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

Diego José Santana¹, Sarah Mângia¹, Suélen da Silva Alves Saccol², Tiago Gomes dos Santos³

¹ Universidade Federal de Mato Grosso do Sul, Instituto de Biociências, 79070-900, Campo Grande, MS, Brazil
² Programa de Pós-Graduação em Biodiversidade Animal, Universidade Federal de Santa Maria, Departamento de Ecologia e Evolução, 97105-900, Santa Maria, RS, Brazil
³ Universidade Federal do Pampa, Campus São Gabriel, 97300-162, São Gabriel, RS, Brazil

http://zoobank.org/20976993-E3E7-40FF-B602-0D3E515F9DA0

Corresponding authors: Diego José Santana (jose.santana@ufms.br)

**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. christiceps, P. minuta*, and *P. redacta*), the *P. concavitympanum* clade (*P. ararype, P. concavitympanum, P. moratoi, P. salvatori*, and *P. strussmannae*) distributed in Amazonia,
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 (Giarretta 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 taxon-omy. 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 Rêpteis 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 I).

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); intermaxillary 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: 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 59°C for 1 min, and 72°C for 1 min, and a final 5 min extension at 72°C. 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 angata* 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: LSID:urn:lsid:zoobank.org:pub:20976993-E3E7-40FF-B602-0D3E515F9DA0.

Results

Proceratophrys kaingang sp. nov.

http://zoobank.org/220976993-E3E7-40FF-B602-0D3E515F9DA0

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 Guaratêla 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 ZUFMS 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. phyllostomus, P. pombali, P. sanctaritae, P. subguttata, and P. tunipamha by lacking a rostral appendage (present in those species). Proceratophrys kaingang sp. nov. differs from P. ararype, P. bagnoi, P. branti, P. carranca, P. concavitýmpaman, P. cristiceps, P. cururu, P. dibernardo, 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 postocular 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 postocular 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 postocular 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; frontotemporal crests well developed; region between frontotemporal 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
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
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-
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...
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 4. Dorsal and ventral color variation in preservative among female specimens from type series. (A) dorsal and (B) ventral views of ZUFMS11027 (holotype); (C) dorsal and (D) ventral views of ZUFMS11132; (E) dorsal and (F) ventral views of ZUFSM-AMP14526. Scale bar = 10 mm
same breeding sites used by *Proceratophrys kaingang* sp. nov. (i.e., *Aplastodiscus perviridis*, *Boana albipunctata*, *B. prasina*, *Dendropsophus minutus*, *Leptodactylus furcarius*, *L. fuscus*, *L. plauamanni*, *Melanophryniscus vilavelhensis*, *Physalaemus aff. gracilis*, *Scinax fuscovarius*, *S. rossaferesi*, 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.
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. bigibbosa* 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 7. Geographic distribution of species from *Proceratophrys bigibbosa* species group in South America.
Acknowledgments

DJS and TGS thank Conselho Nacional de Desenvolvimento Científico e Tecnológico for the research fellowships (CNPq 309420/2020-2 and CNPq 308687/2016-7, respectively). SSAS is grateful to CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for doctoral fellowship. We also thank the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio) and Instituto Ambiental do Paraná (IAP) for issuing the collecting permit, as well as to the Neotropical Grassland Conservancy and the Programa de Pesquisa em Biodiversidade (PPBio) – Bioma Campos Sulinos (CNPq/MCTIC 457503/2012-2) for financial support. We thank all the landowners who granted access to the study sites, and to Sonia Z. Cechin (UFSM) for allowing us to examine specimens under her care.

References

Amaro RC, Pavan D, Rodrigues MT (2009) On the generic identity of Odontophrynus moratoi Jim & Caramaschi, 1980 (Anura, Cycloramphidae). Zootaxa 2071: 61–68. https://doi.org/10.11646/zootaxa.2071.1.5

Amaro RC, Rodrigues MT, Yonenaga-Yassuda Y, Carnaval AC (2012) Demographic processes in the montane Atlantic rainforest: Molecular and cytogenetic evidence from the endemic frog Proceratophrys boiei. Molecular Phylogenetics and Evolution 62: 880–888. https://doi.org/10.1016/j.ympev.2011.11.004

Baldo D, Araujo-Vieira K, Cardozo D, Borteiro C, Leal F, Pereyra MO, Kolene F, Lyra ML, Garcia PCA, Haddad CFB, Faivovich J (2019) A review of the elusive bicolored iris Snouted Treefrogs (Anura:

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 Guaíba 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.
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 Zooloigia da Universidade Federal da Bahia (MUFBA), 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: Ubá-tuba: 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. Quebranguio: MNRJ 9972. RIO DE JANEIRO: Teresópolis: MNRJ 37328–32.

**Proceratophrys branti.** BRAZIL: TOCANTINS: Palmas: Taquaruussu: 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 crispiceps.** BRAZIL: CEARÁ: Serra de Ibiapaba: UFPE 6117-26. Ubajara, Parque Nacional de Ubajara: AAGARDA 10672, 10695, 10698-99, 10703, 10707-09, 10782, 10907, 10909, 10911-14, 10961, 10974, 10981, 10983. Várzea da Conceição: UFPE 9661, 9665, 9667. PIAUÍ: Florianópolis: UFPB 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, 9554–9556, UFMT 11697–11699; Colniza: UFMT 6808; Juina: 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: RIO GRANDE DO SUL: São Francisco de Paula: MCP 3686, 3769, 12940.

**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: B. 60085.

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

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

Appendix II

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

| Species                          | Genbank Accession | Reference                          |
|----------------------------------|-------------------|------------------------------------|
| Cycloramphus boraciensis         | 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)                |
| Odontophrynus 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 bezebul           | KF214154          | Dias et al. (2013)                 |
| Proceratophrys bezebul           | KF214155          | Dias et al. (2013)                 |
| Proceratophrys bezebul           | KF214156          | Dias et al. (2013)                 |
| Species               | Genbank Accession | 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 concavitypyanum | 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 itamari | KF214157 | Dias et al. (2013)  |
| Proceratophrys izecksohni | KU495483 | Lyra et al. (2017)  |
| 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)  |
| Thompa miliaris | FJ685682 | Amaro et al. (2009) |