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A new species of *Proceratophrys* Miranda-Ribeiro, 1920 (Anura, Odontophrynidae) of the *P. bigibbosa* species group from Southern Brazil

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Abstract

The monophyletic *Proceratophrys bigibbosa* species group has a stable taxonomic history. Despite the increasing number of *Proceratophrys* described in the last decades, the *P. bigibbosa* group, for a long time, has consisted of four species distributed in south of South America; except for *P. palustris* that occurs in southeastern Brazil. Herein, based on concordant evidence of morphology and mtDNA barcoding, we describe a new species of *Proceratophrys* for specimens assigned to the *P. bigibbosa* group collected in Tibagi municipality, Paraná state, Brazil. The new species is diagnosed by its small size, snout rounded in dorsal view, palpebral ridge with small and rounded tubercles, small postocular swellings, presence of a line of small and round tubercles on dorso-lateral region of body, and dorsal region covered by small, sparse, and rounded tubercles. We also highlight the potential occurrence of the new species in other areas along the Campos Gerais of Paraná, given the strong association of the species with this vegetational formation in South Brazil.

Key words

Morphology, mtDNA, *Proceratophrys kaingang* sp. nov., Southern Horned-Frogs

Introduction

The knowledge on the systematics and phylogenetic relationship among species in the genus *Proceratophrys* have rapidly increased in the last decades (Dias et al. 2013; Mângia et al. 2018, 2020; Magalhães et al. 2020). Nevertheless, the traditional morphological groups of *Proceratophrys* still lacks phylogenetic support. Despite that most of the groups recovered are paraphyletic, three clades were recurrently consistent with available phylogenetic analysis: a clade of restricted Caatinga species (*P. cristiceps*, *P. minuta,* and *P. redacta*), the *P. concavitympanum* clade (*P. ararype*, *P. concavitympanum*, *P. moratoi*, *P. salvatori*, and *P. strussmannae*) distributed in Amazonia,

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Cerrado, and Brejos de Altitude within the Caatinga, and the *P. bigibbosa* species group, composed of *P. avelinoi*, *P. bigibbosa*, *P. brauni*, and *P. palustris* (Teixeira-Jr et al. 2012; Mângia et al. 2018, 2020; Magalhães et al. 2020). Although the *P. bigibbosa* group has been recovered as monophyletic with high support, *P. palustris* still lacks DNA information and is placed in this group based on putative morphology basis.

The number of species of *Proceratophrys* has increased by 55% in the last two decades. However, the *P. bigibbosa* species group (revised 20 years ago by Kwet and Faivovich 2001) has a stable taxonomic history. Species within this group are mainly diagnosed by the presence of post ocular swellings and being distributed in southern of South America, except for *P. palustris* which is only known from its type locality in Poços de Caldas Plateau, south of Minas Gerais state, southeastern Brazil (Giaretta and Sazima 1993; Kwet and Faivovich 2001; Caldart et al. 2010).

During field expeditions in the Tibagi municipality, Paraná state, South Brazil, we collected specimens of *Proceratophrys* belonging to the *P. bigibbosa* species group, presenting one of the most northern distribution (except for *P. palustris*) that aroused questions about their taxonomy. We combined morphological and mtDNA barcoding evidence to elucidate its status and concluded that no available name could be applied to this population. Herein, we describe the population from Tibagi as a new taxon.

Material and Methods

Sampling

We conducted visual surveys at Tibagi municipality, in the Guartelá Canyon region from Campos Gerais of Paraná state, Brazil, in February 2016. All specimens were captured manually and killed using 5% lidocaine, fixed in 10% formalin, and transferred to 70% ethanol for permanent storage (following Conselho Federal de Biologia-CFBio No 148/2012, 2012). Voucher specimens are housed in the Coleção de Anfíbios do Laboratório de Anfíbios e Répteis da Universidade Federal de Santa Maria (acronym ZUFSM), Santa Maria, Brazil, and Coleção Zoológica da Universidade Federal de Mato Grosso do Sul (acronym ZUFMS-AMP), Campo Grande, Brazil (Appendix I). We state here that appropriate protocols for the collection and handling of the individuals were followed for the present research according to Brazilian federal law. Collect permit was issue by ICMBio (SISBIO #49876-1) and IAP (#03.15).

Morphology

Specimens used in the description of the new species, as well as specimens examined for comparisons, are housed in 11 herpetological collections in Brazil (Appendix 1). Terminology for morphological characters follows Kwet and Faivovich (2001), Prado and Pombal Jr. (2008), and Brandão et al. (2013). We follow Kwet and Faivovich (2001) for the ten morphometric variables: snout-vent length (SVL); head length, defined as the diagonal distance from the tip of the snout to the right angle of the jaw (HL); head width, defined as the distance between the angles of the jaw (HW); horizontal eye diameter (ED); eye-nostril distance (EN); nostril-snout distance (NS); internarial distance (IN); tibia length (TL); foot length (FL); inner metatarsal tubercle length (ML). All measurements were taken by DJS using a digital caliper (0.01 mm precision). We determined the sex of each individual by the presence of vocal slits in males and their absence in females.

Phylogenetic inference and genetic distances

We extracted genomic DNA from liver samples using the phenol-chloroform protocol (Sambrook and Russell 2001) and sequenced fragments of the 16S ribosomal RNA mitochondrial gene from three individuals of the new species (genbank numbers provided in Appendix II). We used the 16Sa/16Sb primer pair (Palumbi et al. 1991), following the polymerase chain reaction (PCR) conditions described by Costa et al. (2016). PCR reactions consisted of 1× buffer, dNTPs at 0.2 mM, each primer at 0.2 µM, MgCl2 at 2 mM, 1 U Taq polymerase, and 2 µl of template DNA, in a total reaction volume of 25 µl. We used the following PCR cycling program: 94ºC for 2 min, followed by 35 cycles of 94ºC for 30 s, 59ºC for 1 min, and 72ºC for 1 min, and a final 5 min extension at 72ºC. We purified PCR products with Ethanol/Sodium Acetate and sequenced them on an ABI 3730XL DNA Analyzer (Applied Biosystems, Foster City, California). Resulting sequences were edited and aligned using Geneious v9.1.2 with the MUSCLE algorithm using default parameters (Edgar 2004). We aligned our 16S sequences along with 16S sequences of 28 species of *Proceratophrys* and with the outgroups *Odontophrynus* spp., *Macrogenioglottus alipioi*, *Cycloramphus acangatan* and *Thoropa miliaris*, which were available in GenBank (Appendix II). The final aligned dataset used in all analyses comprised 498 base pairs (bp) of 16S. We used the Bayesian Information Criterion in jModelTest (Darriba et al. 2012) to determine that HKY+I+G was the best model of nucleotide substitution for our 16S data set.

We performed a Bayesian phylogenetic analysis of 16S using BEAST v.2.6.3 (Bouckaert et al. 2019) for 20 million generations, sampling every 2,000 steps using a Yule Process tree prior. We checked for stationarity by visually inspecting trace plots and ensuring that all values for effective sample size were above 200 in Tracer v1.7.1 (Rambaut et al. 2018). The first 10% of sampled genealogies were discarded as burn-in, and the maximum clade credibility tree with median node ages was calculated with TreeAnnotator v.2.6.3 (Bouckaert et al. 2019). We also calculated sequence divergence (uncorrected p-distance, Appendix II) among species/individuals using MEGA v10.1.1 (Kumar et al. 2018).

Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The LSID (Life Science Identifier) for this publication is: LSIDurn:lsid:zoobank.org:pub:20976993-E3E7-40FF-B602-0D3E515F9DA0.

Results

Proceratophrys kaingang sp. nov.

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Figs. 1–3

Holotype. ZUFSM 11127, adult female, collected at the Reserva Particular do Patrimônio Natural *Rancho Sonho Meu* (RPPN; Private Reserve of Natural Heritage), Tibagi municipality, in the Guartelá Canyon region from Campos Gerais of Paraná state, South Brazil (–24,559588, –50,275243), on 24 February 2016 by T. G. Santos, S. S. A. Saccol and A. A. B. Portela.

Paratypes. ZUFSM 11123, ZUFSM 11126, ZUFSM 11131, ZUFMS-AMP14527–14530 (all adult males), ZUFSM 11132 and ZUFMS-AMP14526 (adult females), collected with the holotype. ZUFSM 11079, ZUFSM 11080, ZUFSM 11081, and ZUFSM 11082 (adult males), collected at a private farmland (–24.559588, –50.275243) on 22 February 2016, by the same collectors.

Diagnosis. *Proceratophrys kaingang* sp. nov. is diagnosed by the following combination of characters: (1) small size for *P. bigibbosa* group (SVL 22.97–27.10 mm in adult males, 33.46–39.36 mm in adult females); (2) snout rounded in ventral and dorsal views, obtuse in profile; (3) upper eyelid border with small, rounded tubercles of similar size, and fused; (4) small postocular swellings; (5) yellowish blotches on the venter (in life); (6) toe webbing poorly developed; (7) distinct tympanic membrane, bordered by rounded tubercles.

Comparison with other Species. *Proceratophrys kaingang* sp. nov. readily differs from *P. appendiculata*, *P. belzebul*, *P. boiei*, *P. gladius*, *P. itamari*, *P. izecksohni*, *P. laticeps*, *P. mantiqueira*, *P. melanopogon*, *P. moehringi*, *P. paviotii*, *P. phyllostomus*, *P. pombali*, *P. renalis*, *P. rondonae*, *P. sanctaritae*, *P. subguttata*, and *P. tupinamba* by the absence of a single unicuspidate palpebral appendage (a single and long unicuspidate palpebral appendage in all species, except in *P. rondonae*, which has a single and short multi-cuspidate palpebral appendage). In addition, *P. kaingang* sp. nov. can be distinguished from *P. appendiculata*, *P. belzebul*, *P. gladius*, *P. itamari*, *P. izecksohni*, *P. laticeps*, *P. mantiqueira*, *P. melanopogon*, *P. moehringi*, *P. phyllostomus*, *P. pombali*, *P. sanctaritae*, *P. subguttata*, and *P. tupinamba* by lacking a rostral appendage (present in those species). *Proceratophrys kaingang* sp. nov. differs from *P. ararype*, *P. bagnoi*, *P. branti*, *P. carranca*, *P. concavitympanum*, *P. cristiceps*, *P. cururu*, *P. dibernardoi*, *P. goyana*, *P. huntingtoni*, *P. minuta*, *P. moratoi*, *P. redacta*, *P. rotundipalpebra*, *P. salvatori*, *P. schirchi*, *P. strussmannae* and *P. vielliardi* by the presence of post-ocular swellings (absent in these species).

Among the species from *P. bigibbosa* group, *P. kaingang* sp. nov. differs by (1) its smaller size (mostly in males): 22.97–27.10 mm in males, and 33.46–39.36 mm in females (*P. brauni*: 30.0–34.6 mm in males and 38.9–39.8 mm in females; *P. bigibbosa*: 35.5–43.8 mm in males and 51.2–53.4 mm in females; Kwet and Faivovich, 2001; and *P. palustris*: 27.3–33.8 mm in males, Giaretta and Sazima, 1991), except from *P. avelinoi* that presents similar sizes (23.9–29.2 males and 30.2–36.5 in females); (2) snout rounded in dorsal view (*P. brauni*: pointed tip of the snout); (3) upper eyelid border with small, rounded tubercles of similar size, and fused (*P. avelinoi*: small and triangular tubercles of varying sizes, and fused; *P. bigibbosa*: enlarged and pointed tubercles of varying sizes, not fused; *P. brauni*: long and triangular pointed tubercles of varying sizes, not fused); (4) small postocular swellings (*P. bigibbosa*, *P. brauni* and *P. palustris*: presence of two well-developed, bulbous, bony post-ocular swellings); (5) toe webbing poorly developed (*P. bigibbosa*: well-developed toe webbing); (6) yellowish blotches on the venter (*P. avelinoi* and *P. brauni*: venter with orange reddish blotches; *P. bigibbosa*: venter red irregularly spotted with black; *P. palustris*: venter dark-grey with small beige blotches); and (7) distinct tympanic membrane, bordered by rounded tubercles (*P. avelinoi*: tympanic membrane indistinct, covered with minute homogeneous tubercles).

Description of the Holotype. Head wider than long (HL/HW = 0.70), head length 32% of SVL, snout rounded in dorsal and ventral views, obtuse in profile; nares elliptical and prominent, canthal crests well marked, prominent, and covered by small tubercles; no preocular crests; eyes directed anterolaterally, eye diameter 38% of head length; eyelid with distinct, rounded tubercles, with the contact point between the ocular-dorsal ridge of warts and the external eyelid margin tubercles in a tubercle posterior to the post-ocular swellings, six warts on the border of the left eyelid and five on the right; sparse tubercles on the eyelid; distinct tympanum; vomerine teeth in two short rows between and below the choanae; frontoparietal crests well developed; region between frontoparietal crests shallow; interocular ridge of warts not organized in a row, with sparse small rounded tubercles; ocular-dorsal ridge of warts incomplete, and discontinued to the coccyx region. Dorsal surface, including flanks, arms and legs, with various warts of different sizes and shapes, a single row of tubercles in different sizes bordered with some sparse tubercles on the forearm; ventral surfaces, except

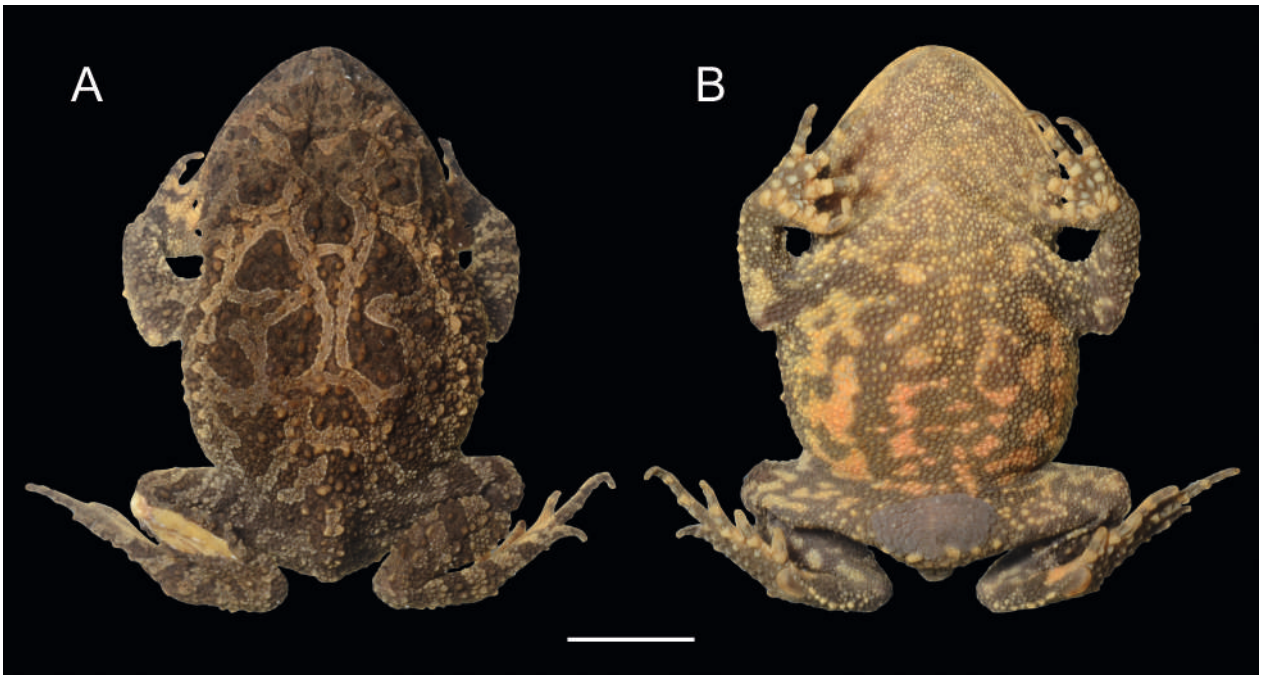

Figure 1. Holotype of *Proceratophrys kaingang* sp. nov. (ZUFSM11127). (A) Dorsal view of the body; and (B) ventral view of the body. Scale bar = 10 mm.

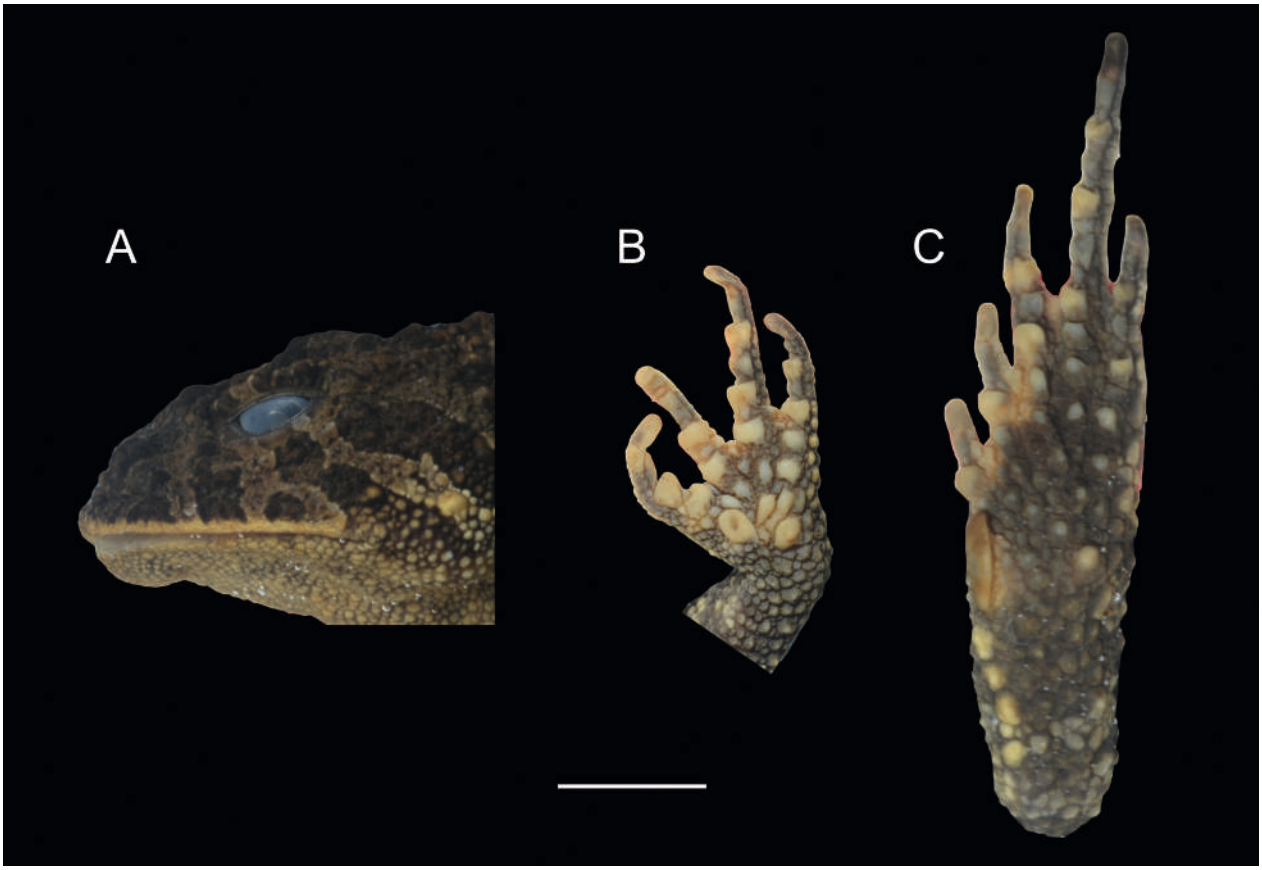

Figure 2. Holotype of *Proceratophrys kaingang* sp. nov. (ZUFSM11127). (A) Head lateral view; (B) ventral view of right hand; and (C) ventral view of right foot. Scale bar = 5 mm

hands and feet and cloacal region, covered by numerous small, rounded, uniform warts. Finger lengths IV > II > I > III (Fig. 2b); interdigital webbing absent; inner metacarpal tubercle rounded; single outer metacarpal small, both internal and external are rounded; scarce small, rounded supernumerary tubercles; subarticular tubercles large, rounded, but grooved anteriorly and posteriorly. Toe lengths I > II > V > III > IV; inner metatarsal tubercle

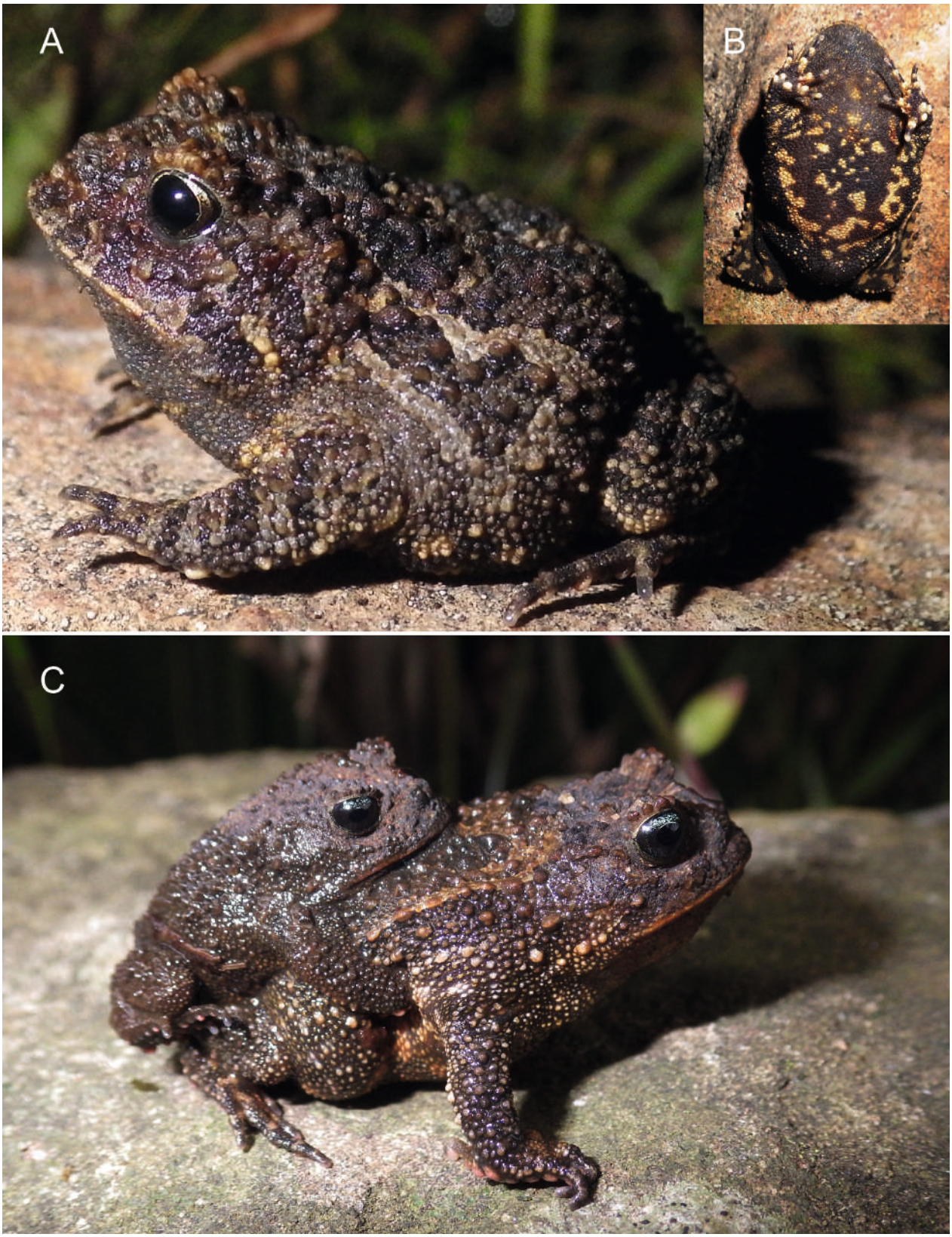

Figure 3. Live specimens of *Proceratophrys kaingang* sp. nov. from Parque Estadual do Guartelá (type locality), Tibagi municipality, Pará state, Brazil. (A) a live male (ZUFSM11127); (B) the ventral coloration in life of an adult male (ZUFSM11127); and (C) a couple in amplexus.

long, elliptical, poorly spatulated; outer metatarsal tubercle small, rounded; scarce small, rounded supernumerary tubercles; subarticular tubercles large, nearly rounded, grooved anteriorly and posteriorly.

Color Pattern in Life (Fig 3). Dorsal coloration overall in variable shades of brown, with regular patterns of dark brown blotches in the dorsum. Presence of longitudinal irregular stripes of light brown in dorsolateral region. Gu-

Table 1. Morphometric measurements (mm) for the type series of *Proceratophrys kaingang* sp. nov. Measurement acronyms: snout-vent length (SVL); head length, defined as the diagonal distance from the tip of the snout to the right angle of the jaw (HL); head width, defined as the distance between the angles of the jaw (HW); horizontal eye diameter (ED); eye-nostril distance (EN); nostril-snout distance (NS); internarial distance (IN); tibia length (TL); foot length (FL); inner metatarsal tubercle length (ML).

Measurement	Holotype ZUFSM11127	Males			Females		
		Average	SD	Range	Average	SD	Range
SVL	39.36	25.18	1.54	22.97–27.10	35.87	3.10	33.46–39.36
HL	12.40	8.58	0.95	7.31–10.25	11.71	0.66	11.08–12.40
HW	17.77	11.84	0.81	10.15–12.86	16.13	1.42	15.30–17.77
ED	3.85	3.19	0.38	2.42–3.95	3.70	0.39	3.25–3.99
EN	3.90	2.85	0.41	2.24–3.64	3.72	0.20	3.51–3.90
NS	3.28	2.51	0.22	2.09–2.76	3.34	0.35	3.03–3.72
IN	2.63	1.94	0.20	1.61–2.35	2.57	0.08	2.48–2.63
TL	13.99	9.12	0.65	8.24–10.09	12.70	1.15	11.79–13.99
FL	22.56	14.45	0.96	12.88–16.45	20.30	1.96	19.00–22.56
ML	2.67	1.71	0.14	1.51–1.99	2.30	0.38	1.91–2.67
HL/HW	0.70	0.72	0.05	0.65–0.80	0.73	0.03	0.70–0.76
HL/SVL	0.32	0.34	0.03	0.31–0.38	0.33	0.01	0.32–0.33

Table 2. Unconrrected sequence divergence (p-distance) among *Proceratophrys* species within *P. bigibbosa* species group.

		1	2	3	4	5	6	7	8	9	10
1	FJ685692 *P. bigibbosa*	—									
2	MG798659 *P. bigibbosa*	0.008	—								
3	MG798660 *P. bigibbosa*	0.008	0.000	—							
4	DQ283039 *P. avelinoi*	0.047	0.047	0.047	—						
5	KP295643 *P. avelinoi*	0.047	0.047	0.047	0.000	—					
6	FJ685691 *P. avelinoi*	0.042	0.042	0.042	0.004	0.004	—				
7	KU495472 *P. brauni*	0.047	0.051	0.051	0.038	0.038	0.034	—			
8	ZUFSM 11080 *P. kaingang* sp. nov.	0.056	0.056	0.056	0.045	0.045	0.040	0.021	—		
9	ZUFSM 11127 *P. kaingang* sp. nov.	0.056	0.056	0.056	0.045	0.045	0.040	0.021	0.000	—	
10	ZUFSM 11082 *P. kaingang* sp. nov.	0.056	0.056	0.056	0.045	0.045	0.040	0.021	0.000	0.000	—

lar region cream colored with mottling dark brown. Belly dark brown to black, irregularly spotted with yellow. Ventral surface of limbs dark brown to black, spotted with yellowish marks. Palm, fingers, soles of foot and toes are black, with two to three transverse dark-brown bars on fingers and toes.

Color Pattern in Preservative. Overall coloration about the same as in life. However, the color became faded, and the light tones became darker. The longitudinal irregular stripes are brown in dorsal-lateral region. Gular region color beige with mottling dark brown. Belly dark brown irregularly spotted with beige.

Variation. The main variation within this species relies on the sexual size dimorphism, with females (Fig. 4; 33.46–39.36 mm) bigger than males (Fig. 5; 22.97–27.10 mm); in addition, males have a darker gular region (Fig. 5). Overall, the tubercles on the dorsum can vary in size, and some can be rounded to triangular. The variation of the dorsal coloration is more prominent in the dark brown blotches that border the dorsal row of tubercles. The ventral pattern varying slightly on shape and size of yellowish blotches (Fig. 4 and 5).

Phylogenetic Inferences and Mitochondrial DNA Divergences. Our 16S tree (Fig. 5) confidently recovered *P. kaingang* sp. nov. nested within the *P. bigibbosa* species group, and as the sister taxon of *P. brauni* (pp > 0.95). All nodes for species in the *P. bigibbosa* species group are well supported (pp > 0.95); however, some deeper nodes within *Proceratophrys* had low posterior probabilities, probably due to the single based gene tree. Average sequence divergence between the new species and its congeners within the *P. bigibbosa* species group ranges from 2.1% (*P. brauni*) to 5.6% (*P. bigibbosa*) (Appendix II).

Distribution and Natural History. *Proceratophrys kaingang* sp. nov. is known only from its type locality, the Guartelá Canyon region, Tibagi municipality, in the *Campos Gerais* of Paraná state, Brazil (Fig. 7). Grassland physiognomies (e.g., rocky vegetational refuge, hygrophilous steppe, and grassy-woody steppe) are predominant in this region (Fig. 8), consisting of relictual vegetation that include Mixed Ombrophilous Forest and Cerrado mosaics (Carmo et al. 2012; Souza et al. 2018). Calling males and a female were found in temporary puddles and slow running waters associated to flooded grasslands in the Private Reserve of Natural Heritage (RPPN *Rancho*

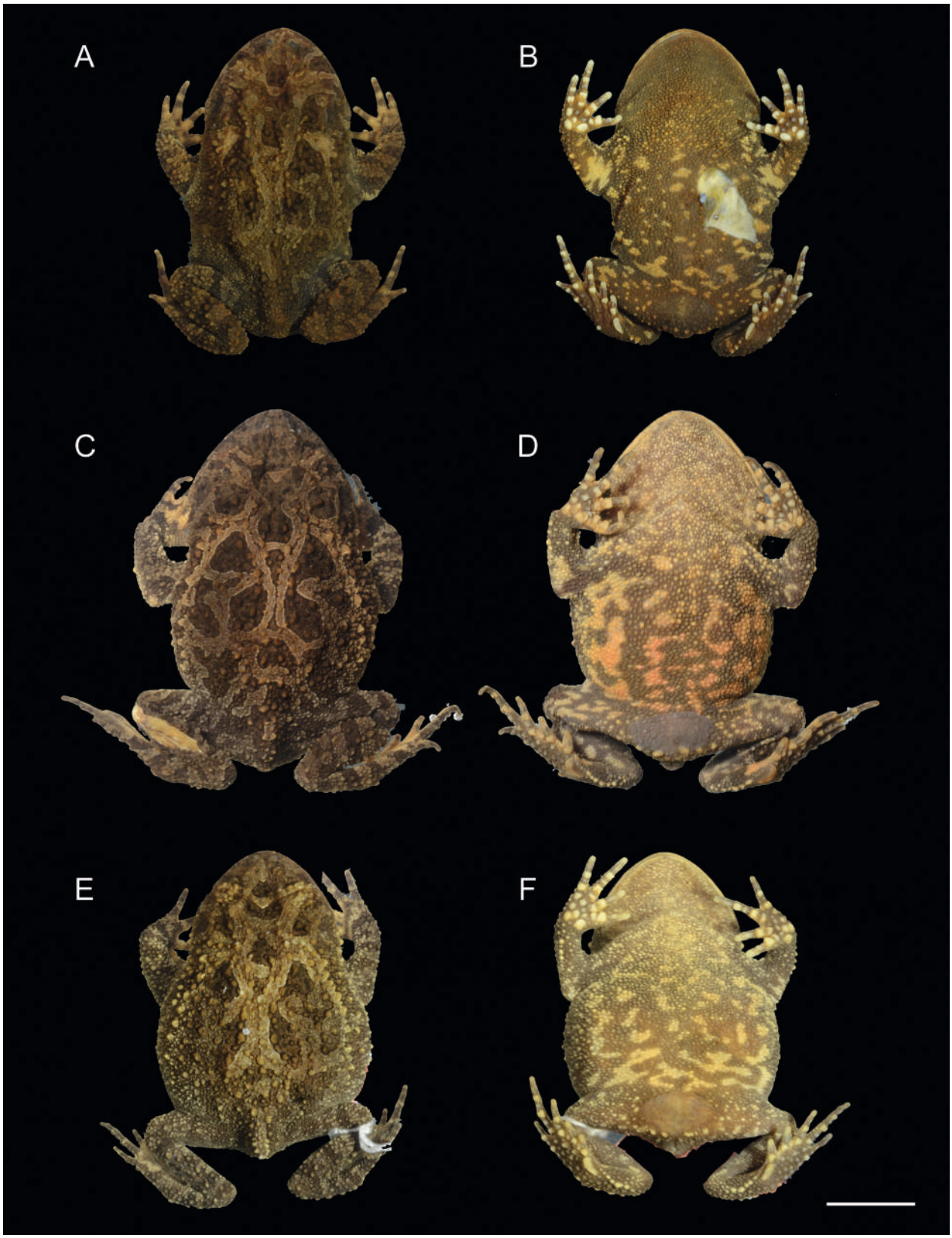

Figure 4. Dorsal and ventral color variation in preservative among female specimens from type series. (A) dorsal and (B) ventral views of ZUFSM11027 (holotype); (C) dorsal and (D) ventral views of ZUFSM11132; (E) dorsal and (F) ventral views of ZUFMS-AMP14526. Scale bar = 10 mm

Sonho Meu) and in a wetland in agricultural landscape. Calling activity was recorded in both diurnal and nocturnal periods (from early afternoon until at least ~11:00 h pm) during a historic event of heavy rains in the Paraná state. Males called from moist soil, exposed at the muddy edges of puddles as well as partially submerged in shallow flowing water, hidden among hygrophilous vegetation. Males of at least 12 other species were calling in the

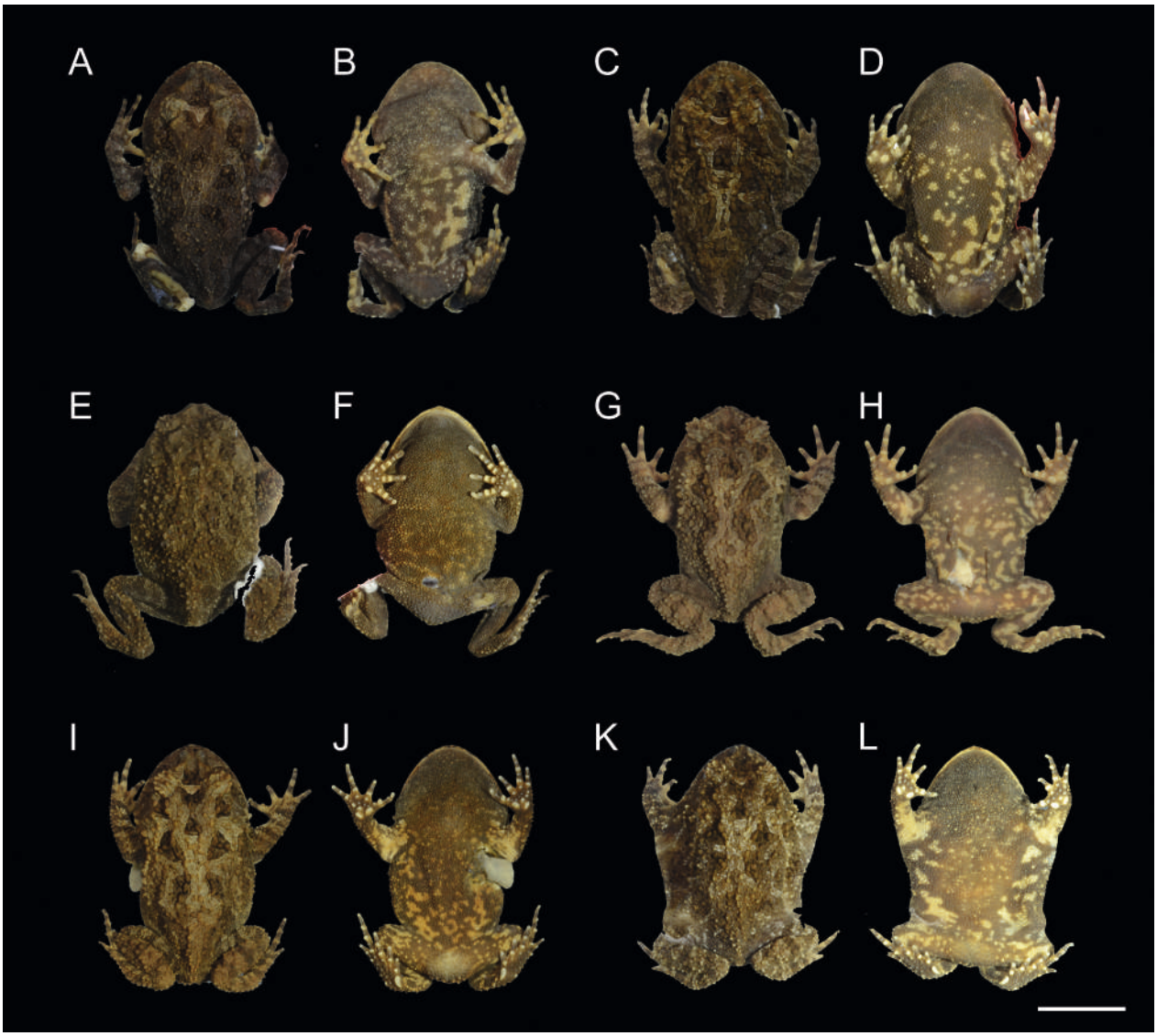

Figure 5. Dorsal and ventral color variation in preservative among male specimens from type series: (A) dorsal and (B) ventral views of ZUFSM11079; (C) dorsal and (D) ventral views ZUFSM11080; (E) dorsal and (F) ventral views of ZUFSM11131; (G) dorsal and (H) ventral views of ZUFMS-AMP14528; (I) dorsal and (J) ventral views of ZUFMS-AMP14529; (K) dorsal and (L) ventral views of ZUFMS-AMP14530.

same breeding sites used by *Proceratophrys kaingang* sp. nov. (i.e., *Aplastodiscus perviridis*, *Boana albpunctata*, *B. prasina*, *Dendropsophus minutus*, *Leptodactylus furnarius*, *L. fuscus*, *L. plaumanni*, *Melanoprhyniscus vilavelhensis*, *Physalaemus* aff. *gracilis, Scinax fuscovarius, S. rossaferesae,* and *S. squalirostris*).

Etymology. The specific epithet *kaingang* is a noun in apposition referring to the Kaingang (or Caingangue) ethnic group, which inhabits the plateau regions of the states of Paraná, São Paulo, Rio Grande do Sul and Santa Catarina, Brazil. We suggest the following Portuguese vernacular names "sapo-de-chifre-dos-caingangue" or "sapo-de-chifre-do-guartelá".

Discussion

Proceratophrys kaingang sp. nov. is the fifth species of the *P. bigibbosa* species group. Similarly to other species within this group, the new species also occurs in open formations in ecotone areas with subtropical forests (Giaretta and Sazima 1993; Kwet and Faivovich 2001; Caldart et al. 2010). The geographical distribution of *P. bigibbosa* species group is similar to those reported for the genus *Julianus* that include a disjunct gap of *J. pinima*, i.e., a northern relictual distribution associated to rock outcrops of the Espinhaço Mountain Range of Minas Gerais state, and a southern distribution along the highland grasslands

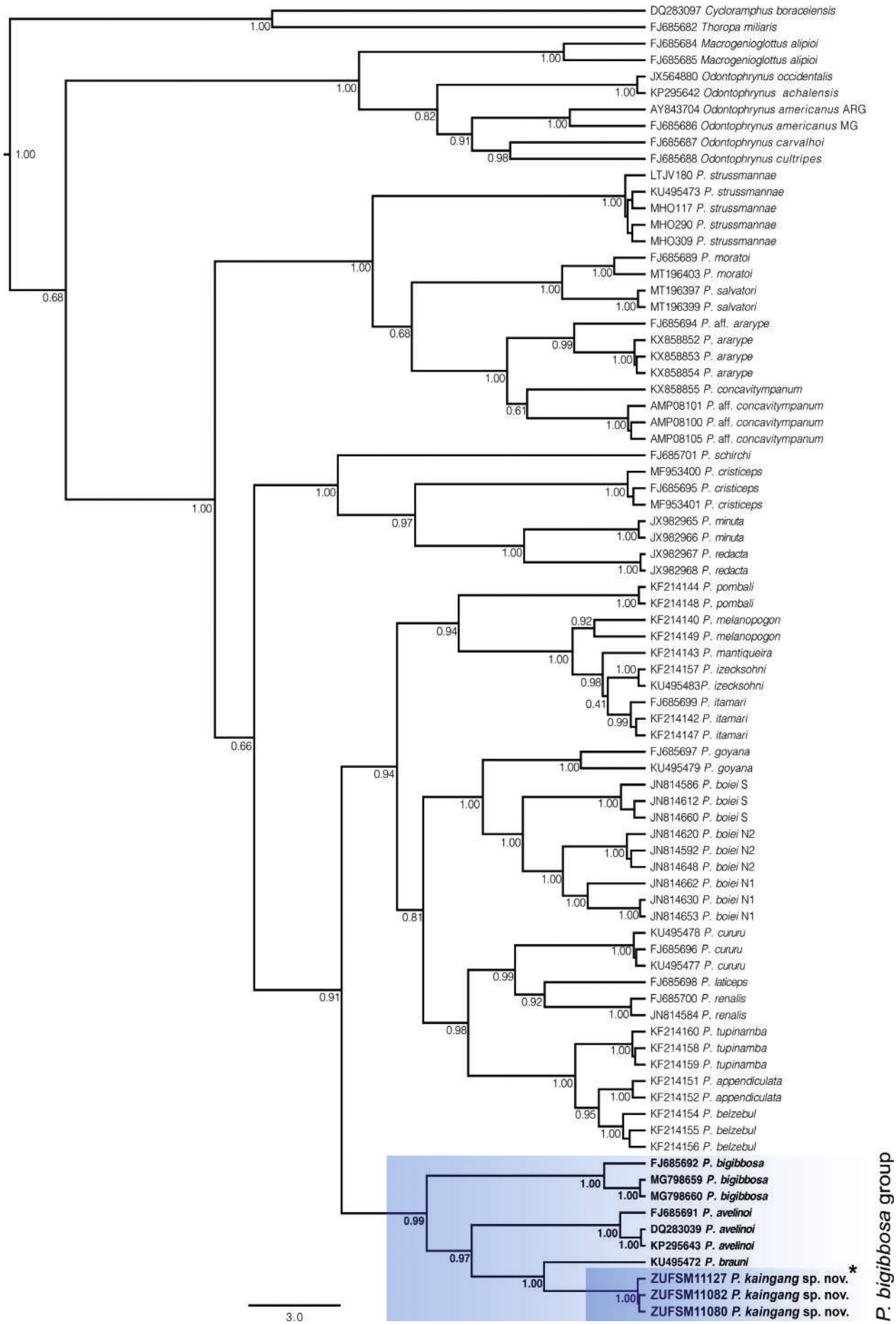

Figure 6. Gene tree based on phylogenetic analysis of the 16S mtDNA gene for the 28 species of the genus *Proceratophrys*. Scale indicates rate of base substitutions per site.

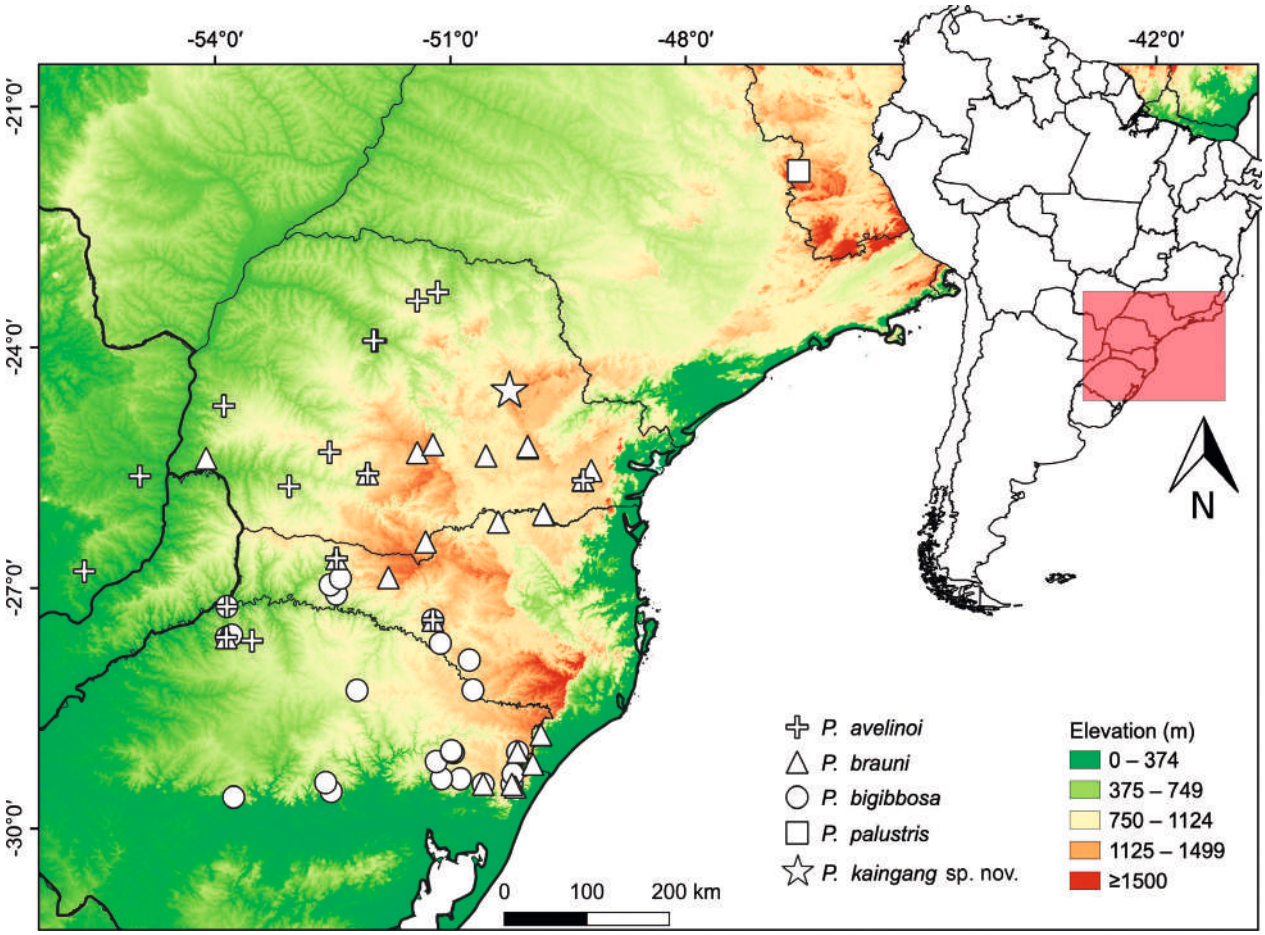

Figure 7. Geographic distribution of species from *Proceratophrys bigibbosa* species group in South America.

of Paraná, Santa Catarina and Rio Grande do Sul states (Baldo et al. 2019). In South Brazil, there is open formations along different vegetational ecotones. In the Tibagi area, the Guartelá Canyon region is a landscape of the Campos Gerais corresponding to the second plateau of Paraná state. The native landscape is characterized as a vegetational relict associated to southern grasslands (Campos Sulinos), Araucaria Forest (Floresta Ombrófila Mista), and Tropical savannah (Cerrado) (Carmo et al. 2012; da Maia and Goldenberg 2014; Souza et al. 2018). At least two other species occur in this region, *Melanophryniscus vilavelhensis* and *Scinax rossaferesae*, with distributions strongly associated to the region (Crivellari et al. 2014; Conte et al. 2016). These species are found in several areas along the Campos Gerais region of the second plateau of Paraná, we expect the occurrence of *P. kaingang* sp. nov. in different locations along this region. Historically, this open grasslands in Southern Brazil have been replaced by agricultural land (MMA 2000; Carmo et al. 2012), particularly soy bean and silviculture (mainly *Pinus* and *Eucalyptus*). Our search for anuran fauna in ponds in the region, among crops and silviculture, but did not find any individual. However, we found the new species inside a conservation unit, where the population was in breeding activity.

Despite the stable taxonomic history of the *P. bigibbosa* species group in the last two decades, the description of *P. kaingang* sp. nov. and the rapid increase of species discovered in the genus highlights the need to study biogeographic and evolutionary patterns to better understand the species distribution. In addition, recent integrative approaches used in *Proceratophrys* taxonomy (Teixeira-Jr et al. 2012; Mângia et al. 2018, 2020; Magalhães et al. 2020;) should be applied to populations assigned to the *P. bigibbosa* group recently (Santos et al. 2009; Caldart et al. 2010; Carosini et al. 2010). Although *P. kaingang* sp. nov. is known only from its type locality, other populations belonging to the *P. biggibosa* group along the Campos Gerais of Paraná may reveal the real distribution of the new species and improved our knowledge to understand its conservational status.

Figure 8. Habitat in which *Proceratophrys kaingang* sp. nov. was found. (A) General view of the Private Reserve of Natural Heritage (RPPN Rancho Sonho Meu), Tibagi municipality, in the Guartelá Canyon region from Campos Gerais of Paraná state, Brazil. (B) The predominant grassland physiognomies in the region, and (C) the rocky vegetational refuge, hygrophilous steppe and grassy-woody steppe where the new species occurs.

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Appendix I

Additional Specimens Examined

Acronyms: Coleção Herpetológica da Universidade Federal do Rio Grande do Norte (UFRN), Coleção Herpetológica da Universidade Federal de Pernambuco (CHUFPE), Museu de Zoologia da Universidade Federal da Bahia (MZUFBA), Coleção Zoológica da Universidade Federal de Mato Grosso (UFMT), Coleção Célio F. B. Haddad, Universidade Estadual Paulista (CFBH), Museu de Zoologia Prof. Adão José Cardoso, Universidade Estadual de Campinas (ZUEC), Museu de Zoologia da Universidade Estadual de Feira de Santana (MZFS), Coleção Herpetológica da Universidade Federal de Minas Gerais (CHUFMG), Museu de Ciências Naturais, Pontifícia Universidade Católica de Minas Gerais (MCNAM), Museu Nacional do Rio de Janeiro, Universidade Federal do Rio de Janeiro (MNRJ), and Coleção de Herpetologia da Universidade Regional do Cariri (URCAH).

Proceratophrys appendiculata. BRAZIL: RIO DE JANEIRO: Angra dos Reis: MNRJ 34016. Guapimirim: MNRJ 30983. Nova Friburgo: MNRJ 34017. Rio de Janeiro: MNRJ 31547.

***Proceratophrys avelinoi*.** BRAZIL: RIO GRANDE DO SUL: Dois Irmãos das Missões: MCP 9772. Bom Progresso: MCP 13066.

***Proceratophrys belzebul*.** BRAZIL: SÃO PAULO: Ubatuba: MNRJ 87144.

Proceratophrys bigibbosa. BRAZIL: RIO GRANDE DO SUL: São Francisco de Paula: MCP 2419, 3204.

***Proceratophrys boiei*.** BRAZIL: ALAGOAS: Murici: MNRJ 9719–20, 9726–29, 9732. Passo de Camaragibe: MNRJ 9817, 9862, 9863, 9864. Quebrangulo: MNRJ 9972. RIO DE JANEIRO: Teresópolis: MNRJ 37328–32.

***Proceratophrys branti*.** BRAZIL: TOCANTINS: Palmas: Taquarussu: UFMS AMP 5536–5538, 8118–8120; Novo Acordo: 8106.

***Proceratophrys brauni*.** BRAZIL: SANTA CATARINA: Timbé do Sul: MNRJ 25003–04. RIO GRANDE DO SUL: São Francisco de Paula: MCP 3686, 3769, 12940.

***Proceratophrys carranca*.** BRAZIL: MINAS GERAIS: Buritizeiro: MNRJ 86440–42.

***Proceratophrys cristiceps*.** BRAZIL: CEARÁ: Serra de Ibiapaba: UFPB 6117-26. Ubajara, Parque Nacional de Ubajara: AAGARDA 10672, 10695, 10698-99, 10703, 10707-09, 10782, 10796, 10907, 10909, 10911-14, 10961, 10974, 10981, 10983. Várzea da Conceição: UFPB 9661, 9665, 9667. PIAUÍ: Floriano: UFPI 214-16, 222, 236. Piripiri: UFPB 10340, 10342-46. RIO GRANDE DO NORTE: Serra Negra do Norte, Estação Ecológica do Seridó: AAGARDA 5447, 5528, 5583, 5689, 6061, 6790. João Câmara: AAGARDA 8913-15, 9806-11; URCA 422, 427, 483-85, 487-88, 493, 498, 501. Macaíba, Escola Agrícola de Jundiaí: AAGARDA 1013-14, 1019-20, 1753-71, 1773, 1776, 1778, 1786-91, 1935, 2495-96, 2583, 3757, 5447, 5528, 5554, 5583, 5689, 6061, 6790, 8866-71, 8913-15, 9806-11.

***Proceratophrys concavitympanum*.** BRAZIL: MATO GROSSO: Aripuanã: MZUFV 9552, 9554–95556, UFMT 11697–11699; Colniza: UFMT 6808; Juína: UFMT 6996, 7825. RONDÔNIA: Espigão D'Oeste: CFBH 5135, 5136; Ministro Andreazza: CFBH 19815, CFBH 19818.

***Proceratophrys cururu*.** BRAZIL: MINAS GERAIS: Santana do Riacho: MNRJ 17905.

***Proceratophrys gladius*.** BRAZIL: SÃO PAULO: São José do Barreiro: MNRJ: 82577–79.

***Proceratophrys goyana*.** BRAZIL: GOIÁS: Colinas do Sul: MNRJ 68292–95. Minaçu: MNRJ 17309–14. Rio São Miguel: MNRJ 47902. Veadeiros: MNRJ 47901, 47903– 04.

***Proceratophrys itamari*.** BRAZIL: SÃO PAULO: Campos do Jordão: MNRJ 82580–84.

***Proceratophrys izecksohni*.** BRAZIL: RIO DE JANEIRO: Parati: MNRJ 88985–86.

***Proceratophrys laticeps*.** BRAZIL: BAHIA: Ilhéus: MNRJ 4124–26, 13950–55. ESPÍRITO SANTO: Conceição da Barra: MNRJ 27946, 27949.

***Proceratophrys mantiqueira*.** BRAZIL: MINAS GERAIS: Ervália: MNRJ 82573–76.

***Proceratophrys melanopogon*.** BRAZIL: RIO DE JANEIRO: Resende: MNRJ 51654–705.

***Proceratophrys minuta*.** BRAZIL: BAHIA: Miguel Calmon, Parque Estadual das Sete Passagens: MNRJ 75410–17.

***Proceratophrys moehringi*.** BRAZIL: ESPÍRITO SANTO: Santa Teresa: MNRJ 46804.

***Proceratophrys moratoi*.** BRAZIL: SÃO PAULO: Botucatu: MNRJ 60085.

***Proceratophrys paviotii*.** BRAZIL: ESPÍRITO SANTO: Santa Teresa: MNRJ 84079–80; Aracruz: MNRJ 40182–84.

***Proceratophrys renalis*.** BRAZIL: ALAGOAS: Passo de Camaragibe: MNRJ 9817.

***Proceratophrys rondonae*.** BRAZIL: RÔNDONIA: Bacia do Rio Branco: MNRJ 40906.

***Proceratophrys sanctaritae*.** BRAZIL: BAHIA: Amargosa: MNRJ 62354–62357.

***Proceratophrys schirchi*.** BRAZIL: BAHIA: Guaratinga: MNRJ 26459–60; Jussari: MNRJ 26456–58; Nilo Peçanha: MNRJ 26461. ESPÍRITO SANTO: Santa Teresa: MNRJ 18445–46, 56000–01.

***Proceratophrys strussmannae*.** BRAZIL: MATO GROSSO: Vale de São Domingos: UFMT 1834, 1836, 7882, 7885, 8319,8320, 8377, 8380; Araputanga: UFMT 7879.

***Proceratophrys subguttata*.** BRAZIL: SANTA CATARINA: Brusque: MNRJ 18282; São Bento do Sul: MNRJ 18281.

***Proceratophrys tupinamba*.** BRAZIL: RIO DE JANEIRO: Angra dos Reis: MNRJ 25101–18, 38938.

***Proceratophrys vielliardi*.** BRAZIL: GOIÁS: Caldas Novas: MNRJ 83314–15.

Appendix II

Genbank acession number and references of the sequences used in the present work.

Species	Genbank Acession	Reference
Cycloramphus boraceiensis	DQ283097	Frost et al. (2006)
Macrogenioglottus alipioi	FJ685684	Amaro et al. (2009)
Macrogenioglottus alipioi	FJ685685	Amaro et al. (2009)
Odontophrynus occidentalis	JX564880	Murphy et al. (2013)
Odontophrynus achalensis	KP295642	Faivovich et al. (2014)
Odontophrynus americanus MG	FJ685686	Amaro et al. (2009)
Odontophrynus carvalhoi	FJ685687	Amaro et al. (2009)
Odontophrynus cultripes	FJ685688	Amaro et al. (2009)
Odontpphrynus americanus ARG	AY843704	Faivovich et al. (2005)
Proceratophrys aff. *ararype*	FJ685694	Amaro et al. (2009)
Proceratophrys appendiculata	KF214151	Dias et al. (2013)
Proceratophrys appendiculata	KF214152	Dias et al. (2013)
Proceratophrys ararype	KX858852	Mângia et al. (2018)
Proceratophrys ararype	KX858853	Mângia et al. (2018)
Proceratophrys ararype	KX858854	Mângia et al. (2018)
Proceratophrys avelinoi	DQ283039	Frost et al. (2006)
Proceratophrys avelinoi	FJ685691	Amaro et al. (2009)
Proceratophrys avelinoi	KP295643	Faivovich et al. (2014)
Proceratophrys belzebul	KF214154	Dias et al. (2013)
Proceratophrys belzebul	KF214155	Dias et al. (2013)
Proceratophrys belzebul	KF214156	Dias et al. (2013)

Species	Genbank Acession	Reference
Proceratophrys bigibbosa	FJ685692	Amaro et al. (2009)
Proceratophrys bigibbosa	MG798659	Dias et al. (2018)
Proceratophrys bigibbosa	MG798660	Dias et al. (2018)
Proceratophrys kaingang sp. nov. ZUFSM11080	MW916088	This study
Proceratophrys kaingang sp. nov. ZUFSM11082	MW916090	This study
Proceratophrys kaingang sp. nov. ZUFSM11127	MW916089	This study
Proceratophrys boiei N1	JN814630	Amaro et al. (2012)
Proceratophrys boiei N1	JN814653	Amaro et al. (2012)
Proceratophrys boiei N1	JN814662	Amaro et al. (2012)
Proceratophrys boiei N2	JN814592	Amaro et al. (2012)
Proceratophrys boiei N2	JN814620	Amaro et al. (2012)
Proceratophrys boiei N2	JN814648	Amaro et al. (2012)
Proceratophrys boiei S	JN814586	Amaro et al. (2012)
Proceratophrys boiei S	JN814612	Amaro et al. (2012)
Proceratophrys boiei S	JN814660	Amaro et al. (2012)
Proceratophrys brauni	KU495472	Lyra et al. (2017)
Proceratophrys concavitympanum	KX858855	Mângia et al. (2018)
Proceratophrys cristiceps	FJ685695	Amaro et al. (2009)
Proceratophrys cristiceps	MF953400	Mângia et al. (2018)
Proceratophrys cristiceps	MF953401	Mângia et al. (2018)
Proceratophrys cururu	FJ685696	Amaro et al. (2009)
Proceratophrys cururu	KU495477	Lyra et al. (2017)
Proceratophrys cururu	KU495478	Lyra et al. (2017)
Proceratophrys goyana	FJ685697	Amaro et al. (2009)
Proceratophrys goyana	KU495479	Lyra et al. (2017)
Proceratophrys itamari	FJ685699	Amaro et al. (2009)
Proceratophrys itamari	KF214142	Dias et al. (2013)
Proceratophrys itamari	KF214147	Dias et al. (2013)
Proceratophrys izecksohni	KF214157	Dias et al. (2013)
Proceratophrys izecksohni	KU495483	Lyra et al. (2017)
Proceratophrys sp.	MW889930	This study
Proceratophrys sp.	MW889928	This study
Proceratophrys sp.	MW889929	This study
Proceratophrys laticeps	FJ685698	Amaro et al. (2009)
Proceratophrys mantiqueira	KF214143	Dias et al. (2013)
Proceratophrys melanopogon	KF214140	Dias et al. (2013)
Proceratophrys melanopogon	KF214149	Dias et al. (2013)
Proceratophrys minuta	JX982965	Teixeira Jr et al. (2012)
Proceratophrys minuta	JX982966	Teixeira Jr et al. (2012)
Proceratophrys moratoi	FJ685689	Amaro et al. (2009)
Proceratophrys moratoi	MT196403	Magalhães et al. (2020)
Proceratophrys pombali	KF214144	Dias et al. (2013)
Proceratophrys pombali	KF214148	Dias et al. (2013)
Proceratophrys redacta	JX982967	Teixeira Jr et al. (2012)
Proceratophrys redacta	JX982968	Teixeira Jr et al. (2012)
Proceratophrys renalis	FJ685700	Amaro et al. (2009)
Proceratophrys renalis	JN814584	Amaro et al. (2012)
Proceratophrys salvatori	MT196397	Magalhães et al. (2020)
Proceratophrys salvatori	MT196399	Magalhães et al. (2020)
Proceratophrys schirchi	FJ685701	Amaro et al. (2009)
Proceratophrys strussmannae	KU495473	Lyra et al. (2017)
Proceratophrys strussmannae	MZ264854	This study
Proceratophrys strussmannae	MZ264855	This study
Proceratophrys strussmannae	MZ264856	This study
Proceratophrys strussmannae	MZ264857	This study
Proceratophrys tupinamba	KF214158	Dias et al. (2013)
Proceratophrys tupinamba	KF214159	Dias et al. (2013)
Proceratophrys tupinamba	KF214160	Dias et al. (2013)
Thoropa miliaris	FJ685682	Amaro et al. (2009)