanum diameter.

Arm robust, forearm hypertrophied, lacking axillary membrane; ulnar fold formed by small tubercles extending along external margin of forearm ventrally; fingers robust, long, bearing round discs; finger IV disc diameter and tympanum diameter almost equal (TD/4FD = 0.98); finger II disc smaller than others; relative finger lengths II < III < V < IV; webbing formula II–III2^{+}–3IV3^{-}–2V; fingers bearing small lateral fringes; subarticular tubercles distinct, non-bifid, rounded in ventral view; subarticular tubercles of fingers IV and V flatter than those of fingers II and III; supernumerary tubercles present; inner metacarpal tubercle flat, elongated, curved to encase the spine-shaped prepollex ventrally; outer metacarpal tubercle bifid, flat, small, indistinct (Fig. 2). Nuptial pad absent; prepollex enlarged, pointed, forming bony spine.

Hind limbs long, slender (THL/SVL = 51; TL/SVL = 0.53); thigh slightly shorter than tibia (THL/TL = 0.97); tarsal fold present, extending from inner metatarsal tubercle to heel; ventrolateral tarsal fringe absent; calcar absent. Toes robust, bearing well-developed, round discs, toe disc diameter approximately the same as finger disc diameter (4TD/4FD = 1.02); relative toe lengths I < II < V = III < IV; webbing formula I$1^{1/2}$–2II1^{+}–2III$1^{1/2}$–$2^{1/2}$IV$2^{1/2}$–1V; lateral fringes on toes present; thickened layer of tissue at midline of webbing between toes IV and V absent. Subarticular tubercles moderately large, round in ventral view, flatter in toes IV and V than in I, II, and III; supernumerary tubercles present but indistinct; outer metatarsal tubercle absent; inner metatarsal tubercle distinct, flat, small, elliptical in ventral view (Fig. 2).

Skin smooth except on pectoral and abdominal areas, and ventral surfaces of thighs, where it is granular/areolate. Pectoral fold absent. Cloacal opening at upper level of thighs, directed posteroventrad by a small cloacal sheath partially covering the aperture; supracloacal dermal ridge absent; cloacal tubercles present, delimiting a narrow median canal, directed ventrad. Tongue slightly cordiform, barely free behind; dentigerous processes of vomers prominent, forming two nearly straight, approximately transverse series that converge medially, separated by the distance of ~2 teeth. Right and left processes bearing 6 and 5 teeth, respectively. Choanae almost round, 4.0 mm apart. Vocal slits moderately long, extending from angle of jaws anteriad along approximately 2/3 of mandible length. Vocal sac single, median, subgular. Dorsum bearing several scars (Fig. 2).

Measurements (mm). SVL 50.0, HL 17.9, HW 16.5, ED 5.7, TD 2.3, EW 4.7, END 4.3, IOD 5.4, NSD 2.4, IND 3.4, AMD 9.5, FAL 10.2, HAL 17, 4FD 2.3, THL 25.7, TL 26.5, TAL 14.3, FL 22.9, 4TD 2.4.

Coloration in preservative. In alcohol, the dorsum is uniformly bluish brown. A light brown loreal stripe extends from the tip of the snout and continues behind the eye, passing over the tympanum, merging with the coloration of the flank. The upper lip is delimited by a thin dark brown line. Above it, a white supralabial stripe extends posteriad to the arm insertion. A white dorsolateral line extends from the posterior margin of the eye until the leg insertion. The flank is light brown with a series of rounded white blotches arranged longitudinally. The dorsum of the arms and legs is the same color as the body dorsum. The forearm and shank both have a lateral white line (from elbow to proximal finger V on the forearm; ankle to knee on the shank), delimiting dorsally a wide stripe darker than body dorsum. The dorsum of the thigh and shank has a series of dark rounded blotches, barely notable. The anterior surface of thigh is light brown, whereas the posterior surface is light brown with scattered white spots. There is a transversal white line above cloacal opening. The body venter is cream, with the mental region bearing dark pigmentation.

Variation. See Table 3 for measurements of the type series. There is variation in both hand webbing [II—III(2^{-}–2^{+})—(3^{-}–3^{+})IV($2^{1/2}$–3)—(2–$2^{1/2}$)V] and toe webbing [I(1–2^{+})—(2–2^{+})II(1–$1^{1/2}$)—(2–3^{-})III(1–2)—(2^{+}–3)IV(2–3)—(1–1^{+})V]. There are 3–7 vomerine teeth on the right process, 4–9 on the left one. The snout can be truncated in dorsal (UFMG 4564) and lateral (FURB 3617, 3625, 3645, UFMG 4564) views, and slightly sloping (MZUSP 138801) or slightly protruding (FURB 3308) in lateral view. The tubercles on the external margin of te forearm can be absent (FURB 3362, UFMG 4565), indistinct (FURB 3363), or fused to form a continuous fold (CHUFSC 5051, FURB 3467, UFMG 4564, 5735). The tarsal fold can be absent (FURB 3307) or indistinct (FURB 3363). In preservative, the dorsal background color varies between cream (UFMG 5735), beige (FURB 3301, 3374, 3362, 3363), light brown (FURB 3307, 3613, 3617, 3622, 3625, MZUSP 138801, UFMG 4565), dark brown (CHUFSC 5050, FURB 3645), and grey (CHUFSC 5051, FURB 3308, 3467, UFMG 4562, 4564, 4566). Dorsal coloration can be uniform, lacking blotches or spots (FURB 3301, 3308, 3363, 3467, 3645, UFMG 4564), or bear blotches of variable shape, size, and number. In some specimens, a large, central blotch is present, sometimes forming an hourglass shape (FURB 3374, 3625, UFMG 4562). UFMG 4566 has an incomplete dark brown vertebral line. The dorsal blotches are usually darker than the background color, but they can also be lighter (FURB 3362, UFMG 4562). In UFMG 4564, the dorsolateral white line merges with some white blotches of the flank. Dorsal limb blotches vary in number and are absent in some specimens (FURB 3301, UFMG 4562). The mental region is unpigmented in some specimens (FURB 3301, 3307, 3308, 3363, 3613, 3617, UFMG 4564, 4565, 5735).

The female specimen (UFMG 4565) has a poorly developed prepollical spine medial to the first finger (it is swollen and presumably at least partially cartilaginous). Its forearm is not hypertrophied, being slender compared to males. Its body is more slender than males. Cloacal tubercles do not delimit a ventrally directed narrow median canal. Whereas the female has no scars on dorsum, 10 of the 20 males in the type series do (CHUFSC 5052, FURB 3307, 3308, 3363, 3466, 3617, 3625, 3645, UFMG 4564, 4566).

Coloration in life. The following description of variation is based on field notes and photographs of some paratypes in life. The background color of the dorsum and dorsal surfaces of limbs varied from uniform dark brown to beige or dark olive-green, with or without dark brown blotches of different sizes and shapes (UFMG 4564, 4565; Fig. 3). Rounded blotches on the dorsum of the forearm, thigh, and shank can be very evident. The loreal region can present green markings (UFMG 4564). The supralabial stripe and blotches on flanks varied from cream to white. The posterior surfaces of the thighs were dark brown. Flank's background color varies from grey to dark brown. The venter of the body and limbs varied from light grey to cream. The iris was golden.

Vocalization. Three recordings from the type locality were analyzed. Two are from an unvouchered specimen (24 October 2009, air temperature 18.8°C, 20:41 daylight saving time; CBUFMG 1079 and 1080), and one is from voucher UFMG 4564 (25 October 2009, air temperature 16.3°C and, at 21:25 daylight saving time; CBUFMG 1081). Males were recorded along small rivulets, perched on branches above the water. Many individuals were calling along the rivulets (Fig. 3C).

Only one type of call was detected, interpreted as the advertisement call. It is a compound call consisting of one A note followed by a series of B notes (Fig. 4). Note A was usually longer and had lower relative amplitude than B notes (i.e., it reaches lower ku levels in the waveform; *n* = 15). It was followed by a series of 2–16 (7.67 ± 4.76; *n* = 15), usually shorter and more intense, B notes (see means below in description of A and B notes). Isolated A notes were recorded eight times, whereas isolated B note were only recorded once. The duration of entire calls, composed of one A note followed by a series of B notes, was 1.82–14.02 s (6.55 ± 4.12; *n* = 15), with variation clearly related to the variation in the number of B notes per call. Intercall intervals were irregular. The interval between two typical calls (i.e., one composed of an A note + series of B notes) was 1.1–155.3 s (52.3 ± 63; *n* = 11). In a typical call, the interval between note A and the first B note was 195–251 ms (215 ± 19; *n* = 15), and the interval between two consecutive B notes was 183–321 ms (231 ± 29; *n* = 100). Isolated A notes preceded the following call by 0.7–46.8 s (13.2 ± 19.8; *n* = 8). The isolated note B preceded the following call by 2.6 s.

Note A duration was 498–781 ms (595 ± 74; *n* = 23) and it was formed by 43–79 (75 ± 19.8; *n* = 23) regularly spaced pulses with little amplitude variation (Fig. 4B). The fundamental frequency was dominant and covered 672.5–1898.6 Hz (*n* = 23), with peak frequencies at 1,125 (*n* = 8), 1,312.5 (*n* = 7), and 1,500 Hz (*n* = 8). Up to seven additional higher-frequency, lower energy bands were observed, not associated with harmonics.

Note B duration was 47–748 ms (533–115; *n* = 116) and it was formed by 4–81 (44.3 ± 14; *n* = 116) irregularly spaced pulses with great variation in intensity (Fig. 4C). Note that a single B note with only four pulses recorded, another note comprised five pulses, and among the remaining 114 notes, the minimum number of pulses was 21; thus, we believe that four and five pulses are abnormal. The dominant frequency coincided with the fundamental. The dominant frequency range was between 737.6–1,925 Hz (*n* = 116). The peak frequency was found at 1,125 (*n* = 3), 1,312.5 (*n* = 54), and 1,500 Hz (*n* = 59). Up to four extra higher bands with lower energy were able to be found, not associated with harmonics.

Natural history. Known populations of *Boana itajahy* **sp. nov.** are distributed across submontane and montane rainforest areas (*sensu* Klein, 1979) in the Parque Nacional da Serra do Itajaí. Individuals occur in rivulets up to 5 m wide within the eroded plains of valleys. The rivulet bottoms varied, being either rocky or covered by deposited sand. Air humidity was not lower than 85%, and air temperature was normally 14–19°C, with water temperature ca. 17°C when males were found calling. Males began their vocal activity just before sunset, with peak activity ca. 2 h later. They were found calling perched on vines, shrubs, exposed roots, rocks, and slopes on the rivulet margins 0.2–1.2 m from water, always above backwaters, never in rapids. Males were usually found calling in groups of 3–4 individuals distributed along the rivulet (Fig. 3C). Sometimes, 1–2 females were found near these groups. Some amplectant pairs were observed 6–7 h after the onset of calling. A female would approach a calling male, which would leap over and amplex her, and then the amplectant pair would jump into the water. Spawning was not observed. We observed tadpoles on the sandy bottom of backwater portions of rivulets.

The only female voucher (UFMG 4565), collected 24 October 2009, lacks mature oocytes. As males can be found calling throughout the year, it could be that this female either was not yet sexually active or had already laid her eggs. The high number of males presenting scars on dorsum (10 out of the 20 collected) suggests that territorial combat between males is common.

Aplastodiscus ehrhardti, Bokermannohyla hylax, and, rarely, *Boana bischoffi* (Hylidae), *Vitreorana parvula* (Centrolenidae), and *Hylodes perplicatus* (Hylodidae) were also found calling in the same rivulets.

Male CHUFSC 5051 was infected by at least nine larvae of a parasitic dipteran. As those larvae were fixed at the second instar stage of development, their precise identification is difficult. They probably belong to Calliphoridae or Sarcophagidae. Larvae were distributed on wounds on the right forearm, posterior to the right eye, posterior to the right tympanum, and at mid-length of the left side of the dorsum.

Geographic distribution. *Boana itajahy* **sp. nov.** is known exclusively from forest rivulets within the Serra do Itajaí region at 316–753 m a.s.l. (Fig. 5).

Conservation status. *Boana itajahy* **sp. nov.** is known only from the type locality within the protected area of Parque Nacional da Serra do Itajaí (PNSI; National Park of Itajaí Mountain Range; Fig. 5). Brazilian Law and IUCN category II define national parks as "large natural or near-natural areas, set aside to protect large-scale ecological processes along with the complement of species and ecosystems characteristic of the area" (Brazil, 2000; Lausche, 2011). In this regard, PNSI ensures the conservation of *Boana itajahy* **sp. nov.**

Etymology. The specific epithet, *itajahy,* alludes to the region where the species is found, the Serra do Itajaí (Itajaí Mountain Range), as well as to the Parque Nacional da Serra do Itajaí. The Portuguese name "Itajaí" has an uncertain origin. It could have originated from the Tupi name *tajahy,* which means Taiás' river, with Taiá being an edible plant of the family Araceae. Another explanation from the Tupi suggests that it means "water that runs over stones," derived from the junction of the terms *ita* (stone), *j-* (belongs to), and *-y* (water). A third hypothesis, proposed by journalist and writer Magru Floriano, posits that the name alludes to Mount Tayó, a locality within the Itajaí Valley, desired for its potential gold during colonization in the 17th and 18th centuries (Floriano, 2012). The name *itajahy* is used here as a noun in apposition.

***Boana quiriri,* new species**
Figures 5, 6, 7, 8; Table 4

Boana sp. 2 – Faivovich et al., 2021: 2, 12.

Holotype. CHUFSC 5053, adult male, DNA voucher specimen (as UFMG 20876; GenBank: MT823971,

MT824110, MT824482, MT824335, MT824566), APA (Environmental Protected Area) Campos do Quiriri, Fazenda de Preservação Alto Quiriri, Garuva, Santa Catarina, Brazil, 26°1′35.98″S, 48°58′20.10″W, datum SAD 69, 1333 m elevation, P.D.P. Pinheiro, E.J. Comitti, P.C.A. Garcia, T.H. Condez, J.P. Monteiro, B. Zaidan, D. Baêta, and I. Borel, 27 November 2013.

Paratypes. Ten specimens, UFMG 20883, one female, CHUFSC 5054−5056 and UFMG 20880−20882, 20884−20886, nine males, same collection data as for holotype. Male CHUFSC 5054 (previously UFMG 20877) is a DNA voucher specimen (GenBank: MT823972, MT824111, MT824483, MT824336, MT824522).

Diagnosis. A species of the *Boana pulchella* group, nested within the *B. semiguttata* clade (Faivovich et al., 2021). The new species shares the absence of a scapular origin of the *m. depressor mandibulae* (CHUFSC 5054) with species of the group (Pinheiro et al., 2018). *Boana quiriri* **sp. nov.** can be diagnosed by the following combination of characters: (1) dorsum of forearm and leg with irregular dark pigmented blotches; (2) supralabial stripe yellowish green in life, not surpassing tympanum level posteriorly; (3) posterior surfaces of thighs with uniform pale green coloration in life; (4) flanks usually with series of yellowish green rounded spots in life; (5) advertisement call composed of a series of 3−10 notes of the same type; (6) decreasing inter-note intervals as call progresses; (7) increasing relative amplitude (i.e., intensity) of notes as call progresses, (8) increasing number of pulses per note as call progresses, and (9) increasing frequency among notes as call progresses (i.e., ascending modulation); (10) call duration 324−1,250 ms; (11) male SVL 36.7−41.5 mm; (12) dorsum light to moss green in life, with large light to dark brown blotches forming longitudinal stripes or not; (13) dorsolateral stripe, when present, yellowish green, interrupted or not; (14) ventral surfaces of hands and feet pale green; (15) forearm hypertrophied in adult males.

Comparisons with species of the* Boana pulchella *group. Among species of the *Boana semiguttata* clade, the irregular dark blotches on the forearm and dorsal leg of *Boana quiriri* **sp. nov.** differentiate it from *B. caipora, B. curupi, B. joaquini, B. poaju, B. semiguttata,* and *B. stellae* (forearm and dorsal leg without blotches, or with rounded, well-delimited dark blotches in those species; Garcia et al., 2003, 2007, 2008; Antunes et al., 2008; Kwet, 2008; material examined in this study). The yellowish green coloration (in life) of the supralabial stripe of *B. quiriri* **sp. nov.** sets it apart from *B. caipora, B. curupi, B. itajahy* **sp. nov.**, *B. joaquini, B. poaju, B. semiguttata,* and *B. stellae* (supralabial stripe cream to golden in *B. caipora,* white or cream in the remaining species; Garcia et al., 2003, 2007, 2008; Antunes et al., 2008; Kwet, 2008; present work). Additionally, in *B. quiriri* **sp. nov.** the supralabial stripe does not surpass the tympanum posteriorly, whereas in *B. itajahy* **sp. nov.** it does (present study). The uniform pale green color in life (light grey in preservative) of the posterior surface of thighs of *B. quiriri* **sp. nov.** differentiates it from *B. curupi, B. itajahy* **sp. nov.**, *B. joaquini, B. semiguttata,* and *B. stellae* (in life dark brown, light brown, or light grey, in preservative cream, brown, or light brown, sometimes with white flecks, spots, or reticulations in those species; Garcia et al., 2003, 2007; Kwet, 2008; PDPP pers. obs.). The presence of a series of yellowish green (in life) rounded spots on the flanks distinguishes *B. quiriri* **sp. nov.** from *B. caipora* (cream to golden; Antunes et al., 2008), *B. curupi, B. itajahy* **sp. nov.**, *B. joaquini, B. semiguttata* (cream or white; Garcia et al., 2003, 2007; present study), and *B. stellae* (yellow; Kwet, 2008). In comparison to *B. itajahy* **sp. nov.**, the advertisement call of *B. quiriri* **sp. nov.** exhibits a distinct general structure, characterized by a series of 3−10 notes of the same type (*B. itajahy* **sp. nov.** advertisement call composed of one note A followed by a series of 2−16 notes B; present work). The decreasing values in internotes interval observed in the advertisement call of *B. quiriri* **sp. nov.** differentiate it from *B. caipora, B. curupi, B. itajahy* **sp. nov.**, and *B. poaju* (regular intervals in those species; Garcia et al., 2007, 2008; Antunes et al., 2008; present work). The advertisement call of *B. quiriri* **sp. nov.** is characterized by a gradual increase in both relative amplitude (i.e., intensity) and the number of pulses per note, setting it apart from the calls of *B. poaju* and *B. stellae* (uniform note intensity, with regular pulses per note in *B. poaju* and *B. stellae;* Garcia et al., 2008; Kwet, 2008). It also exhibits ascending frequency modulation among notes that differentiates it from the call of *B. stellae* (frequency modulation among notes absent; Kwet, 2008). The call duration of 324−1,250 ms in *B. quiriri* **sp. nov.** differentiates it from *B. caipora* (149.3−408 ms; Antunes et al., 2008) and *B. curupi* (132−276 ms; Garcia et al., 2007). The bioacoustic parameters of *B. quiriri* **sp. nov.** and *B. semiguttata* overlap (see Discussion). The adult male SVL of *B. quiriri* **sp. nov.** (36.7−41.5 mm) is smaller than that of *B. itajahy* **sp. nov.** (42.3−54.6 mm; this study) and overlaps slightly with the largest male *B. caipora* (29.5−38.9 mm in our measurements; 29.7−39.5 mm in Antunes et al., 2008). The average male SVL of *B. quiriri* **sp. nov.** (39.6 ± 1.5; 36.7−41.5; mm; *n* = 10) is smaller than those of *Boana joaquini* (41.3 ± 6.8; 29.5−55.1 mm; *n* = 27 in our measurements; 47.8 ± 3.8; 40.3−56.4 mm; *n* = 36 in Garcia et al., 2007) and of *B. stellae* (42.2 ± 6.1; 37.1−50.9 mm, *n* = 4 in our measurements; 46.6 ± 2.9, 40.7−49.9, *n* = 9; Kwet, 2008). The light- to moss-green dorsal background color (in life) of *B. quiriri* **sp. nov.** differentiates it from *B. caipora, B. curupi,* and *B. stellae* (background color light brown, almost golden, tan, dark beige, dark brown, or coffee brown in those species; Garcia et al., 2007; Antunes et al., 2008; Kwet, 2008). The yellowish green dorsolateral stripe of *B. quiriri* **sp. nov.**, in life, when present, differentiates it from *B. curupi, B. poaju,* and *B. semiguttata* (dorsolateral stripe black with adjacent white line, or just as a white line or stripe in those species; Garcia et al., 2007, 2008). The pale green ventral surfaces of hands and feet in *B. quiriri* **sp. nov.** differentiates it from *B. stellae* (surfaces dark brown; Kwet, 2008).

From other species of the *Boana pulchella* group, the hypertrophied forearms in males of *B. quiriri* **sp. nov.** differentiates it from *B. aguilari, B. bischoffi, B. botumirim,*

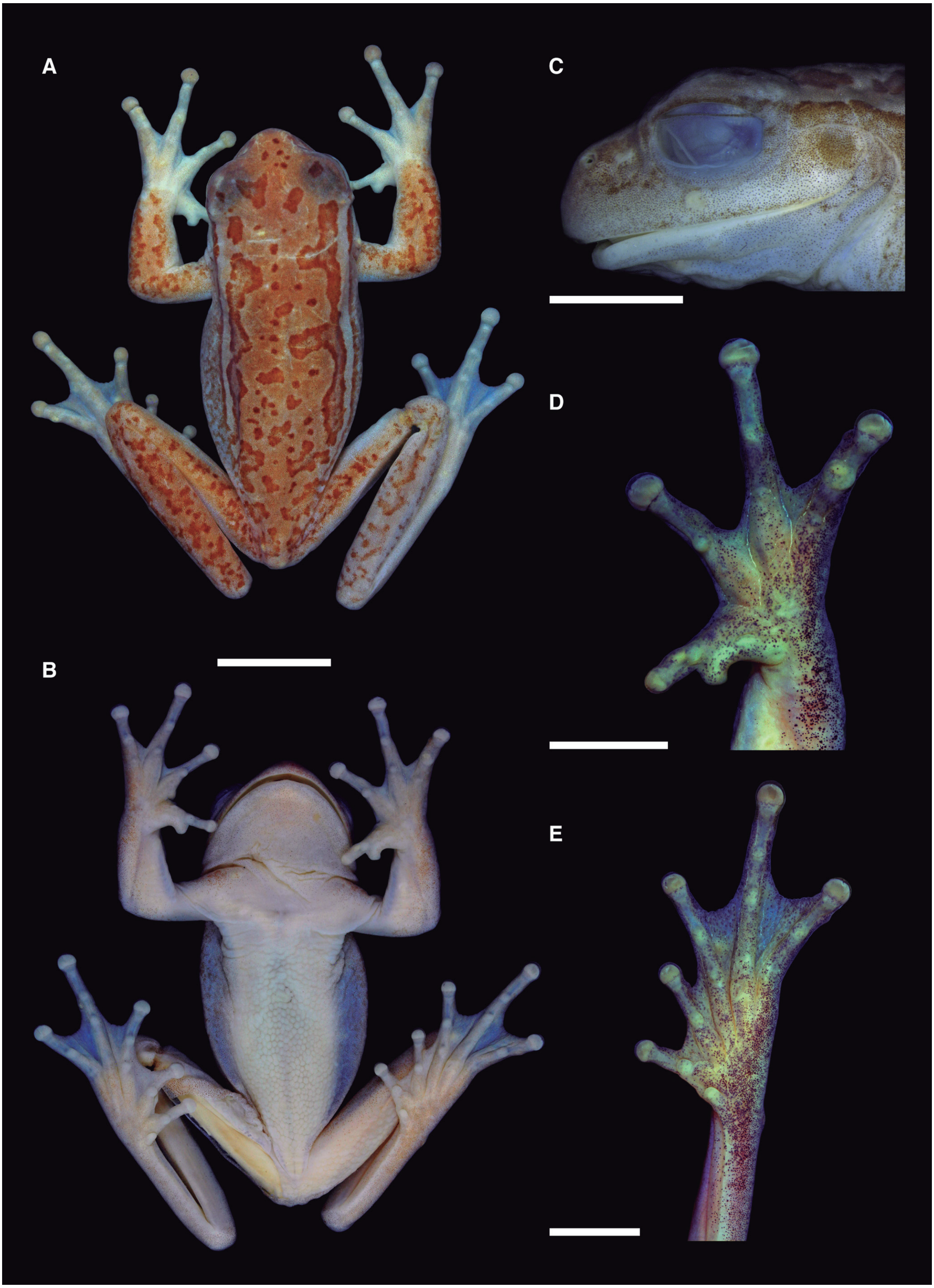


Figure 6. Holotype of *Boana quiriri* **sp. nov.** (CHUFSC 5053). **(A)** dorsal and **(B)** ventral views; **(C)** lateral profile of head; **(D)** palmar surface of left hand; **(E)** plantar surface of tarsal region of left foot. Scale bars of A–B 10 mm; C–E 5 mm.

B. buriti, B. caingua, B. cambui, B. cipoensis, B. ericae, B. goiana, B. guarinimirim, B. guentheri, B. leptolineata, B. melanopleura, B. polytaenia, B. prasina, and *B. pulchella* (forearms not hypertrophied in those species; Braun and Braun, 1977; Caramaschi and Cruz, 1999, 2000; Lehr and von May, 2004; Toledo et al., 2007; Garcia and Haddad, 2008; Lehr et al., 2010; Pinheiro et al., 2016; Marinho et al., 2022; PDPP and PCAG pers. obs.). The dorsal pattern formed by large blotches, light to dark brown on a green background easily differentiates *Boana quiriri* **sp. nov.** from *B. botumirim, B. buriti, B. cipoensis, B. guarinimirim, B. jaguariaivensis, B. leptolineata, B. polytaenia, B. stenocephala, B. bischoffi* (populations of state of São Paulo; see Marcelino et al., 2009), *B. caingua,* and *B. goiana* (dorsal pattern formed by well-marked longitudinal stripes of different brown tones and/or white in those species; Cope, 1870; Lutz, 1968b; Braun and Braun, 1977; Heyer et al., 1990; Carrizo, 1991; Cruz and Caramaschi, 1998; Caramaschi and Cruz, 1999; Caramaschi et al., 2009, 2010; Faivovich et al., 2021; Marinho et al., 2022); from *Boana cambui* and *B. freicanecae* (dorsal pattern consisting of two dorsolateral stripes connected to a dorsal triangle anterior to the eyes forming a dorsal frame in those species; Carnaval and Peixoto, 2004; Pinheiro et al., 2016); and from *B. aguilari, B. gladiator, B. melanopleura,* and *B. palaestes* (dorsal blotches absent in those species; transversal marks present in *B. aguilari* and *B. palaestes;* Duellman et al., 1997; Lehr and von May, 2004; Köhler et al., 2010; Lehr et al., 2010). The pale green coloration of the posterior surfaces of thighs in *B. quiriri* **sp. nov.** promptly differentiates it from *B. balzani, B. bischoffi, B. caingua, B. callipleura, B. cordobae, B. cymbalum, B. gladiator, B. guentheri, B. marianitae, B. prasina, B. pulchella,* and *B. riojana* (posterior surfaces of thighs presenting heterogenous pattern consisting of vertical bars, blotches, spots, or reticulations, of distinct colors in those species; Bokermann, 1963; Barrio, 1965; Heyer et al., 1990, Carrizo, 1991, 1992; Langone, 1997; Köhler et al., 2010). The flanks of *B. quiriri* **sp. nov.** normally present a series of yellowish green spots, while in *B. aguilari, B. balzani, B. ericae, B. gladiator, B. marianitae, B. melanopleura,* and *B. palaestes* those spots are white to cream (Caramaschi and Cruz, 2000; Köhler et al., 2010; Lehr et al., 2010). *Boana quiriri* **sp. nov.** might have a dorsolateral yellowish green stripe (in life), that distinguishes it from *B. marginata* (conspicuous dorsolateral blackline, with an adjacent white stripe; Garcia et al., 2001a). The yellowish green supralabial stripe of *B. quiriri* **sp. nov.** differentiates it from *B. botumirim, B. buriti, B. cipoensis, B. guarinimirim, B. jaguariaivensis, B. leptolineata, B. polytaenia, B. stenocephala, B. bischoffi, B. caingua, B. cymbalum, B. goiana, B. guentheri, B. marginata, B. marianitae, B. prasina,* and *B. pulchella* (supralabial stripe white in those species; Bokermann, 1963; Barrio, 1965; Lutz 1968b; Heyer et al., 1990; Carrizo, 1991, 1992; Langone, 1997; Cruz and Caramaschi, 1998; Caramaschi and Cruz, 1999; Garcia et al., 2001a; Caramaschi et al., 2009, 2010; Marinho et al., 2022).

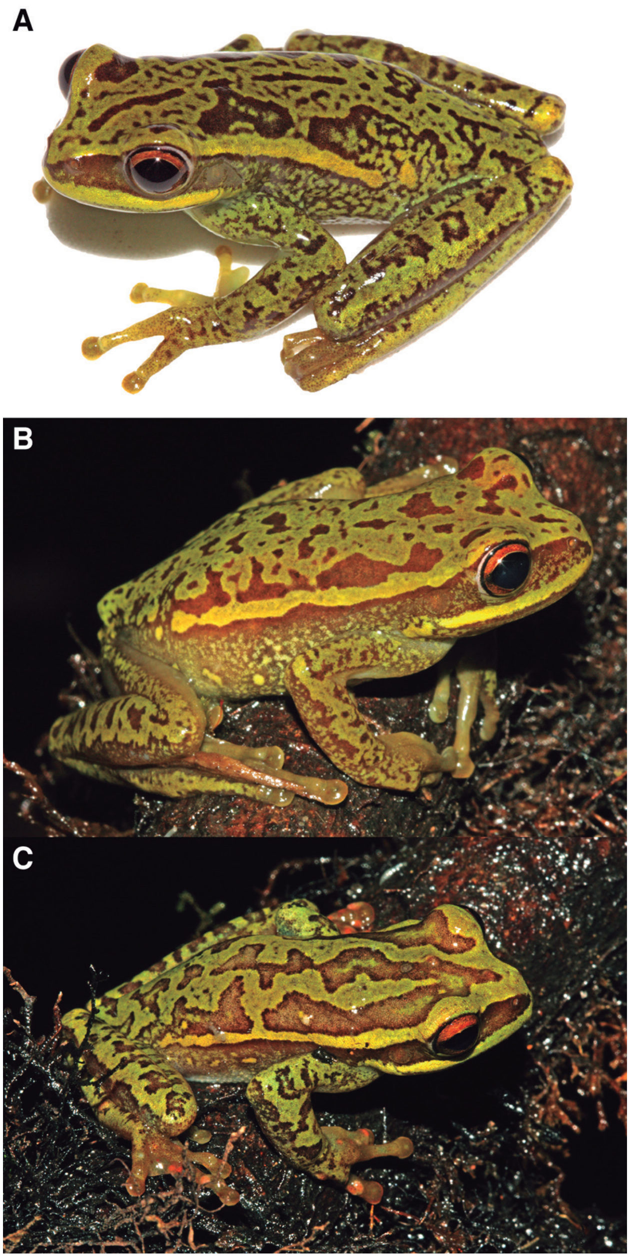


Figure 7. Coloration in life of *Boana quiriri* **sp. nov. (A)** Male paratype UFMG 20882; **(B)** female paratype UFMG 20883; **(C)** male paratype UFMG 20884—notice the orange trumbiculid acari spread across the specimen's limbs.

Description of the holotype. Adult male; SVL 40.2 mm; body robust; head as wide as body at arm level, nearly as wide as long (HW/HL = 1.03); snout rounded in dorsal view, slightly rounded in lateral view (Fig. 6), shorter than eye diameter (END/ED = 0.69); *canthus rostralis* distinct, curved in dorsal view, rounded in cross-section; loreal region slightly concave; lips thin, not flared; internarial region not depressed; nostrils not protuberant, directed dorsolaterad; posterior margin of nasal fossa forming thin aperture partially contouring nostril. Interorbital area flat, shorter than eye diameter (IOD/ED = 0.82), more than three times shorter than head width (IOD/HW = 0.29). Eyes large (ED/HL = 0.36; ED/HW = 0.35), protuberant, lateral, directed slightly anteriad; pupil horizontal; palpebral membrane translucent, without reticulations. Supratympanic fold present, covering upper portion of tympanic ring, starting behind the upper eyelid, extending to arm insertion. Tympanum small (TD/ED = 0.44), distinct, directed laterad, distance to eye approximately equal to tympanum diameter.

Arm robust, slightly hypertrophied, lacking an axillary membrane. Forearms lacking a ventrolateral fringe or a row of small and juxtaposed ulnar tubercles. Fingers slender and long, bearing round discs; disc diameter on finger IV slightly narrower than tympanum (TD/4FD = 1.09); disc on finger II smaller than the others; relative finger length II < III < V < IV; webbing formula II—III2—3^{+}IV3^{-}—2V; presence of small and lateral fringes on fingers; subarticular tubercles distinct, nonbifid, and rounded in ventral view; subarticular tubercles conical in profile on fingers II and III and flat on IV and V; supernumerary tubercles present; inner metacarpal tubercle flat and elongated, curved prepollex; outer metacarpal tubercle bifid, flat, small, and barely distinct (Fig. 6). Nuptial pad absent; prepollex enlarged and pointed as a bony spine.

Hind limbs long and slender (THL/SVL = 0.50; TL/SVL = 0.50); thigh slightly shorter than tibia (THL/TL = 0.99); tarsal fold present, extending from inner metatarsal tuber-

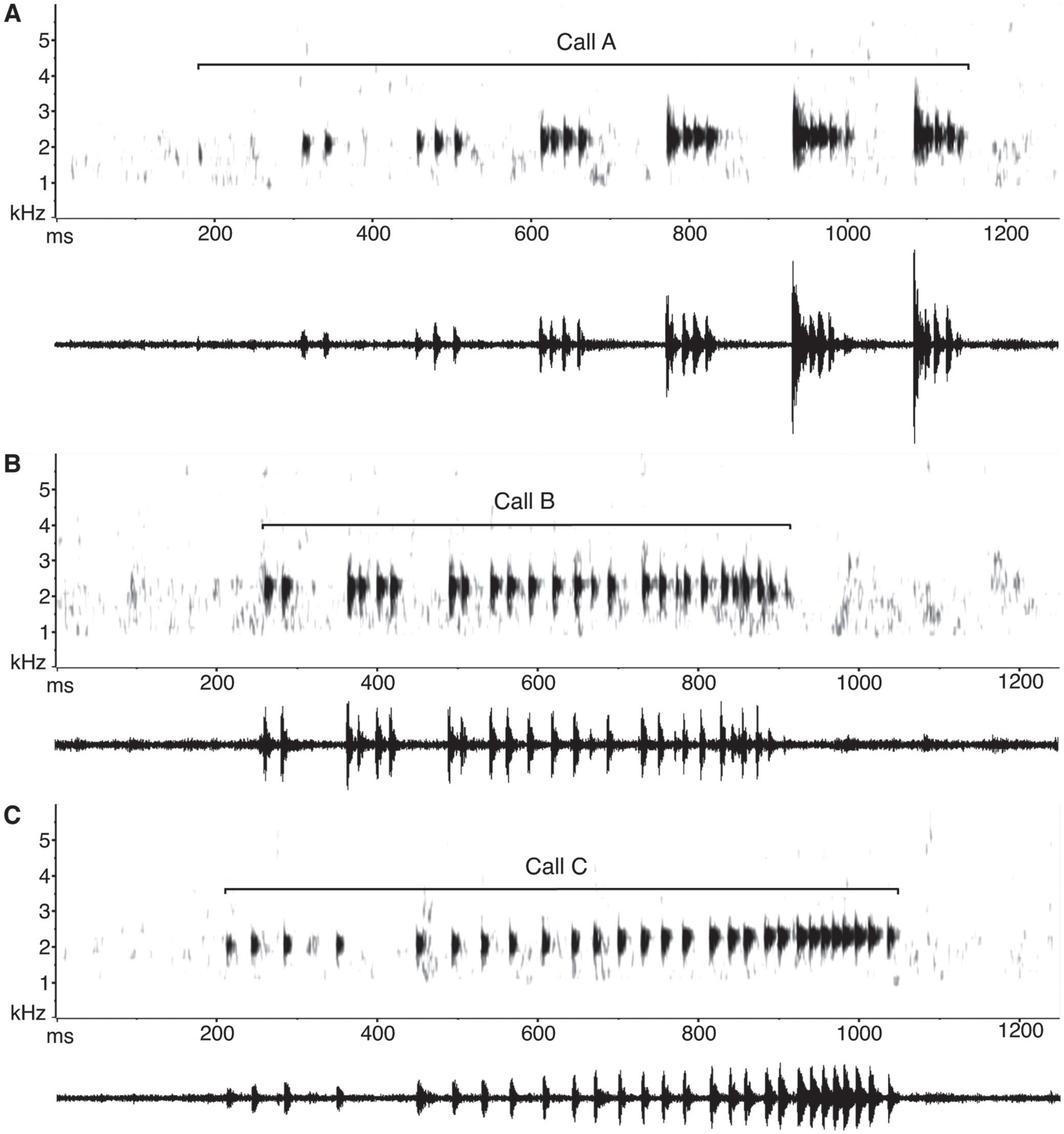


Figure 8. Calls of *Boana quiriri* **sp. nov.** from recording CBUFMG 1082 (voucher CHUFSC 5053), Fazenda de Preservação Alto Quiriri, APA Campos do Quiriri, Garuva, state of Santa Catarina, Brazil; 27 November 2013, air temperature 14°C, 22:10 Brazilian daylight saving time. **(A)** Call A (advertisement call); **(B)** Call B; **(C)** Call C. Spectrogram is shown on top and the waveform on bottom.

Table 4. Some measurements and proportions of the type-series of *Boana quiriri* **sp. nov.** Values (in mm) are reported as minimum–maximum ($\bar{x}$ ± SD). See text for measurement abbreviations.

Measurements		
	Males (n = 10)	**Female (*n* = 1)**
SVL	36.7–41.5 (39.6 ± 1.5)	44.1
HL	10.8–13.2 (12.3 ± 0.7)	13.5
HW	10.6–12.9 (12.2 ± 0.7)	13.6
ED	3.5–4.5 (4.1 ± 0.3)	4.4
TD	1.7–2.2 (1.9 ± 0.2)	2.0
EW	2.8–3.9 (3.4 ± 0.3)	3.3
END	2.9–3.2 (3.0 ± 0.1)	3.4
IOD	3.3–3.9 (3.6 ± 0.2)	3.7
NSD	1.3–2.1 (1.8 ± 0.2)	2.1
IND	2.1–2.7 (2.4 ± 0.2)	2.6
AMD	6.3–7.4 (6.9 ± 0.4)	7.5
FAL	8.0–9.3 (8.8 ± 0.4)	9.5
HAL	10.9–12.8 (12.0 ± 0.6)	12.7
4FD	1.4–1.8 (1.7 ± 0.1)	1.9
THL	17.1–21.2 (19.6 ± 1.4)	21.6
TL	17.8–21.8 (20.1 ± 1.1)	22.6
TAL	10.9–12.9 (11.8 ± 0.5)	12.9
FL	16.1–18.8 (17.5 ± 0.8)	19.3
4TD	1.6–2.0 (1.7 ± 0.1)	1.9

Proportions		
	Males (*n* = 10)	**Female (*n* = 1)**
HW/HL	0.92–1.03 (0.99 ± 0.03)	1.01
END/ED	0.69–0.82 (0.74 ± 0.04)	0.77
ED/HL	0.32–0.36 (0.33 ± 0.01)	0.32
ED/HW	0.31–35 (0.34 ± 0.01)	0.32
IOD/ED	0.78–0.98 (0.88 ± 0.07)	0.85
IOD/HW	0.27–0.32 (0.30 ± 0.01)	0.27
TD/ED	0.44–0.51 (0.47 ± 0.02)	0.46
TD/4FD	1.04–1.29 (1.15 ± 0.10)	1.08
FAL/SVL	0.20–0.24 (0.22 ± 0.01)	0.22
HAL/SVL	0.29–0.33 (0.31 ± 0.01)	0.29
THL/SVL	0.45–0.51 (0.49 ± 0.02)	0.49
TL/SVL	0.46–0.53 (0.51 ± 0.02)	0.51
4TD/4FD	0.95–1.17 (1.04 ± 0.06)	1.03
TL/THL	0.98–1.08 (1.03 ± 0.03)	1.05
FL/SVL	0.42–0.47 (0.44 ± 0.01)	0.44

cle to the heel; tarsal ventrolateral fringe absent; calcar absent. Toes long and slender, bearing round discs, slightly smaller than those on fingers (4TD/4FD = 0.98); relative toe length I < II < III = V < IV; webbing formula I2^{-}—2II1^{+}—2^{+}III1$^{1/2}$—3IV3^{-}—1V; presence of small and lateral fringes on toes; thickened layer of tissue at midline of webbing between toes IV and V absent. Subarticular tubercles moderately large, round in ventral view, slightly conical in profile on toes I–III and flat on toes IV and V; supernumerary tubercles present but barely notable; outer metatarsal tubercle absent; inner metatarsal tubercle distinct, flat, small, and elliptical in ventral view (Fig. 6).

Skin smooth except pectoral and abdominal areas, and ventral surfaces of thighs, where it is granular/areolate. Pectoral fold absent. Cloacal opening at upper level of thighs, directed posteroventrad; cloacal sheath small, only partially covering cloaca opening; supracloacal dermal ridge absent; cloacal tubercles present, but barely distinct. Tongue slightly cordiform shaped, barely free behind; dentigerous processes of vomers prominent, in two separate, nearly straight series, almost transverse, converging medially, bearing four (right) and five (left) teeth. Choanae large, almost rounded, spaced 3.0 mm from each other. Vocal slits moderately long, extending from midlateral base of tongue, almost reaching the angle of jaws. Vocal sac single, median, subgular. Dorsum with scars (Fig. 6).

Measurements (mm). SVL 40.2, HL 12.4, HW 12.8, ED 4.5, TD 2.0, EW 3.7, END 3.1, IOD 3.7, NSD 2.1, IND 2.6, AMD 7.3, FAL 8.8, HAL 12.8, 4FD 1.8, THL 20.1, TL 20.3, TAL 12.0, FL 18.8, 4TD 1.8.

Coloration in preservative. The dorsum is greyish brown, with several irregular dark brown blotches spread over it. A whitish dorsolateral stripe of almost uniform width throughout its entire length extends from the posterior margin of the eye to the leg insertion. Medially and adjacent to this stripe, there is a large longitudinal blotch, with dark brown margins and filled with greyish brown. This blotch exhibits irregular margins, with occasional expansions directed towards the vertebral region. Positioned laterally and adjacent to the whitish dorsolateral stripe is a thin, dark brown stripe extending from the snout, along the *canthus rostralis* and external margin of the upper eyelid, dorsad to the supratympanic fold, and along the lateral dorsum to groin. The loreal region exhibits dense, dark brown pigmentation forming broad a loreal stripe. The upper and lower lips are cream-colored. The tympanic membrane shows a high density of melanophores. The flanks display several scattered, dark brown flecks, with some areas having densely organized flecks forming indistinct, irregular rings around cream spots. The inguinal region is cream and has no markings. The dorsum of arms, forearms, thighs, and shanks present a greyish-brown hue adorned with several irregular dark brown blotches. On the forearms, these blotches are densely arranged, creating two irregular longitudinal stripes, one pre-axial, and the other post-axial. The external margin of the shank features an irregular dark brown longitudinal stripe. The hands and feet are cream, with several tiny melanophores evenly distributed over their dorsal and ventral surfaces. The dorsal surface of the tarsal region has some dark brown flecks. The anterior and posterior surfaces of the thighs share a similar, albeit lighter, color with the dorsum and display numerous scattered melanophores. The right shank and feet appear to have slightly faded colors compared to the left, possibly due to the knot of the field tag tied around the specimen's right knee. The ventral surfaces of the gular region, arms, belly, thighs, and shanks are cream-colored and immaculate.

Variation. Some measurements and body proportions are provided in Table 4. Webbing formulae on hands vary as follows: II−III2−(3−3^+)IV(3−3^-)−(2−2^+)V, whereas webbing on feet varies as I(1^+−2^-)−2II(1−$1^{1/2}$)(2^+−3^-)III($1^{1/2}$−2^-)−($2^{1/2}$−3)IV(2^-−3^-)−1V. On three specimens (UFMG 20882, 20884, and 20886), the external metacarpal tubercle is completely indistinct. Vomerine teeth number varies between 3−5 teeth on the right dentigerous process, and 3−6 on the left one.

The dorsolateral whitish stripe is absent in CHUFSC 5054 and UFMG 20880. CHUFSC 5054−5046, and UFMG 20883 lack the large, longitudinally arranged dorsal blotches (instead, these specimens have only irregular blotches scattered over the dorsum). UFMG 20880 exhibits large, fragmented longitudinal blotches. UFMG 20880 and UFMG 20882 have an irregular and interrupted vertebral line, extending from near the snout to the sacral region. In UFMG 20884, rather than a vertebral line, dorsal blotches are arranged in a medial line over the dorsum, forming a large longitudinal blotch. CHUFSC 5054, 5055, UFMG 20885, and 20886, possess a well-defined loreal stripe extending to the inguinal region as a lateral brown stripe passing over the tympanum. In UFMG 20880−20882, the loreal stripe is absent. UFMG 20886 exhibits a well-defined whitish labial stripe ventral to the loreal stripe. In UFMG 20880−20883, the tympanic membrane lacks densely organized melanophores. Additionally, in UFMG 20885, the left tympanic membrane is unpigmented, while the right one is dark brown as the lateral stripe passing over it. UFMG 20887 lacks the dark thin stripe laterally adjacent to the whitish dorsolateral one. The cream spots on the flanks of CHUFSC 5056, UFMG 20880−20882 and UFMG 20886 lack dark rings. The irregular blotches of the dorsal surface of the forearm do not form longitudinal stripes in CHUFSC 5054, UFMG 20880−20882, or 20884. Blotches are present on both the forearms and the dorsal surfaces of fingers IV and V in CHUFSC 5054, 5056, UFMG 20883−20886. The ventral surfaces of the tarsal region are dark in CHUFSC 5054, 5055, UFMG 20885, and 20886.

The only female specimen (UFMG 20883) is larger (SVL 44.1 mm) than the largest male (SVL 41.5 mm; CHUFSC 5054), its forearm is not hypertrophied, and its prepollex is poorly developed (presumably at least partially cartilaginous). The tubercles around the cloaca are more pronounced than in males. Vocal slits and the vocal sac are absent.

Coloration in life. The following description of variation is based on field notes and photographs of some paratypes in life (Fig. 7). Individuals exhibited an overall greenish background coloration on the dorsum, venter surfaces of limbs, belly, and throat. The dorsum of the body and dorsal surfaces of limbs were light green to moss green; blotches and flecks are light to dark brown, and in UFMG 20884, the filling of these blotches was green to light gray. Flanks typically had a longitudinal series of yellowish green spots that varied in number. In some individuals, melanophores spread over the flanks formed a brown reticulation. The supralabial and dorsolateral stripes (when present) were yellowish green. The loreal stripe was dark brown. The iris was almost homogenously copper, with indistinct brown reticulations. A black ring surrounded the iris. Hidden surfaces of arms and legs, as well as dorsal and ventral surfaces of hands and feet, throat, belly, and inguinal region were pale green, likely due to high concentrations of biliverdin in the lymph.

Vocalization. We recorded 51 calls (CBUFMG 1082−1085) from four individuals (CHUFSC 5053, UFMG 20881, 20884, 20886) on 27 November 2013 (air temperature 14°C, between 21:50 and 22:25 Brazilian daylight saving time). The vocal repertoire consists of three types of call (A, B, and C; Fig. 8). Calls were emitted either in isolation or in distinct combinations (see below). Among the three types, Call A was most common (*n* = 25), followed by Call B (*n* = 16) and call C (*n* = 10). Consequently, we believe Call A functions as the advertisement call and used it for the species diagnosis. Calls B and C require further study to understand their biological role(s).

Advertisement call (Call A) duration was 324−1,250 ms (899.4 ± 211.5; *n* = 25). It is formed by a series of 3−10 pulsed notes (6.4 ± 1.2; *n* = 25). Throughout call emission, notes increase in relative amplitude, number of pulses, and duration, along with a slightly ascending frequency modulation. Each note contains 2−9 pulses (4.0 ± 1.8; *n* = 128; notes from recording CBUFMG 1085, voucher UFMG 20886, were excluded because they presented a pulsatile structure, making pulse counting difficult). In three notes, there was only one pulse, characterizing it as a tonal note, lasting 6−19 ms (17 ± 10.1). The first note of the call was usually composed of 2−3 pulses, while the last note comprised 4−6 pulses. Similarly, the first notes of the call were usually shorter than the last ones. Note duration was 20−101 ms (57.9 ± 17.8; *n* = 160), with shorter notes composed of fewer pulses. Inter-note intervals were 16−172 ms (98.9 ± 31.4; *n* = 136), with larger intervals usually at the beginning of the call, gradually decreasing until the end of the call. As mentioned above, the dominant frequency had a slight ascending modulation among notes. In the first two notes, the dominant frequency was 1,380.5−2,842.2 Hz and the peak frequency was 2,062.5 (*n* = 13 calls), 2,067.2 (*n* = 6 calls), 2,239.5 (*n* = 4 calls), 2,411.7 (*n* = 1 call), or 2,584 Hz (*n* = 1 call). In the last note of the call the dominant frequency was 1,711.8−3,090.8 Hz and the peak frequency was 2,250 (*n* = 13 calls), 2,411.7 (*n* = 1 call), or 2,584 Hz (*n* = 11 calls). No harmonic bands were detected.

The Call B differs from Call A by presenting uniform relative amplitude and frequency among notes. Given the irregular spacing between pulses, it is interpreted as a single trilled note, in which the pulses are usually more concentrated toward the end of the call. Call B duration was 151−910 ms (439.9 ± 170.5; *n* = 16) and it was composed of 9−86 pulses (24.4 ± 17.6; *n* = 16). The dominant frequency was 1,555.6−2,977.9 Hz and the peak frequency was 2,250 (*n* = 13 calls), 2,584 (*n* = 2 calls), or 2,411.7 Hz (*n* = 1 call). No harmonic bands were detected.

Call C exhibited a progressive increase in relative amplitude during the first two thirds, with stabilization occurring only in the final third, as well as ascending frequency modulation. Similar to Call B, it was composed of irregularly spaced pulses, usually more concentrated at the end of the call. Call C duration was 497−1,050 ms (757.3 ± 173.5; *n* = 10) and it was composed of 27−44 pulses (34.5 ± 6.5; *n* = 10). In the initial pulses of the call, the dominant frequency was 1,549.4−2,586.3 Hz and the peak frequency was 1,722.7 Hz (*n* = 1 call), 1,894.9 Hz (*n* = 5 calls), 2,062.5 Hz (*n* = 3 calls), or 2,411.7 Hz (*n* = 1 call). In the last pulses of the call, the dominant frequency was 1,868.6−2,935.4 Hz and peak frequency was 2,250 (*n* = 3 calls), 2,411.7 (*n* = 2 calls), or 2,584 Hz (*n* = 5 calls). No harmonic bands were detected.

When calls were emitted in sequences with short intervals between them (interval < 1 s), they were observed in the following sequences: A + B, A + C, A + A, C + A. In those cases, inter-call intervals varied as follows: A + B: 53−260 ms (190 ± 47; *n* = 15); A + C: 83−869 ms (540 ± 410; *n* = 3); A + A: 179 ms (*n* = 1); C + A: 79 ms (*n* = 1). When inter-call intervals were long (interval > 2 s), calls were observed in the following sequences: B + A, C + A, B + C, A + A, A + B, A + C, C + C. In those cases, inter-call intervals varied as follows: B + A: 2.45−14.48 (6.86 ± 4.28; *n* = 11); C + A: 2.11−7.47 s (4.67 ± 1.85; *n* = 8); B + C: 2.07−3.22 (2.67 ± 0.58; *n* = 3); A + A: 3.06−10.26 s (6.66 ± 5.09; *n* = 2); A + B: 8.18 s (*n* = 1); A + C: 3.52 s (*n* = 1); C + C: 11.41 s (*n* = 1).

***Natural history. Boana quiriri* sp. nov.** specimens were found in small gallery forest fragments within highland (1,333 m a.s.l.) open fields with small rocky outcrops. They were perched on small branches from a few centimeters to 1 m above the water level of a small creek. The marginal vegetation entirely covered the creek and extended no more than 2 m beyond its margins. Despite the creek's small proportions, it was extremely loud, making it difficult to record vocalizations. Males were only heard within this grove. Out of it, no other individuals were found in uncovered sections of the same creek, or in other similar groves in the area. Although no combat between males was observed, some males showed several scars on their dorsum, indicating aggressive behavior. Many specimens were parasitized by orange (in life) trumbiculid mites under their skin (Fig. 7C). Eggs and tadpoles were not observed, but the female paratype (UFMG 20883) presented many mature oocytes with a pigmented animal pole, visible through her skin.

Geographic distribution. Known only from the type locality at Serra do Quiriri, northeastern Santa Catarina (Fig. 5).

***Conservation status. Boana quiriri* sp. nov.** occurs in an area that today is within the Campos do Quiriri Environment Protected Area (APA, from the Portuguese *Área de Proteção Ambiental,* similar to IUCN Category VI: protected area with sustainable use of natural resources; Brazil, 2000; Lausche, 2011). According to Brazilian law, it is possible to exploit and alter the environment in an APA in a sustainable way, ensuring the perennity of renewable natural resources (Brazil, 2000). However, recent visits to the original collection site revealed that the area has been significantly modified (EJC pers. obs.). Extensive *Pinus* cultivation is driving the rapid spreading of this important invasive species throughout the region (Garrote et al., 2011). Other activities, such as wood and kaolin extraction for the pottery industry, along with uncontrolled trails from pickup trucks and motorcycles, threaten the headwaters by promoting erosion and silting of rivulets and streams. Additionally, the use of fire for pasture management in cattle breeding poses a significant environmental threat, particularly during dry seasons. These factors clearly indicate that the current exploitation of the area is unsustainable, rendering the sole known population of *B. quiriri* **sp. nov.** highly vulnerable. Also, as the region embraces several headwaters, the threats to the rivulets could also bring serious consequences for the water of the municipalities in the region.

Etymology. Quiriri is a Portuguese word derived from the Tupi *kyrirĩ,* meaning silence or quietness. In the state of Santa Catarina, it is the name of the mountain range where the new species was collected, and also the river that drains from this mountain, the Quiriri River. A preservation area in the region also bears the same name, *Área de Proteção Ambiental Campos do Quiriri* (Campos do Quiriri Environment Protected Area). The name *quiriri* is used as a noun in apposition.

DISCUSSION

The existence of several candidate species closely related to *Boana semiguttata* was previously emphasized by Garcia et al. (2007) and Kwet (2008). One of them, *Boana poaju,* was described shortly thereafter by Garcia et al. (2008). Faivovich et al. (2021) considered the taxonomy of the species in the *B. semiguttata* clade to be complex, marked by high genetic diversity and challenging species delimitation. They concluded that our understanding of the diversity within this clade is still far from complete and identified two candidate species in the clade, referring to them as *Boana* sp. 1 and sp. 2, on the basis of DNA sequence data in a phylogenetic approach (Fig. 1; Table 2). *Boana* sp. 1 had already been noted by Kwet (2008; as *Hypsiboas* sp. 2) and Vasconcellos et al. (2021; as *Boana* sp.).

The uncorrected pairwise genetic distances for the 16S fragment employed by Faivovich et al. (2021) show that *Boana* sp. 1 differs 2.14−3.56% from its sister clade (including *B. caipora, B. semiguttata,* and *Boana* sp. 2) in the 16S fragment, while *Boana* sp. 2 differs 2.31−2.49% from its sister taxon, *B. semiguttata* (Fig. 1; Table 2). The values for *Boana* sp. 1 and *Boana* sp. 2 in relation to closely related species are slightly below the threshold of 3% proposed by Fouquet et al. (2007) as "preferable" for distinguishing species. However, Fouquet et al. (2007) identified a range between 1.9 and 4.9% difference between the pairs of species analyzed by them. Other groups of frogs are also known to have low genetic distance between species. Within *Hyloscirtus* (also Cophomantini), for example, values as low as 1.3% have been reported for unambiguously distinct species (e.g., *H. lindae* and *H. pantostictus;* Rivera-Correa et al., 2024). In this regard, the values reported for *Boana* sp. 1 and *Boana* sp. 2 are consistent with their delimitation as distinct species.

In addition to genetic distances, the morphological and bioacoustic diagnostic traits support the recognition of both taxa as distinct from the other species of the *Boana semiguttata* clade, leading us to described them as *B. itajahy* **sp. nov.** and *B. quiriri* **sp. nov.**, respectively. With the recognition of these species the content of the *Boana semiguttata* clade rises to eight species, and the *B. pulchella* group includes 36 species.

The advertisement call of *Boana itajahy* **sp. nov.** is similar to that of *B. marginata* (see species comparisons above). The calls of both species share a unique structure compared to the other species in the *B. pulchella* group: one A note followed by a series of B notes. However, *B. marginata,* which is distributed in southern Brazil and shares a similar natural history with *B. itajahy* **sp. nov.**, exhibits a distinct coloration pattern (Garcia et al., 2001a). Despite the similarities in their calls and ecological traits, these two species are not closely related, with *B. marginata* being nested in the *B. prasina* clade (see Faivovich et al., 2021). The selective pressures that led to the convergence in advertisement calls between these species are unknown.

Whereas *Boana itajahy* **sp. nov.** can be distinguished from all species in the *B. semiguttata* clade by both external morphology and advertisement call, *B. quiriri* **sp. nov.** closely resembles *B. semiguttata,* its sister species. Preserved specimens show differentiation in the limb dorsal color pattern, and living specimens exhibit differences in color, particularly in the supralabial and dorsolareral stripes, and the blotches on the flanks (see comparisons with species of the *Boana pulchella* group for details). These traits, coupled with the weakly supported phylogenetic relationship between *B. quiriri* **sp. nov.** and *B. semiguttata,* the considerably long branch lengths for each species (Faivovich et al., 2021, Fig. 3; Fig. 1), and the genetic distance between them (Table 2), provide support for recognizing the taxon described here as *B. quiriri* **sp. nov.**

Furthermore, the sole population of *Boana quiriri* **sp. nov.** was discovered in a noisy section of a creek, contrasting with the habitat preferences of male *B. semiguttata,* which are typically found in backwaters of creeks and rivulets where they inhabit calmer environments (i.e., more silent surroundings; see Garcia et al., 2007). Field observations on populations of *B. semiguttata* from São Bento do Sul and Joinville (Santa Catarina) corroborate this pattern of reproduction in calm environments (Garcia et al., 2007; PDPP, EJC, and PCAG pers. obs.). However, whether these environmental differences genuinely correspond to the natural conditions required for each species' occurrence or represent biases from our limited sampling remains to be investigated.

Interestingly, the bioacoustic parameters of these two species, *Boana quiriri* **sp. nov.** and *B. semiguttata,* overlap, meaning that there are no discernible acoustic differences between them—the structure identified as Note A and Note B by Garcia et al. (2007) correspond to Call A and Call B of *B. quiriri* **sp. nov.**, respectively. While bioacoustics is an important tool in anurans taxonomy (see a revision on this topic in Köhler et al., 2017), it does not necessarily provide a conclusive solution in all cases. Similar situations exist in other species pairs in which few or no differences in bioacoustic parameters are observed. Examples from other hylid genera include *Aplastodiscus* (Garcia et al., 2001b) and *Phyllomedusa* (Pombal and Haddad, 1992). Outside of Hylidae, Taucce et al. (2018) found calls very similar to each other among species of *Ischnocnema* (Brachycephalidae). Additional examples can be found in references cited within Köhler et al. (2017).

Males of both species described in this study possess hypertrophied forearms relative to females. Garcia et al. (2001a) noted that hypertrophied forearms and a large prepollical spine are commonly found in males of *Boana* species that breed in montane streams. The association between forearm hypertrophy and breeding in streams was tested and supported by Pinheiro et al. (2022) through the optimization of qualitative characters obtained from the literature. However, a comprehensive test of this hypothesis would require proper measurements of these characters across a broad sample of individuals and taxa within the tribe Cophomantini. Although we did not conduct such a test, life in streams and males with hypertrophied forearms are traits shared by all species of the *Boana semiguttata* clade (Garcia et al., 2003, 2007, 2008; Antunes et al., 2008; Kwet, 2008; Pinheiro et al., 2022; current study). Whether these are synapomorphies for this clade should be further assessed.

Conservation

Boana itajahy **sp. nov.** is found in the strictly protected Parque Nacional da Serra do Itajaí (National Park of Itajaí Mountain Range), whereas the sole known population of *B. quiriri* **sp. nov.** is located within APA Campos do Quiriri. According to Brazilian law, an APA is a category of preservation area that can be altered and exploited in a sustainable way. Subsequent visits to the original collection site revealed drastic alterations of the area that pose a threat to the headwaters, leading to erosion and siltation of the rivulets and streams (for details see Conservation Status, under Boana quiriri **sp. nov.**, above). All these disturbances make the only known population of *Boana quiriri* **sp. nov.** vulnerable. Moreover, given that the region encompasses several headwaters, the threats to the rivulets could have severe consequences for the water supply of municipalities in the area.

The threats to the environment also affect sympatric species, including *Brachycephalus auroguttatus, B. quiririensis* (Brachycephalidae), and *Melanophryniscus biancae* (Bufonidae). The two *Brachycephalus* species are known only from Serra do Quiriri, like *Boana quiriri* **sp. nov.**, whereas *M. biancae* is also documented for the nearby Serra de Araçatuba (Araçatuba Mountain Range) in Paraná (Bornschein et al., 2015, 2019; Pie and Ribeiro, 2015; Nadaline et al., 2019).

Given that only one population of *Boana quiriri* **sp. nov.** is known, it is difficult to evaluate its conservation status. Additional collection efforts should be made to discover other populations and gain a better understanding of its conservation status. However, given that the area where the species occurs is under threat, we cannot rule out the possibility of incorporating it into both the Brazilian list of endangered species and the National Action Plan for the Conservation of Reptiles and Amphibians of Southern Brazil (*Plano de Ação Nacional para a Conservação dos Répteis e Anfíbios do Sul do Brasil*).

We reiterate the suggestion made by Bornschein et al. (2015) that the Instituto do Meio Ambiente de Santa Catarina (IMA)—the environmental agency responsible for the region formerly known as Fundação de Amparo à Tecnologia e Meio Ambiente FATMA)—should refrain from allowing the plantation of *Pinus* spp. in Serra do Quiriri. Additionally, the IMA should cease issuing new permits for kaolin mining in this area and require the mining company to conduct a study on the conservation status of the new species at the type locality before considering any requests to renew licenses for mining operations. While our knowledge about these endemic species is still limited, given their limited geographic distribution and the current threats they suffer, we cannot dismiss the risk of their extinction.

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APPENDIX: MATERIAL EXAMINED

Asterisks (*) identify type material; pound signs (#) identify topotypic material; plus signs ($^{+}$) identify non-type material employed by Garcia et al. (2003) for raising *Hyla pulchella joaquini* to species level and Garcia et al. (2007) for redescribing *Hyla semiguttata.*

Boana caipora (*n* = 21): BRAZIL: **São Paulo:** Capão Bonito, CFBH 40718; Pilar do Sul#, CFBH 5738*−5741*, 5743*, 7312*−7318*, 8292*, 8293*, 8297*, 10873*, 14198; São Miguel Arcanjo, CFBH 38435, 38461, 38462.

Boana curupi (*n* = 16): ARGENTINA: **Misiones:** San Vicente: Departamento Guarany#, CFBH 3444*, 3445*, 4908, 4910. BRAZIL: **Santa Catarina:** São Domingos, CFBH 9558−9561; Xavantina, CFBH 21140, 21141; Xanxerê, CFBH 21128, 21129, 21143−21146.

Boana joaquini (*n* = 28): BRAZIL: **Rio Grande do Sul:** Cambará do Sul, CFBH 3129, 3132, 3347, 3350, 3351, 30362; São Francisco de Paula, CFBH 80, 3065, 6088, 3145, 14536, 14537; São José dos Ausentes, CFBH 3339−3341, 3344, 3345. **Santa Catarina:** Campos Novos, CFBH 20793, 20795, 20798, 20802, 23856; Urubici, 3281^{+}, 3282^{+}, 3284^{+}, 3285^{+}, 3626^{+}, 3627^{+}.

Boana poaju (*n* = 21): BRAZIL: **Santa Catarina:** Anitápolis, CFBH 20263, 20264; Rancho Queimado#, CFBH 3049*, 3104*, 3106*, 3333−3337, 3583*−3586*, 3589*, 3591*, 5398*, 3606, 3608−3610.

Boana semiguttata (*n* = 39): BRAZIL: **Paraná:** Palmeira, CFBH 3195−3197, 3365−3367, 3369−3372; Piraquara, CFBH 3364^{+}, 3579^{+}, 3580^{+}, 3704^{+}−3707^{+}, 5000^{+}, UFMG 19542−19545; Tijucas do Sul, UFMG 16025; **Santa Catarina:** Joinville: Castelo dos Bugres, UFMG 17421, 17422, 18992−18994, 20887−20889; Joinville: APA (Environment Protection Area) Dona Francisca, CFBH 39304, 39306; São Bento do Sul: Alto Rio Vermelho#, UFMG 13164, 13165, 13875, 13888, 14435, 20070.

Boana stellae (*n* = 4): ARGENTINA: **Misiones:** Cainguás: Salto Encantado, CFBH 47974. BRAZIL: **Rio Grande do Sul:** Herveiras, CFBH 25715, 25716; Vacaria, CFBH 12425.

ONLINE SUPPORTING INFORMATION

The following Supporting Information is available for this article online:

Supplementary file 1. List of coordinates employed to generate the map presented in Fig. 5, with their respective references, including corrections to coordinates provided by Faivovich et al. (2021) and Vasconcellos et al. (2021).

SUPPORTING INFORMATION

List of coordinates employed to generate the map presented in Fig. 5, with their respective references.

Species	Longitude	Latitude	Locality	Reference
Boana caipora	-48.017811	-24.094954	Brazil: São Paulo: Capão Bonito	This study (CFBH 40718)
	-47.666667	-23.933333	Brazil: São Paulo: Pilar do Sul	Antunes et al. (2008); Faivovich et al. (2021)
	-47.683333	-23.966667	Brazil: São Paulo: Pilar do Sul	Antunes et al. (2008)
	-47.700000	-23.933333	Brazil: São Paulo: Pilar do Sul	Antunes et al. (2008)
	-47.866667	-23.983333	Brazil: São Paulo: São Miguel Arcanjo	Antunes et al. (2008)
	-47.983333	-24.116667	Brazil: São Paulo: Sete Barras	Antunes et al. (2008)
Boana curupi	-54.160556	-25.588611	Argentina: Misiones: General Manuel Belgrano	Faivovich et al. (2021)
	-54.067222	-26.000278	Argentina: Misiones: General Manuel Belgrano*	Lescano et al. (2013)
	-54.424531	-26.918622	Argentina: Misiones: Guarany	Faivovich et al. (2021)
	-54.430556	-26.917500	Argentina: Misiones: Guarany	Garcia et al. (2007)
	-54.217361	-25.897889	Argentina: Misiones: Iguazú	Faivovich et al. (2021)

-54.877778	-27.008611	Argentina: Misiones: Libertador General San Martín*	López & Prado (2012)
-54.103694	-26.631306	Argentina: Misiones: San Pedro	Faivovich et al. (2021)
-53.927500	-26.468083	Argentina: Misiones: San Pedro	Faivovich et al. (2021)
-54.263333	-26.689167	Argentina: Misiones: San Pedro*	López & Nazer (2009)
-51.586000	-26.534000	Brazil: Paraná: Palmas	Vasconcellos et al. (2021)
-53.864000	-27.610000	Brazil: Rio Grande do Sul: Bom Progresso*	Widholzer & Prieto-Torres (2022)
-51.944111	-28.612278	Brazil: Rio Grande do Sul: Casca	Fontana et al. (2017)
-53.949017	-27.242494	Brazil: Rio Grande do Sul: Derrubadas	Iop et al. (2009)
-52.214153	-28.045665	Brazil: Rio Grande do Sul: Sertão	Zanella et al. (2012)
-52.890833	-27.168444	Brazil: Santa Catarina: Caxambú do Sul	Lucas & Garcia (2011)
-52.116139	-27.289000	Brazil: Santa Catarina: Concórdia	Lucas & Garcia (2011)
-51.955000	-26.771472	Brazil: Santa Catarina: Ponte Serrada	Lucas & Garcia (2011)
-52.539878	-26.573547	Brazil: Santa Catarina: São Domingos	Faivovich et al. (2021)

	-53.394694	-26.744806	Brazil: Santa Catarina: São Miguel do Oeste	Lucas & Garcia (2011)
	-51.756052	-26.945119	Brazil: Santa Catarina: Vargem Bonita*	Pinheiro et al. (2016)
	-52.427534	-27.012338	Brazil: Santa Catarina: Xanxerê	This study (CFBH 21128, 21129, 21143–21146)
	-52.322424	-27.021749	Brazil: Santa Catarina: Xavantina*	Pinheiro et al. (2016)
	-55.623306	-26.109694	Paraguay: Caazapá: Tavaí	Brusquetti & Lavilla (2008)
	-56.219722	-25.847222	Paraguay: Guairá: General Eugenio A. Garay*	Wood et al. (2013)
	-55.633333	-26.583333	Paraguay: Itapúa: Itapúa Poty	Brusquetti & Lavilla (2008)
	-55.333333	-26.415583	Paraguay: Itapúa: Tomás Romero Pereira	Brusquetti & Lavilla (2008)
Boana itajahy	-49.157028	-27.106083	Brazil: Santa Catarina: Apiúna	This study
	-49.158833	-27.107361	Brazil: Santa Catarina: Blumenau	This study; Faivovich et al. (2021; as *Boana* sp. 1)

	-49.084722	-27.058056	Brazil: Santa Catarina: Blumenau	This study; Kwet (2008; as *Hypsiboas* sp. 2)
	-49.111944	-27.060278	Brazil: Santa Catarina: Indaial	This study
	-49,151007	-27.023732	Brazil: Santa Catarina: Indaial	Vasconcellos et al. (2021; as *Boana* sp.)[a]
Boana joaquini	-50.301000	-28.685000	Brazil: Rio Grande do Sul: Bom Jesus	Vasconcellos et al. (2021)
	-49.985694	-29.077361	Brazil: Rio Grande do Sul: Cambará do Sul	Faivovich et al. (2021)
	-50.166828	-29.042300	Brazil: Rio Grande do Sul: Cambará do Sul	Faivovich et al. (2021)
	-50.296000	-29.698000	Brazil: Rio Grande do Sul: Caraá*	Widholzer & Prieto-Torres (2022)
	-50.166667	-29.500000	Brazil: Rio Grande do Sul: Itati*	Kwet et al. (2010)
	-50.455333	-29.408167	Brazil: Rio Grande do Sul: São Francisco de Paula	Faivovich et al. (2021)
	-50.065331	-28.748844	Brazil: Rio Grande do Sul: São José dos Ausentes*	This study (CFBH 3339–3341, 3344, 3345)
	-49.624722	-28.336944	Brazil: Santa Catarina: Bom Jardim da Serra	Garcia et al. (2003)

	-51.265881	-27.385575	Brazil: Santa Catarina: Campos Novos	Faivovich et al. (2021)
	-49.809000	-28.210000	Brazil: Santa Catarina: São Joaquim	Vasconcellos et al. (2021)
	-49.932222	-28.288611	Brazil: Santa Catarina: São Joaquim	Garcia et al. (2003)
	-49.931667	-28.293889	Brazil: Santa Catarina: São Joaquim	Garcia et al. (2003)
	-49.498917	-28.125617	Brazil: Santa Catarina: Urubici	Faivovich et al. (2021)
	-49.478611	-28.122778	Brazil: Santa Catarina: Urubici	Garcia et al. (2003)
	-50.097855	-29.180947	Brazil: Santa Catarina: Praia Grande*	Deiques et al. (2007)
Boana poaju	-48.823611	-27.693889	Brazil: Santa Catarina: Águas Mornas*	Garcia et al. (2008)
	-49.132017	-27.901444	Brazil: Santa Catarina: Anitápolis	Faivovich et al. (2021)
	-48.998056	-27.690000	Brazil: Santa Catarina: Rancho Queimado	Garcia et al. (2008); Faivovich et al. (2021)
	-48.803056	-27.742500	Brazil: Santa Catarina: Santo Amaro da Imperatriz	Garcia et al. (2008); Kwet (2008; as *Hypsiboas* sp. 1)
Boana quiriri	-48.972645	-26.027145	Brazil: Santa Catarina: Garuva	This study; Faivovich et al. (2021; as *Boana* sp. 2)

Boana semiguttata	-50.020758	-25.433622	Brazil: Paraná: Palmeira	Faivovich et al. (2021)
	-49.022078	-25.443117	Brazil: Paraná: Piraquara	Faivovich et al. (2021)
	-48.983889	-25.484722	Brazil: Paraná: Piraquara	Garcia et al. (2007)
	-50.153000	-25.096000	Brazil: Paraná: Ponta Grossa	Vasconcellos et al. (2021)
	-49.198900	-25.928100	Brazil: Paraná: Tijucas do Sul*	This study (UFMG 16025)
	-49.943000	-26.564000	Brazil: Santa Catarina: Itaiópolis*	Widholzer & Prieto-Torres (2022)
	-49.053906	-26.232889	Brazil: Santa Catarina: Joinville	Faivovich et al. (2021)
	-50.726283	-26.886367	Brazil: Santa Catarina: Lebon Régis	Faivovich et al. (2021)
	-49.260972	-26.245333	Brazil: Santa Catarina: São Bento do Sul	Faivovich et al. (2021)[b]
	-49.333333	-26.686111	Brazil: Santa Catarina: Rio dos Cedros	Garcia et al. (2007)
	-49.378611	-26.250278	Brazil: Santa Catarina: São Bento do Sul	Garcia et al. (2007)
	-49.992982	-27.120205	Brazil: Santa Catarina: Taió*	This study (CFBH 14531–14533); Faivovich et al. (2021)[c]
Boana stellae	-54.952778	-27.086667	Argentina: Misiones: Cainguás	Faivovich et al. (2021)

-54.826389	-27.058889	Argentina: Misiones: Cainguás	Faivovich et al. (2021)
-55.036075	-27.038736	Argentina: Misiones: Cainguás	Faivovich et al. (2021)
-54.134000	-25.871000	Argentina: Misiones: Dos de Mayo*	Widholzer & Prieto-Torres (2022)
-54.065000	-26.192000	Argentina: Misiones: Oberá: Villa Bonita	Widholzer & Prieto-Torres (2022)
-53.886000	-26.471000	Argentina: Misiones: San Pedro: Tobuna*	Widholzer & Prieto-Torres (2022)
-52.298000	-28.855000	Brazil: Rio Grande do Sul: Arvorezinha	Widholzer & Prieto-Torres (2022)
-50.998000	-29.033000	Brazil: Rio Grande do Sul: Caxias do Sul:	Widholzer & Prieto-Torres (2022)
-51.842861	-28.984122	Brazil: Rio Grande do Sul: Dois Lajeados	Faivovich et al. (2021)
-52.650905	-29.454120	Brazil: Rio Grande do Sul: Herveiras*	This study (CFBH 25715, 25716)
-53.223000	-29.409000	Brazil: Rio Grande do Sul: Ibarama*	Widholzer & Prieto-Torres (2022)

-51.399000	-28.238000	Brazil: Rio Grande do Sul: Lagoa Vermelha	Widholzer & Prieto-Torres (2022)
-52.602647	-29.449903	Brazil: Rio Grande do Sul: Sinimbú	Faivovich et al. (2021)
-52.558000	-29.392000	Brazil: Rio Grande do Sul: Sinimbú	Vasconcellos et al. (2021)
-52.600000	-29.566667	Brazil: Rio Grande do Sul: Sinimbú	Kwet (2008)
-52.567333	-29.564250	Brazil: Rio Grande do Sul: Sinimbú	Widholzer & Castroviejo-Fisher (2018)
-50.981331	-28.501703	Brazil: Rio Grande do Sul: Vacaria	Faivovich et al. (2021)

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* Localities with approximated coordinates.

[a] The locality of the sample FURB 14100 was wrongly reported by Vasconcellos et al. (2021) as "Itajaí, Santa Catarina, Brazil, -26.965S, -48,656W." Correction: Brazil, state of Santa Catarina, Indaial, Parque Nacional das Nascentes, Rancho do Mono, approximate coordinates 27°01'S, 49°09'W (P.D.P.P. pers. obs.).

[b] This coordinate was wrongly reported by Faivovich et al. (2021) as 23°14'43.2"S / 49°15'39.5"W. Correction: 26°14'43.2"S / 49°15'39.5"W (P.D.P.P. pers. obs.).

[c] The locality of the sample CFBH 14531 was wrongly reported by Faivovich et al. (2021) as "Brazil, Rio Grande do Sul, São Francisco de Paula (with approximate coordinates as 29°27'41.55"S / 50°34'13.26"W). Correction: Brazil, state of Santa Catarina, Taió, approximate coordinates 27°71'2.74"S / 49°59'34.74"W (P.D.P.P. pers. obs.).

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