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Taxonomic revision of the horned frog *Proceratophrys boiei* (Wied-Neuwied, 1824) (Amphibia, Anura, Odontophrynidae) with revalidation of *P. fryi* (Günther, 1873) and *P. intermedia* (Barbour, 1908)

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Abstract

Proceratophrys boiei is a horned frog species that lives camouflaged within leaf litter and inhabits both Atlantic Forest environments and transitional areas with the Cerrado of Brazil. Its distribution covers the states of Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo, Paraná, and Santa Catarina. Previous studies using molecular and cytogenetic data identified three distinct lineages (North 1, North 2, and South) grouped under the taxon *Proceratophrys boiei*. To investigate the possibility that more than one species is currently subsumed under *P. boiei*, a taxonomic review of populations across the entire geographic range was conducted. Morphological and morphometric characters were evaluated in 132 individuals (80 males and 52 females); 708 calls from 66 males were analyzed for acoustic data; and osteological traits were examined in nine males, representing a total of 82 localities. Analyses were conducted based on the three lineages previously identified (N1, N2, and S), and uncorrected mtDNA *p*-distances for the 16S and *Cytb* markers were estimated among them. External morphological and osteological characters and advertisement call parameters varied among these lineages; however, most of the variation overlapped, even among individuals within the same lineage, indicating high polymorphism. Mean uncorrected mtDNA *p*-distances among lineages were relatively similar for 16S, ranging from 2.7% to 2.9%, whereas for *Cytb*, they ranged from 5.6% to 7.3%. Regarding morphometrics, specimens from the South lineage exhibited the smallest snout–vent length (SVL: 46.0 mm in males and 51.6 mm in females). Based on the results, combined with differences previously identified among the three lineages (using molecular markers, cytogenetics, and tadpole external morphology and chondrocranium), *P. fryi* and *P. intermedia*, both formerly considered synonyms of *P. boiei*, are revalidated, and *P. boiei* is redescribed, providing updated diagnostic characters and clarifying the current geographic distribution of each species.

Key Words

Atlantic Forest, geographic distribution, integrative taxonomy, systematics

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PENSOFT

Introduction

Species with wide geographical distributions can present taxonomic challenges, mainly due to polymorphism, as morphology can be influenced by locality and environmental characteristics (Gomes and Peixoto 1991; Orrico et al. 2017; Correa 2019). Historically, taxonomic assessments were primarily based on morphological characters, but they are now increasingly complemented by other methods, such as acoustic and molecular analyses, to support species delimitation (Padial et al. 2009; Da Silva et al. 2020). Recent studies have highlighted the importance of integrating multiple sources of evidence to assess a species' taxonomic status more robustly, including in species descriptions and synonymizations (Dayrat 2005; Ortega-Andrade 2008; Schlick-Steiner et al. 2010; Köhler et al. 2015; Mângia et al. 2020). This combined use of different approaches for species delimitation and identification is known as integrative taxonomy (Padial et al. 2010; Glaw et al. 2010). With the increasing adoption of this framework, numerous studies have addressed the description of new species (Gehara et al. 2013; Mângia et al. 2018; Taucce et al. 2020), revalidations (Cassini et al. 2010; Ortega-Andrade and Rojas-Soto 2017; Magalhães et al. 2020), and synonymizations (Júnior et al. 2004; Ortega-Andrade 2008; Mângia et al. 2020) across various amphibian groups.

Among the several neotropical frogs that have been facing taxonomic rearrangements and for which several new species have been described in recent years is the genus *Proceratophrys* Miranda-Ribeiro, 1920, which belongs to the family Odontophrynidae and currently comprises 42 species (Seger et al. 2025; Frost 2026). Numerous studies have contributed to its taxonomy by utilizing different datasets, including morphology, molecular data, karyotypes, and bioacoustics (Godinho et al. 2013; Magalhães et al. 2020; Mângia et al. 2014, 2020, 2022; Santana et al. 2021; Seger et al. 2025). Species of *Proceratophrys* have been historically organized in phenetic groups and species complexes based on general morphological similarities (Prado and Pombal-Jr. 2008; Cruz and Napoli 2010; Dias et al. 2013b; Brandão et al. 2013; Mângia et al. 2014), including (1) the *P. boiei* complex (Prado and Pombal Jr 2008), which includes species with palpebral appendages and without rostral appendage: *P. boiei* (Wied-Neuwied, 1824), *P. renalis* (Miranda-Ribeiro, 1920), and *P. paviotti* (Cruz et al., 2005); (2) the *P. appendiculata* complex (Izecksohn et al., 1998) includes species with palpebral and rostral appendages: *P. appendiculata* (Günther, 1873), *P. melanopogon* (Miranda-Ribeiro, 1926), *P. laticeps* (Izecksohn & Peixoto, 1981), *P. moehringi* (Weygoldt & Peixoto, 1985), *P. phyllostomus* (Izecksohn et al., 1998), *P. subguttata* (Izecksohn et al., 1998), *P. tupinamba* (Prado & Pombal Jr, 2008), *P. sanctaritae* (Cruz & Napoli, 2010), *P. belzebul* (Dias et al., 2013b), *P. izecksohni* (Dias et al., 2013b), *P. gladius* (Mângia et al., 2014), *P. itamari* (Mângia et al., 2014), *P. mantiqueira* (Mângia et al., 2014), and *P. pombali* (Mângia et al., 2014); (3) the *P. cristiceps* group (Giaretta et al., 2000) includes species without palpebral appendages and postocular swellings: *P. cristiceps* (Müller, 1884), *P. goyana* (Miranda-Ribeiro, 1937), *P. moratoi* (Jim & Caramaschi, 1980), *P. cururu* (Eterovick & Sazima, 1998), *P. concavitympanum* (Giaretta et al., 2000), *P. strussmannae* (Ávila et al., 2011), *P. vielliardi* (Martins & Giaretta, 2011), *P. huntingtoni* (Ávila et al., 2012), *P. bagnoi* (Brandão et al., 2013), *P. branti* (Brandão et al., 2013), *P. dibernardoi* (Brandão et al., 2013), *P. carranca* (Godinho et al., 2013), *P. rotundipalpebra* (Martins & Giaretta, 2013), and *P. ararype* (Mângia et al., 2018); and (4) the *P. bigibbosa* group (Kwet & Faivovich, 2001) includes species with postocular swellings and without palpebral appendages: *P. bigibbosa* (Peters, 1872), *P. avelinoi* (Mercadal de Barrio & Barrio, 1993), *P. palustris* (Giaretta & Sazima, 1993), and *P. brauni* (Kwet & Faivovich, 2001). Seven species were not associated with any species groups: *P. korekore* (Santana et al., 2021), *P. minuta* (Napoli et al., 2011), *P. redacta* (Teixeira Jr et al., 2012), *P. rondonae* (Prado & Pombal Jr, 2008), *P. salvatori* (Caramaschi, 1996), *P. schirchi* (Miranda-Ribeiro, 1937), and *P. velhochico* (Mângia et al., 2022). This lack of assignment was due either to their distinct morphology or to the use of molecular data, which supported alternative phylogenetic relationships (Prado and Pombal Jr 2008; Napoli et al. 2011; Teixeira Jr et al. 2012). Over the years, taxonomic changes involving widely distributed species have generated inconsistencies, as exemplified by *P. boiei*, whose nomenclatural history includes synonymizations and revalidations of previously named populations.

Taxonomic history of *P. boiei* and allied species

Proceratophrys boiei Wied-Neuwied (1824) was originally described as *Ceratophrys boiei*. In 1825, the same author redescribed this species based on two specimens, one from Bahia and the other from Rio de Janeiro. However, the original description lacks information on the collector and the type locality of the species. Bokermann (1966) restricted the type locality to the state of Guanabara (Rio de Janeiro). Morphological analyses by Lynch (1971) later moved *Ceratophrys boiei* to the genus *Proceratophrys*. In 1990, Hoogmoed designated the individual RMNH 24109 of *P. boiei* as the lectotype and restricted the type locality to Bahia, without collection data or details on the type specimen. Subsequently, Prado and Pombal-Jr. (2008) compared populations of *P. boiei* from the northeast and south/southeast with the lectotype designated by Hoogmoed (1990). They found clear evidence that lectotype RMNH 24109 belonged to the south/southeast population, thus restricting the type locality of *Proceratophrys boiei* to Rio de Janeiro. The authors also mapped the geographical distribution of the species,

which is found in Atlantic Forest regions from the state of Santa Catarina to Espírito Santo, with occurrences in some transitional areas with the Cerrado in São Paulo and Minas Gerais. Furthermore, in the same study, the authors removed *P. renalis* from the synonymy of *P. boiei*, including populations from northern Minas Gerais and northeastern Brazil. Regarding its taxonomic history, *Proceratophrys boiei* currently has three junior synonyms: (1) *Ceratophris granosa* Cuvier (1829), which was illustrated by Guérin-Méneville (1838) without a specific locality association; (2) *Ceratophrys fryi* Günther (1873), described from "Serra da Mantiqueira" in Minas Gerais, was synonymized by Prado and Pombal-Jr. (2008); and (3) *Ceratophrys intermedia* Barbour (1908), described from "Santa Katarina, Brazil," but later synonymized by Bokermann in 1966.

Multiple lines of evidence have supported geographic structuring within *Proceratophrys boiei*. In 2005, Izecksohn and colleagues conducted a study of the osteocranium of *Proceratophrys boiei*, *P. appendiculata*, *P. melanopogon* (all from the state of Rio de Janeiro), and *P. laticeps* (from the state of Espírito Santo). However, the study's primary focus was to compare these species rather than to investigate specific variations within *P. boiei*. Three years later, Prado and Pombal Jr (2008) carried out a taxonomic revision of *Proceratophrys* species with palpebral appendages, including *P. boiei*, based on external morphology and osteological characters. The authors found subtle variations in some populations of *P. boiei* from the states of Paraná and Santa Catarina compared to specimens from São Paulo, Minas Gerais, and Espírito Santo. These variations were noted in the posterior region of the frontoparietals and in the internal and external metacarpal tubercles, suggesting the potential existence of distinct lineages within *P. boiei*. These authors synonymized *Proceratophrys fryi* with *P. boiei* due to the lack of distinguishing morphological characteristics between these species. Subsequently, Amaro et al. (2012), using molecular (*Cytb*, 16S, and RAG-1) and cytogenetic approaches, identified three deeply divergent lineages within *P. boiei*: two northern lineages—North 1 (N1, populations from Espírito Santo and Minas Gerais) and North 2 (N2, populations from northern São Paulo, southern Minas Gerais, and Rio de Janeiro)—and one southern lineage (S, populations from southern São Paulo to southern Santa Catarina). This study also revealed that the S lineage has the nucleolar organizer region (NOR) located on the short arm of subtelocentric pair 4, whereas the N1 and N2 lineages have the NOR on the short arm of submetacentric pair 8. More recently, Silva et al. (2021) conducted a study using cytogenetic and cytological approaches for some *Proceratophrys* species and confirmed the NOR pattern observed by Amaro et al. (2012), further reinforcing the differences among the three lineages. Complementing these data, Dias et al. (2013a) demonstrated that chondrocranial morphology also varies geographically: specimens from Minas Gerais (N1) exhibit unique subdivisions of the frontoparietal fontanelle and ventral fusion of suprarostral cartilages, whereas those from Paraná (S) present a reduced hypobranchial plate and a more developed ceratobranchial IV; populations from Rio de Janeiro (N2), in turn, display the typical condition shared with other congeners bearing palpebral appendages.

In addition, the advertisement call of *P. boiei* was described by Heyer et al. (1990) for a population from Boracéia, São Paulo state, approximately 550 km from the type locality [defined as Rio de Janeiro in the work of Prado and Pombal-Jr. (2008)], represented as lineage N2 by Amaro et al. (2012). However, there are no studies that address the variation in the advertisement call across the entire distribution of the species, which is currently recorded in Atlantic Forest areas across six Brazilian states: Minas Gerais, São Paulo, Espírito Santo, Santa Catarina, Paraná, and Rio de Janeiro (Frost 2026).

Over the past 17 years, several studies have focused on taxonomic issues involving *Proceratophrys boiei* (e.g., Prado and Pombal-Jr. 2008; Amaro et al. 2012; Silva et al. 2021). More broadly, knowledge of species-level diversity in *Proceratophrys* has expanded considerably in recent years. Advertisement calls are currently known for 33 species, genetic data are available for 28 species, including the two species revalidated herein, and tadpole morphology has been described for 21 species (Dias et al. 2018; Nascimento et al. 2019; Simões et al. 2020; Mângia et al. 2022; Seger et al. 2025). However, taxonomic questions remain regarding the validity of some species synonymized with *P. boiei*, and, more generally, none of these sources of evidence alone is sufficient to resolve all taxonomic problems in the genus. Broadly distributed species often encounter physical barriers such as rivers (Nascimento et al. 2013; Werneck et al. 2015), mountain ranges (Firkowski et al. 2016; Oswald et al. 2022), and even latitudinal and longitudinal gradients (Marcelino et al. 2009; Fonseca et al. 2018), which can influence genetic structure, morphological variation, and advertisement call parameters (Feio et al. 2006; Menezes et al. 2016; Mângia et al. 2017). These variations among populations along geographic gradients can occur at different scales, and, in general, the greater the geographic distance between populations, the greater the differences tend to be (Futuyma and Morgante 2002). However, the degree of divergence is variable and may complicate the assessment of whether population differences represent intraspecific morphological, acoustic, or molecular variation, or instead indicate a species complex grouped under a single name (Silva et al. 2008; Forti et al. 2015).

Reviewing populations identified as *Proceratophrys boiei* is essential to assess the validity of species that have been synonymized with it and to establish a solid taxonomic framework for these populations. To address these taxonomic issues, the taxonomy of *P. boiei* populations is revised using an integrative approach, evaluating external morphology, morphometrics, bioacoustics, and osteology across the species distribution.

Materials and methods

Materials examined

Populations of *Proceratophrys boiei* across its distribution were analyzed using specimens from the following scientific collections: CCLZU (Coleção Científica do Laboratório de Zoologia, Unitau, Taubaté, São Paulo, Brazil), MZUFV (Museu de Zoologia João Moojen, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil), MCT-PUCRS (Museu de Ciência e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil), ZUEC-AMP (Coleção de Anfíbios do Museu de Zoologia Adão José Cardoso, Universidade Estadual de Campinas, Instituto de Biologia, Campinas, São Paulo, Brazil), and ZUFMS-AMP (Coleção Zoológica da Universidade Federal de Mato Grosso do Sul—Anfíbios, Instituto de Biociências, Campo Grande, Mato Grosso do Sul, Brazil). Type specimens were also analyzed using photographs provided by the following collections: lectotype of *Proceratophrys boiei*: RMNH (Nationaal Natuurhistorisch Museum, Leiden, Netherlands), holotype of *Ceratophrys intermedia*: HU-Zoo (Museum of Comparative Zoology, Harvard University, Cambridge, United States), and type of *Proceratophrys fryi*: NHM-London-Zoo (Zoological Collection, London, United Kingdom).

Specimens were evaluated following the lineages identified genetically in the study by Amaro et al. (2012): N1, N2, and S (see Appendix I). Males and females were analyzed separately, determined by the presence or absence of vocal slits. Individuals were classified as adults if they attained at least half the size of the largest specimen in each sample, as outlined by Prado and Pombal-Jr. (2008).

External morphology and morphometry

Morphological characters of 132 specimens of *Proceratophrys boiei* (80 males and 52 females) from 48 localities in southeastern and southern Brazil (Fig. 1; Appendix I) were analyzed. Among these, for each lineage identified by Amaro et al. (2012), 49 specimens of lineage N1 (25 males and 24 females), 56 specimens of lineage N2 (38 males and 18 females), and 27 specimens of lineage S (17 males and 10 females) were examined.

The characters analyzed were ocular-dorsal ridges, interocular ridge, canthal crest, frontoparietal crests; region between the frontoparietal crests, squamosal crests, pre-ocular crest, commissural tubercles, dorsal skin texture (tubercles), rows of forearm tubercles; ventral color; internal metacarpal tubercle, external metacarpal tubercle, internal metatarsal tubercle; external metatarsal tubercle—the characterization of these structures follows Prado and Pombal-Jr. (2008); palpebral appendages—Mângia et al. (2014); snout shape—Heyer et al. (1990); tympanum can be distinct (defined as a depression in the skin behind the eyes and below the crest of the squamosal bone) or indistinct (not externally visible).

Morphometric data for 126 specimens of the three lineages (N1, N2, and S) of *Proceratophrys boiei* (80 males, 46 females) from 49 localities (Appendix I) were analyzed. The 14 morphometric measurements used were: snout–vent length (SVL), head length (HL), head width (HW), forearm and hand length (FHL), palpebral appendage length (PAL), distance from ocular-dorsal ridge to snout (DOS), internarial distance (IND), eye–nostril distance (END), eye diameter (ED), interorbital distance (IOD), thigh length (THL), tibia length (TBL), foot length (FL), and relative length of fingers and toes (RLFT), following Prado and Pombal-Jr. (2008) and Watters et al. (2016). Only one researcher (L. Castro) took these measurements of each specimen using a 0–150 mm digital caliper, with a precision of 0.01 mm.

Morphometric data were first log-transformed prior to analysis, and differences among lineages and sexes were then assessed using a two-way PERMANOVA implemented in the vegan package in R version 4.4.2 (Oksanen et al. 2025; R Core Team 2025). Non-metric multidimensional scaling (NMDS) was then performed to visualize the PERMANOVA results using the ggplot2 package (Wickham 2016). Because the main goal was to evaluate differences among lineages, pairwise PERMANOVAs between lineages were subsequently conducted separately for females and males. Finally, two Random Forest analyses, one for females and one for males, were performed to identify the most important morphometric variables, using the randomForest package (Breiman et al. 2002).

Osteology

For the clearing and staining process, the protocol adapted from Taylor and Van Dyke (1985) was followed and applied to nine male specimens from seven localities, representing the three lineages: N1 (three individuals from Ervália, Minas Gerais: ZUFMS-AMP 15671, ZUFMS-AMP 15672, and ZUFMS-AMP 15673), N2 (one individual from Nova Friburgo, Rio de Janeiro: ZUFMS-AMP 19393, one from Caçapava, São Paulo: CCLZU 758, and one from Santo Antônio do Pinhal, São Paulo: CCLZU 831), and S (one individual from Azambuja, Pedras Grandes, Santa Catarina: ZUFMS-AMP 19386, one from Testo Salto, Blumenau, Santa Catarina: ZUFMS-AMP 19428, and one from Reserva Betary, Iporanga, São Paulo: ZUEC-AMP 20176).

For the osteological analysis, three adult males from each lineage were cleared and stained, as more male specimens than females were available. Therefore, females were not included in this stage due to the smaller numbers and to standardize the analysis and avoid the effects of sexual dimorphism. The analysis of osteological characters was the same as that used by Prado and Pombal-Jr. (2008): height of the alar process (HAP), depth of the pars palatina of the premaxilla (DPPPm), texture of the external surface of the pars facialis (TESPF), posterior ventral fossae of the maxillae (PVFM), shape of the nasal bone in dorsal view (SNBDV), texture of the nasals (TN),

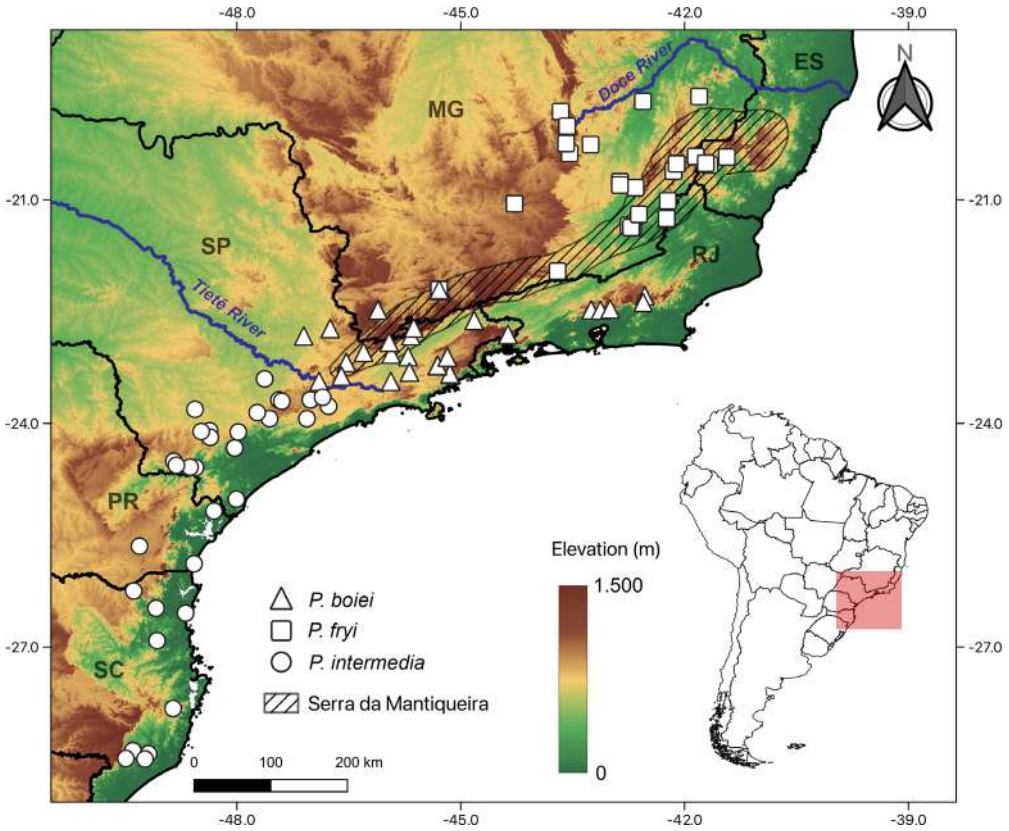

Figure 1. Geographic distribution of each lineage. Squares represent N1: *Proceratophrys fryi*, triangles represent N2: *P. boiei*, and circles represent S: *P. intermedia*.

height of the posterodorsal region of the nasals (HPdRN), contact between nasals (CN), contact between nasals and frontoparietals (CNFp), texture of the frontoparietals (TFp), relief of the frontoparietals in dorsal view (RFpDV), width of the frontoparietals (WFp), posterolateral extension of the frontoparietals (PlEFp), direction of the posterior region of the frontoparietal crest (DPRFpC), length of the otic ramus of the squamosal (LORS), cultriform process of the parasphenoid (CulPPph), and extent of the anterior region of the cultriform process of the parasphenoid (EARCulPPph).

Bioacoustics

A total of 708 advertisement calls from 66 males across the three lineages were analyzed, collected from 27 localities: N1 (seven localities), N2 (seven localities), and S (13 localities). To obtain acoustic data, recordings of the advertisement call of *Proceratophrys boiei* were requested from researchers, and recordings available in the following sound libraries were utilized: FNJV-UEC (Fonoteca Neotropical Jacques Vielliard, Universidade Estadual de Campinas, Departamento de Biologia Animal, Campinas, São Paulo, Brazil) and Fonotropica–UFBA (Fonoteca da Universidade Federal da Bahia, Salvador, Bahia, Brazil).

The acoustic parameters (spectral and temporal) were analyzed using Raven Pro 1.6.1 software (Cornell Lab of Ornithology Bioacoustics Research Program), with the following parameters: FFT window width = 512, frame = 22050, overlap = 50. To construct the spectrogram, R 4.2.1 software (R Core Team 2020) was used with the tuneR (Ligges et al. 2024) and Seewave (Sueur et al. 2008) packages, following the same parameters used in the analysis of the calls. For the selection of acoustic parameters, acoustic studies for the genus *Proceratophrys* were followed (e.g., Mângia et al. 2014; Nascimento et al. 2019; Simões et al. 2020): call duration, number of pulses per call, number of pulses per second, and dominant frequency. The terminology for each parameter followed Köhler et al. (2017). To assess possible differences in acoustic parameters among lineages, PERMANOVA and principal component analysis (PCA) were performed using the vegan package in R version 4.4.2 (Oksanen et al. 2025; R Core Team 2025).

Genetic distance

To estimate uncorrected pairwise genetic distances (*p*-distances) among the N1, N2, and S lineages identified by Amaro et al. (2012), samples of 94 individuals of *Proceratophrys boiei* from 40 localities distributed across the southern Atlantic Forest in Brazil were analyzed in the states of Minas Gerais (MG), Espírito Santo (ES), Rio de Janeiro (RJ), São Paulo (SP), and Santa Catarina (SC). Most sequences were obtained from GenBank (Amaro et al. 2012; Lyra et al. 2017), and three new sequences were generated from additional populations (Suppl. material 1). For these samples, genomic DNA was extracted

from muscle or liver tissue using a phenol–chloroform protocol (Sambrook et al. 1989), and a fragment of the mitochondrial 16S ribosomal gene was amplified (Palumbi et al. 2002). These new sequences were deposited in GenBank (Suppl. material 1). Partial sequences from two mitochondrial genes were used: 93 sequences of 16S rRNA (16S) and 59 sequences of cytochrome *b* (*Cytb*). Sequences were aligned separately for each gene in Geneious 9.1.2 using the MUSCLE algorithm with default settings (Edgar 2004; Kearse et al. 2012). The final alignments comprised 424 bp for 16S and 555 bp for *Cytb*. Uncorrected pairwise genetic distances (*p*-distances) within and among the three lineages were calculated for each gene in MEGA X (Kumar et al. 2018) under default settings, including the outgroup taxa.

Results

External morphology

The external morphology of the three lineages classified under the name *Proceratophrys boiei* is overall similar, with variation observed in all analyzed characters, including individuals in the same lineage, indicating considerable polymorphism (Figs 2–5). Individuals from lineages N1, N2, and S may exhibit either short or long palpebral appendages, a canthal crest that can range from faint to moderate or pronounced, and ocular-dorsal ridges that may be continuous or discontinuous. However, some differences between the lineages are notable: specimens from lineage S do not exhibit highly arched bony crests on the frontoparietals (showing only slightly arched crests, S = 16, 59%, or not arched, S = 11, 41%), whereas specimens from lineages N1 and N2 can have highly arched (N1 = 6, 12%; N2 = 7, 13%), slightly arched (N1 = 26, 53%; N2 = 25, 45%), or not arched (N1 = 17, 35%; N2 = 23, 42%) crests. Furthermore, lineage S lacks the moderate state of the bony crests seen in the frontoparietals, showing only faint (S = 20, 74%) and pronounced (S = 7, 26%) states. Additionally, while all three lineages showed variation in relative finger-length patterns, lineage S was the only one that did not exhibit the IV < I < II < III arrangement in the hands. Lineage N2 was unique in displaying a semicircular snout among the specimens analyzed (N2 = 4, 7%), although it also included specimens with rounded snouts (N2 = 17, 31%) and nearly rounded snouts (N2 = 34, 62%).

Morphometry

Morphological variation was significantly influenced by both lineage (R^2 = 0.11, p = 0.001) and sexual dimorphism (R^2 = 0.23, p = 0.001). In addition, the interaction

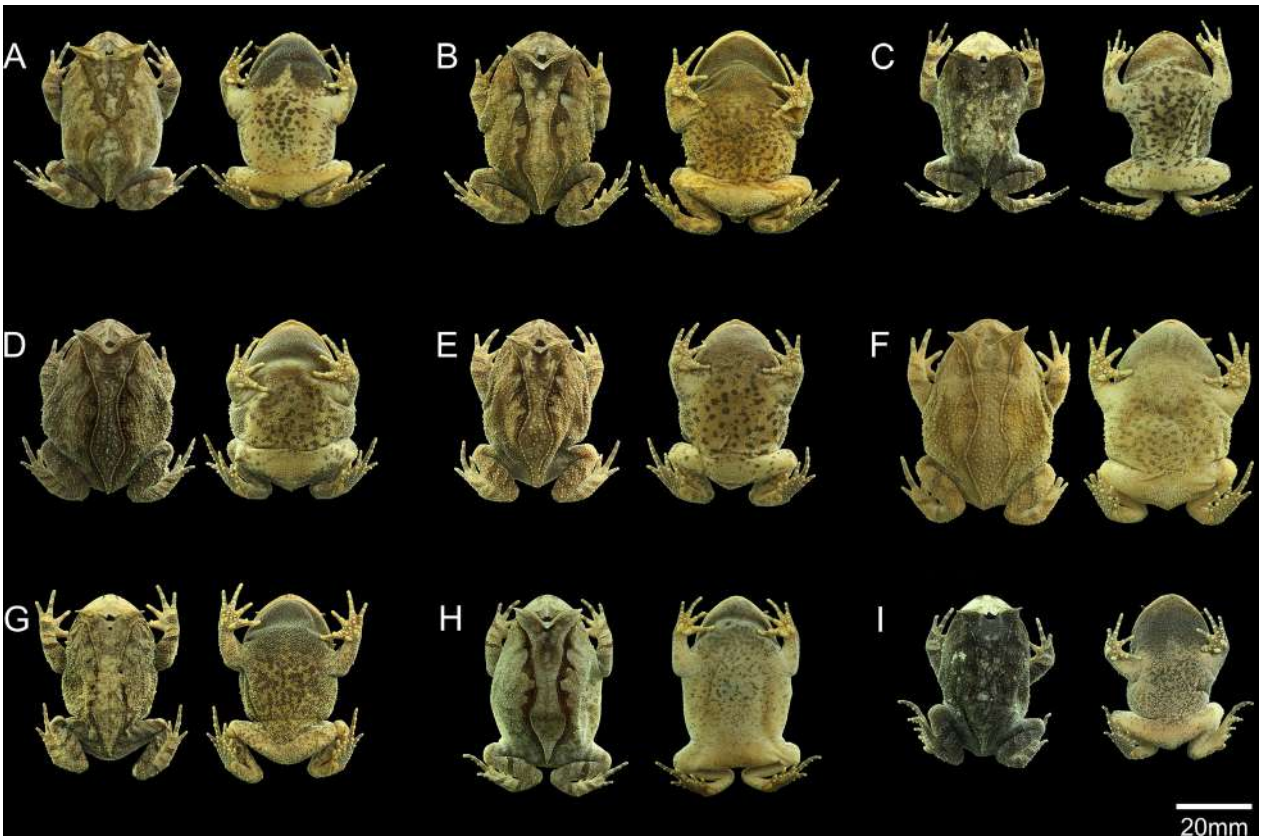

Figure 2. Dorsal and ventral views of males from each lineage. N1: *Proceratophrys fryi* (**A**) MZUFV 8007, (**B**) MZUFV 13304, (**C**) MZUFV 12202; N2: *P. boiei* (**D**) CCLZU 415, (**E**) CCLZU 1109, (**F**) CCLZU 1479; S: *P. intermedia* (**G**) MCP 12319, (**H**) MCP 6355, (**I**) ZUFMS-AMP 19386.

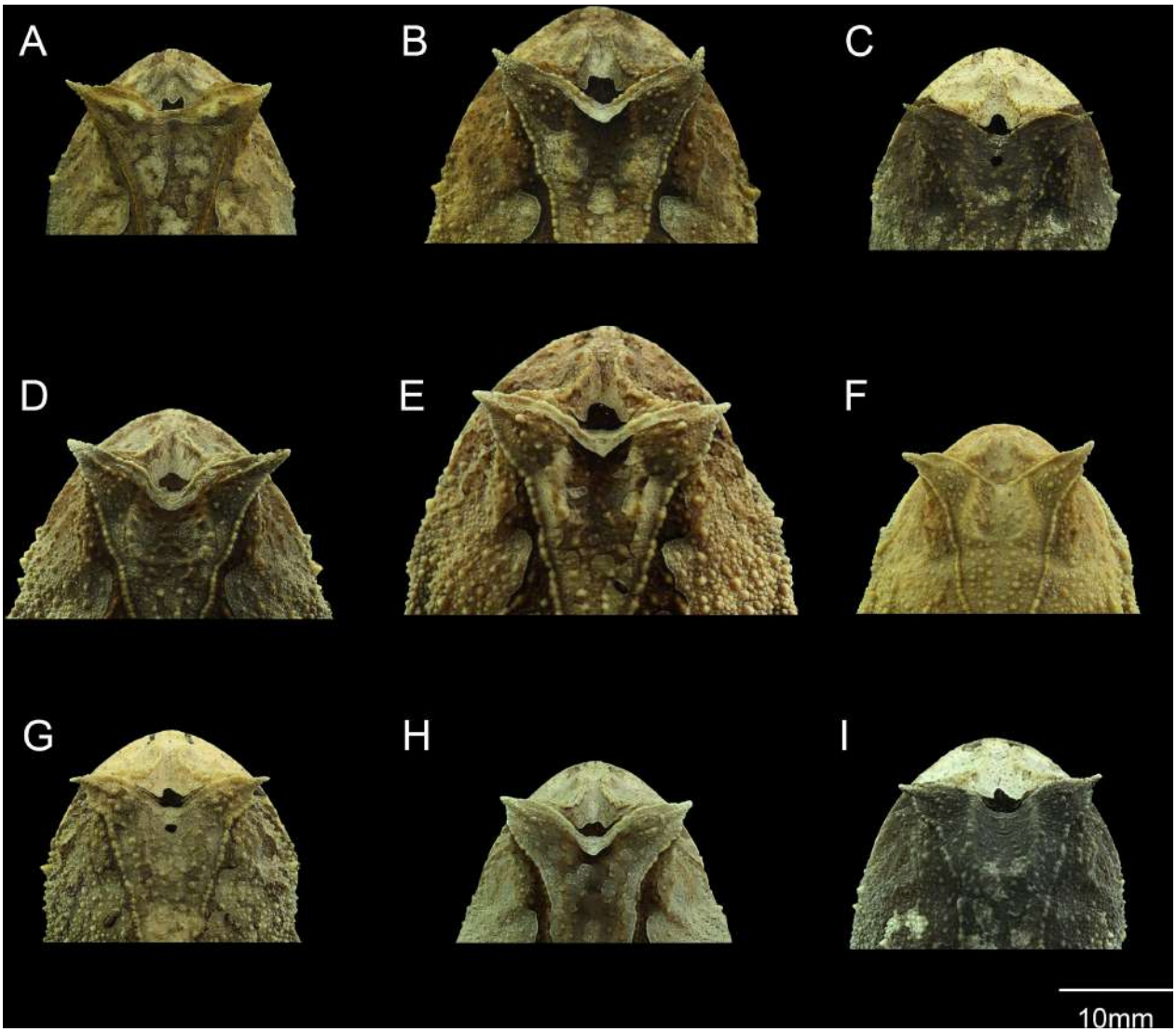

Figure 3. Dorsal views of the palpebral appendage of males of each lineage. N1: *Proceratophrys fryi* (**A**) MZUFV 8007, (**B**) MZUFV 13304, (**C**) MZUFV 12202; N2: *P. boiei* (**D**) CCLZU 415, (**E**) CCLZU 1109, (**F**) CCLZU 1479; S: *P. intermedia* (**G**) MCP 12319, (**H**) MCP 6355, (**I**) ZUFMS-AMP 19386.

between lineage and sex was also significant (R^2 = 0.03, p = 0.048). Overall, sexual dimorphism explained a greater proportion of the variation than lineage. Pairwise comparisons among lineages showed that lineage S differed from both N1 and N2 in males and females, whereas lineages N1 and N2 did not differ from each other (Table 1, Fig. 6).

Among the lineages evaluated, specimens from lineage N1 had the largest mean SVL (51.5 mm in males and 60.3 mm in females; Table 2). Specimens from lineage N2 showed intermediate SVL values (50.0 mm in males and 60.1 mm in females; Table 2), while those from lineage S had the smallest mean SVL (46.0 mm in males and 51.6 mm in females; Table 2). The two most important variables contributing to lineage separation were, for females, interorbital distance (IOD) and eye–nostril distance (END), and for males, interorbital distance (IOD) and palpebral appendage length (PAL) (Fig. 7).

Table 1. Pairwise comparisons of morphological variation among the three lineages recognized by Amaro et al. (2012), performed separately for females and males. *F* corresponds to the *F* statistic, and *p* to the associated probability value. N1: *Proceratophrys fryi*, N2: *P. boiei*, and S: *P. intermedia*.

	Female		Male	
	F	***p***	***F***	***p***
N1-N2	1.73	0.17	1.66	0.18
N1-S	6	0.01	8.99	0.004
N2-S	9.04	0.001	5.59	0.006

Osteology

The osteological characteristics of the three lineages under the name *Proceratophrys boiei* are generally similar, with some variation in the analyzed characters, even among individuals of the same lineage, as observed in the external morphology (Fig. 8).

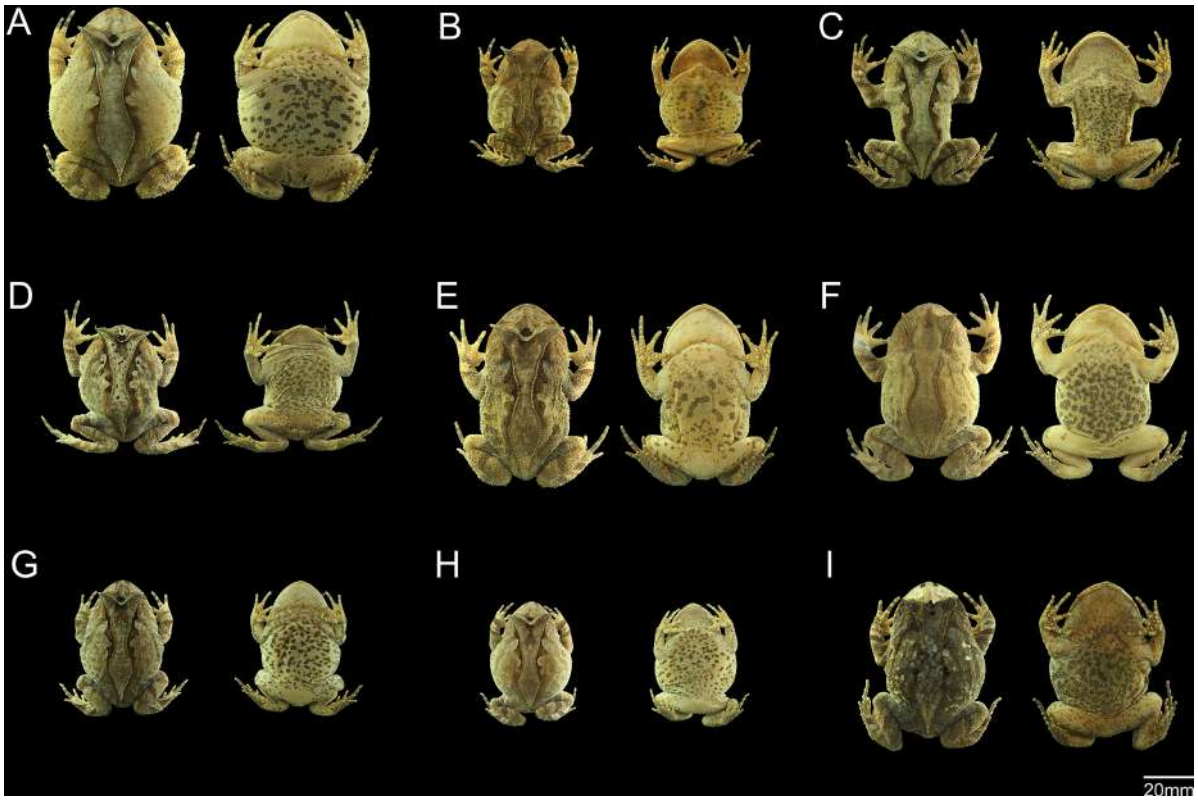

Figure 4. Dorsal and ventral views of females from each lineage. N1: *Proceratophrys fryi* (**A**) ZUFMS-AMP 18903, (**B**) MZUFV 13305, (**C**) MZUFV 15767; N2: *P. boiei* (**D**) ZUFMS-AMP 19392, (**E**) CCLZU 1475, (**F**) CCLZU 2002; S: *P. intermedia* (**G**) ZUEC-AMP 4754, (**H**) MCP 7635, (**I**) ZUFMS-AMP 19390.

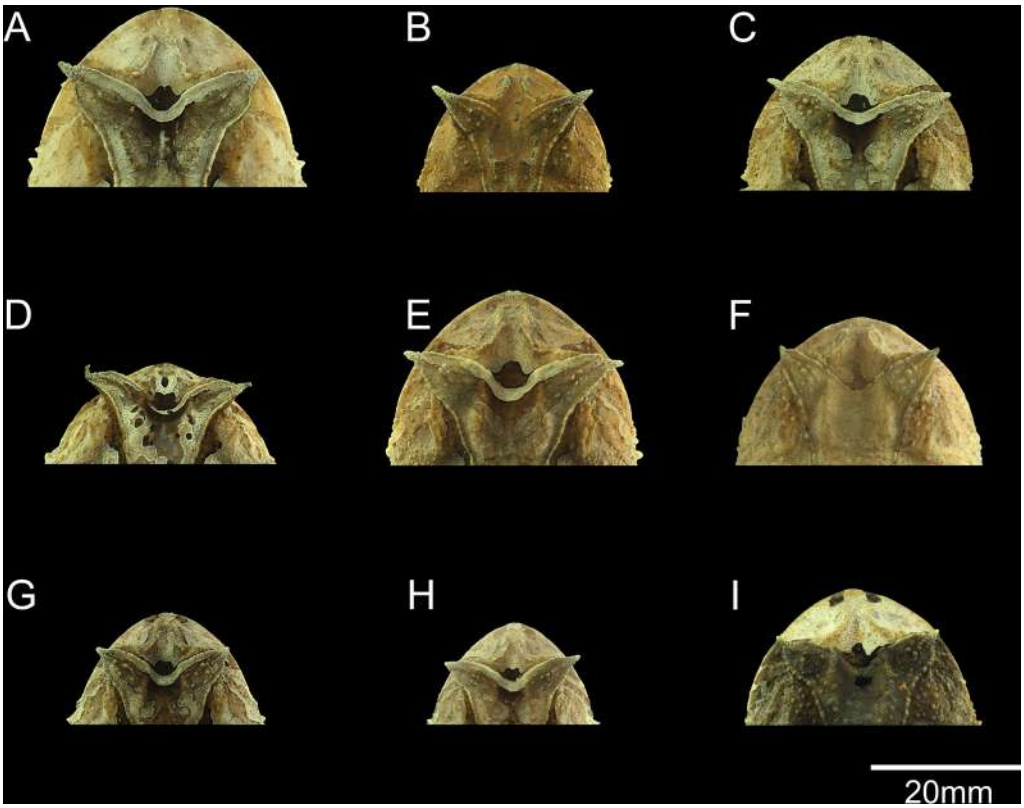

Figure 5. Dorsal views of the palpebral appendage of females of each lineage. N1: *Proceratophrys fryi* (**A**) ZUFMS-AMP 18903, (**B**) MZUFV 13305, (**C**) MZUFV 15767; N2: *P. boiei* (**D**) ZUFMS-AMP 19392, (**E**) CCLZU 1475, (**F**) CCLZU 2002; S: *P. intermedia* (**G**) ZUEC-AMP 4754, (**H**) MCP 7635, (**I**) ZUFMS-AMP 19390.

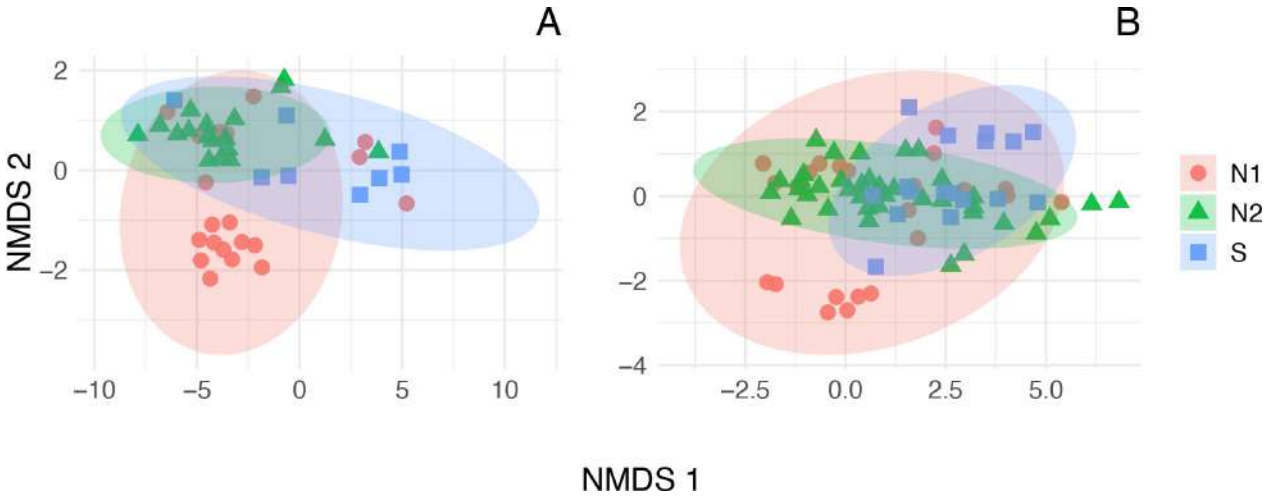

Figure 6. Non-metric multidimensional scaling for the three lineages (N1: *Proceratophrys fryi*, N2: *P. boiei*, and S: *P. intermedia*) separated by females (**A**) and males (**B**).

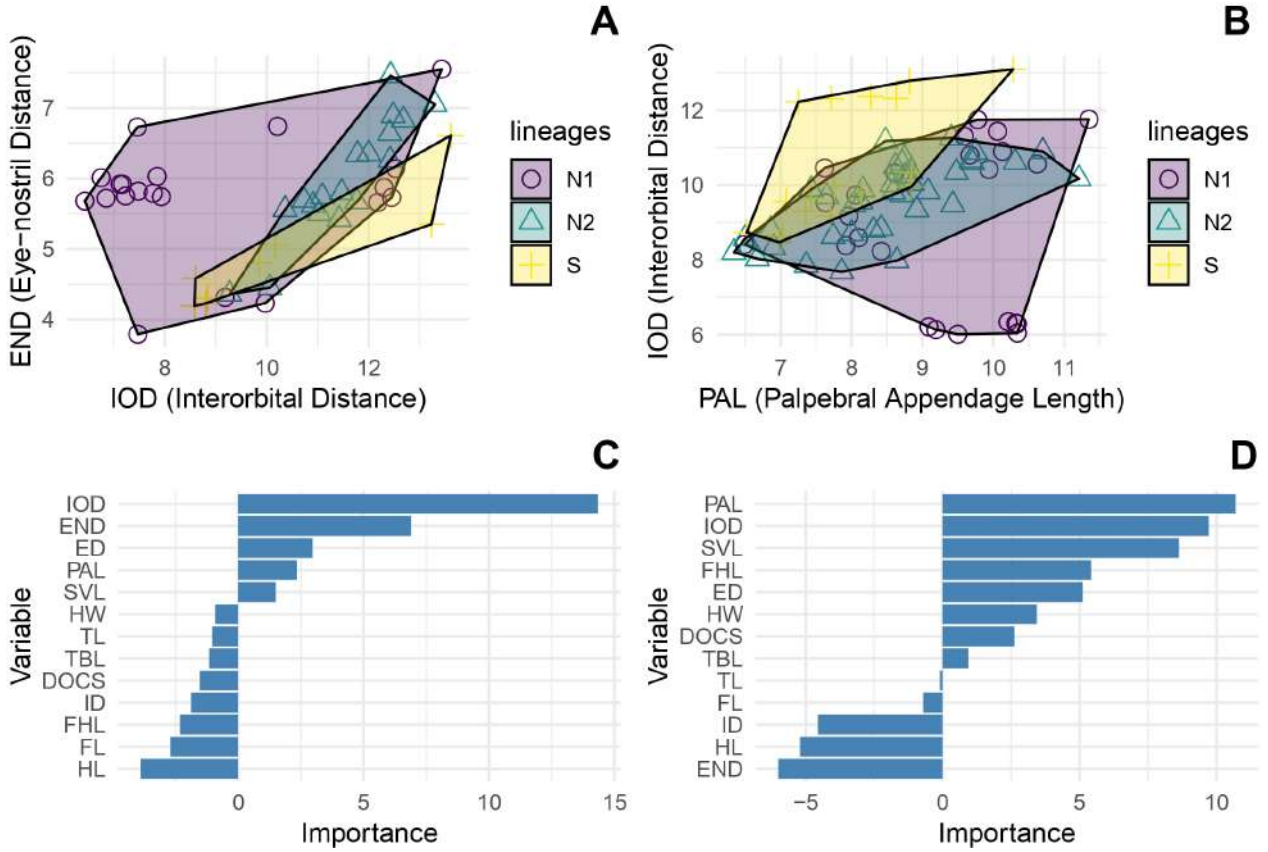

Figure 7. Female (**A**) and male (**B**) variation of the two best predictors among lineages of *Proceratophrys boiei*. Females (**C**) and males (**D**) variable importance scores. Snout–vent length (SVL), head length (HL), head width (HW), forearm and hand length (FHL), palpebral appendage length (PAL), distance from ocular cord to snout (DOCS), internasal distance (ID), eye–nostril distance (END), eye diameter (ED), interorbital distance (IOD), thigh length (TL), tibia length (TBL), foot length (FL), relative length of fingers and toes (RLFT).

Bioacoustics

The advertisement call of all three lineages consists of a multipulsed note that is emitted sporadically (Fig. 9). Overall, there is considerable overlap in the values of the evaluated parameters for the calls of the three lineages (F = 1.8332; p > 0.05) (Fig. 10). However, regarding the dominant frequency, lineage N1 exhibits the highest values (646–904.40 Hz), while lineage S displays the lowest (562.50–861.33 Hz), and lineage N2 has the narrowest range (602.93–818.26 Hz) among the three (Table 3).

Table 2. Morphometry of the three lineages according to Amaro et al. (2012). N1: *Proceratophrys fryi*, N2: *P. boiei*, and S: *P. intermedia*. Measurements are presented in mm: mean, standard deviation, and range.

Measurements/ lineages	North 1		North 2		South	
	Males (*n* = 25)	Females (*n* = 20)	Males (*n* = 38)	Females (*n* = 18)	Males (*n* = 17)	Females (*n* = 8)
Snout-vent length	51.5 ± 5.2	60.3 ± 8.6	50.0 ± 4.3	60.1 ± 10.3	46.0 ± 2.6	51.6 ± 9.3
	41.2–59.4	41.3–71.2	38.4–58.1	42.1–79.1	42.6–51.6	42.8–68.6
Head length	27.1 ± 2.6	30.9 ± 3.9	26.4 ± 2.9	31.5 ± 3.9	23.8 ± 1.5	26.0 ± 5.0
	21.2–31.1	21.5–36.9	19.3–30.8	22.6–38.4	21.1–25.9	20.6–35.7
Head width	21.4 ± 1.9	24.6 ± 2.5	21.3 ± 2.0	25.5 ± 3.0	19.6 ± 1.3	21.5 ± 3.6
	17.7–24.7	18.8–28.7	16.5–24.2	18.0–31.1	17.2–21.4	17.6–28.1
Distance from ocular-dorsal ridge to snout	12.9 ± 1.2	14.8 ± 1.8	12.4 ± 1.2	15.0 ± 1.7	11.7 ± 0.9	12.8 ± 2.4
	10.3–14.6	10.7–17.3	9.6–14.9	10.5–17.8	10.5–13.4	10.0–17.2
Internarial distance	3.0 ± 0.3	3.6 ± 0.5	3.1 ± 0.3	3.9 ± 0.4	2.8 ± 0.3	3.0 ± 0.5
	2.4–3.6	2.4–4.5	2.3–3.7	2.9–4.4	2.2–3.3	2.5–3.9
Eye-nostril distance	4.8 ± 0.5	5.8 ± 0.9	4.8 ± 0.6	6.0 ± 0.8	4.5 ± 0.4	4.9 ± 0.8
	3.9–5.6	3.8–7.6	3.2–6.0	4.4–7.5	3.8–5.5	4.2–6.6
Eye diameter	5.5 ± 0.7	5.8 ± 0.6	5.1 ± 0.6	5.9 ± 0.7	5.1 ± 0.4	5.3 ± 0.7
	4.1–7.0	4.4–6.6	3.7–6.3	4.5–7.8	4.5–6.0	4.1–6.2
Palpebral appendage length	9.1 ± 1.2	9.9 ± 1.2	8.5 ± 1.1	9.9 ± 1.3	7.9 ± 0.9	8.7 ± 2.2
	6.5–11.4	7.4–12.5	6.3–11.2	8.0–12.2	6.5–10.3	6.3–13.4
Interorbital distance	8.9 ± 2.1	9.0 ± 2.3	9.6 ± 1.0	11.5 ± 1.0	7.0 ± 3.4	9.0 ± 2.9
	6.0–11.8	6.4–13.4	7.7–11.3	9.3–13.3	2.2–10.3	3.2–13.6
Thigh length	20.8 ± 2.0	24.3 ± 3.4	20.4 ± 2.2	25.8 ± 3.2	19.0 ± 1.4	21.4 ± 3.7
	17.4–24.9	16.2–28.4	15.7–24.2	18.3–30.8	16.7–21.7	17.6–28.1
Tibia length	19.4 ± 1.6	23.0 ± 2.9	19.2 ± 1.8	24.2 ± 2.9	18.0 ± 1.2	19.4 ± 3.4
	16.3–22.0	16.1–26.5	14.9–22.3	18.3–28.5	16.3–20.9	16.2–25.9
Foot length	29.4 ± 2.4	35.2 ± 4.5	29.0 ± 2.7	36.4 ± 4.1	27.0 ± 1.7	29.1 ± 4.7
	24.3–33.6	25.1–40.2	22.7–33.7	27.2–43.4	24.5–29.8	23.9–38.4
Forearm and hand length	25.3 ± 2.3	30.1 ± 3.8	24.4 ± 2.7	31.0 ± 3.9	23.6 ± 1.8	25.0 ± 4.4
	20.0–29.1	22.2–35.4	17.9–29.2	19.9–36.9	21.0–26.8	20.7–34.0

Table 3. Acoustic parameters of the advertisement call of the three lineages according to Amaro et al. (2012). N1: *Proceratophrys fryi*, N2: *P. boiei*, and S: *P. intermedia*. Values are presented as mean, standard deviation, and range.

Lineages/acoustic parameters	Call duration (s)	Pulses/call	Pulses/sec	Dominant frequency (Hz)
North 1; 298 calls; 24 males	0.831 ± 0.211; 0.58–1.68; (*n* = 93)	41.011 ± 13.534; 28–97; (*n* = 93)	48.892 ± 4.113; 42.87–57.93; (*n* = 93)	770.316 ± 51.859; 646.00–904.40; (*n* = 298)
North 2; 183 calls; 12 males	0.973 ± 0.177; 0.50–1.32; (*n* = 183)	51.206 ± 9.570; 32–71; (*n* = 141)	53.072 ± 6.977; 40.96–66.26; (*n* = 141)	717.424 ± 50.003; 602.93–818.26; (*n* = 183)
South; 227 calls; 30 males	0.951 ± 0.121; 0.36–1.35; (*n* = 116)	47.973 ± 6.374; 21–63; (*n* = 111)	50.016 ± 4.572; 41.46–62.15; (*n* = 111)	750.358 ± 67.568; 562.50–861.33; (*n* = 224)

Genetic distance

The mean uncorrected mtDNA *p*-distances among the three lineages were relatively similar for 16S, ranging from 2.7% to 2.9%, whereas for *Cytb*, they ranged from 5.6% to 7.3% (Table 4).

Table 4. Uncorrected *p*-distances of the three lineages according to Amaro et al. (2012) based on 16S (lower diagonal) and *Cytb* (upper diagonal) gene fragments. N1: *Proceratophrys fryi*, N2: *P. boiei*, and S: *P. intermedia*.

Lineages	*P. fryi* (N1)	*P. boiei* (N2)	*P. intermedia* (S)
P. fryi (N1)	–	0.062	0.073
P. boiei (N2)	0.027	–	0.056
P. intermedia (S)	0.028	0.029	–

Taxonomic implications

Based on the evaluation of morphological, morphometric, acoustic, and osteological characteristics and genetic distances, along with the results of previous studies based on molecular data (Amaro et al. 2012), cytogenetics (Amaro et al. 2012; Silva et al. 2021), tadpole chondrocranium (Dias et al. 2013a), and tadpole morphology (Pezzuti et al. 2021; Rossa-Feres et al. 2024), it was determined that there are three valid species currently grouped under the name *Proceratophrys boiei*. The type locality historically associated with the species corresponds to the populations

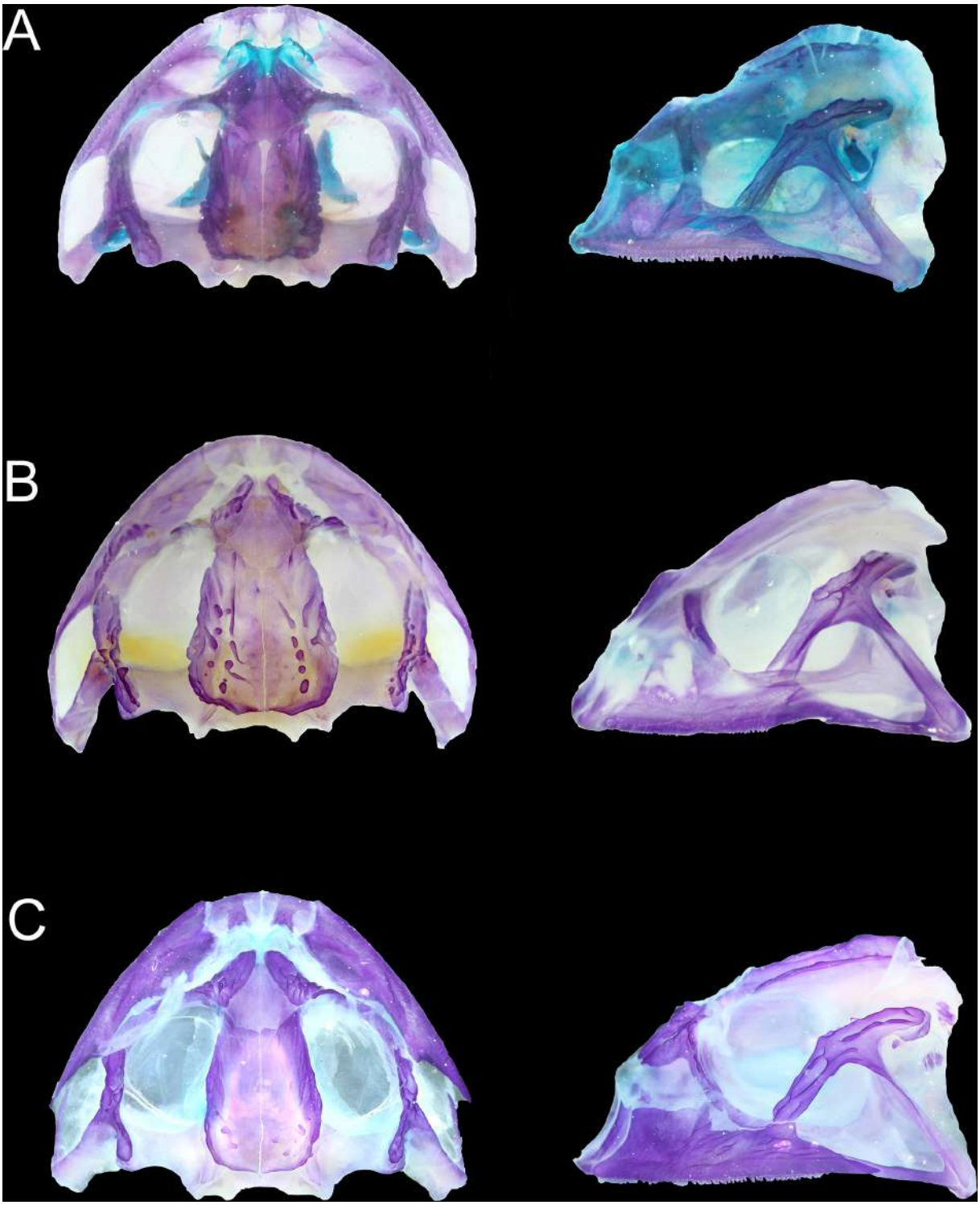

Figure 8. Cranial morphology of adult males of the three lineages in dorsal (left) and lateral (right) views. (**A**) N1: *Proceratophrys fryi* (ZUFMS-AMP 15672 and ZUFMS-AMP 15671, respectively), (**B**) N2: *P. boiei* (CCLZU 758 and CCLZU 831, respectively), and (**C**) S: *P. intermedia* (ZUEC-AMP 20176 and ZUFMS-AMP 19386, respectively).

of lineage N2, which should therefore be recognized as *Proceratophrys boiei*. Lineage N1, with a more northern distribution in Brazil (Espírito Santo and Minas Gerais, excluding the southern regions), is associated with *P. fryi*, previously considered a synonym of *P. boiei*. Lineage S, occurring further south in Brazil (Santa Catarina, Paraná, and the southern state of São Paulo, with the Tietê River acting as a geographic barrier), is linked to *P. intermedia*, another synonym of *P. boiei*. These two latter species were synonymized with *P. boiei* 15 and 50 years ago, respectively (Bokermann 1966; Prado and Pombal Jr 2008). Thus, the redescriptions of *Proceratophrys boiei* and the revalidation of *P. fryi* and *P. intermedia* are presented below.

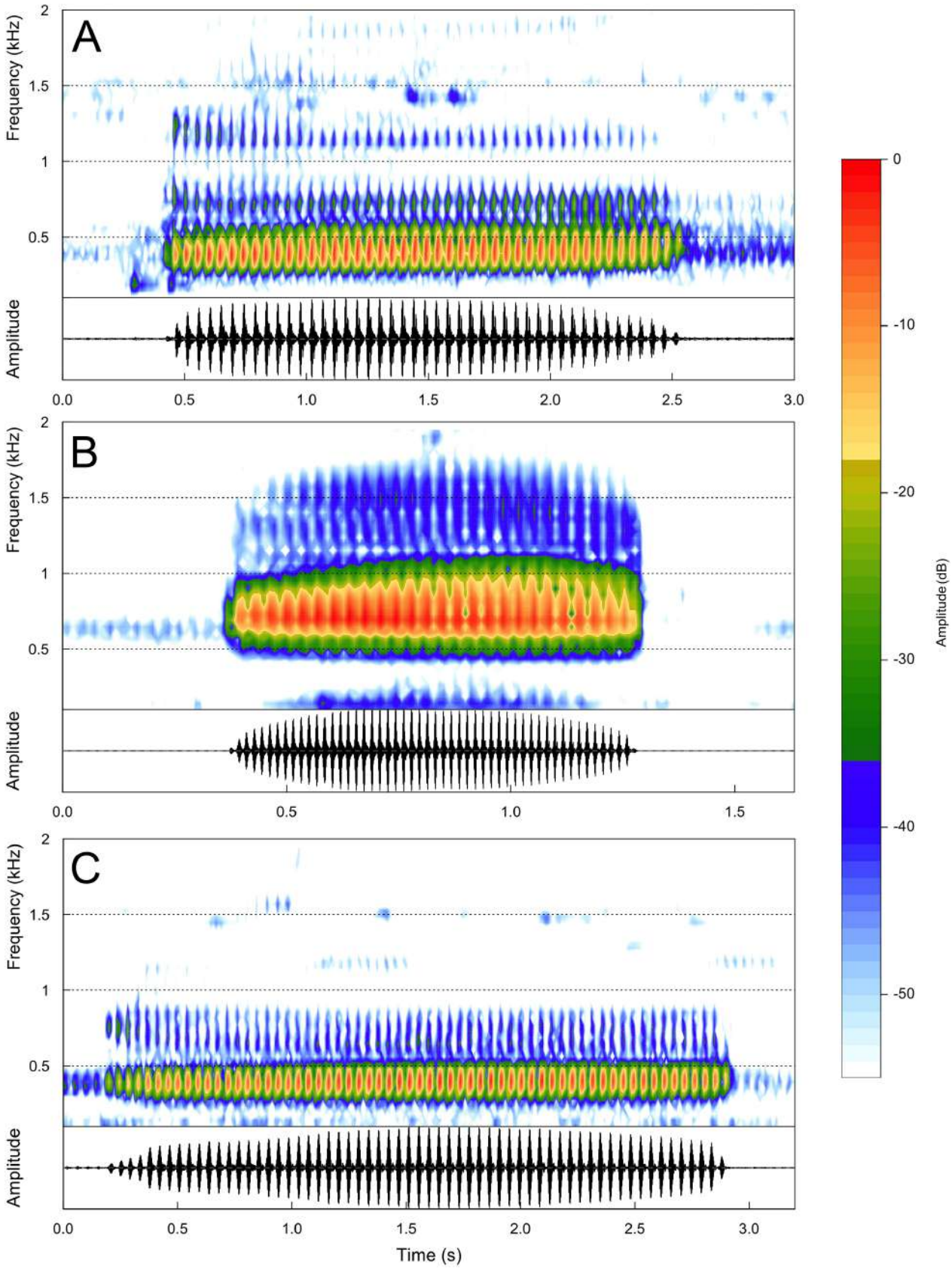

Figure 9. Advertisement call of each lineage. (**A**) N1: *Proceratophrys fryi* (recording Mascate2_01112008, no air temperature data available), (**B**) N2: *P. boiei* (FNJV_0033758, air temperature 19.4 °C), and (**C**) S: *P. intermedia* (FNJV_0012954, air temperature 22.0 °C). Audiospectrogram (above) and oscillogram (below).

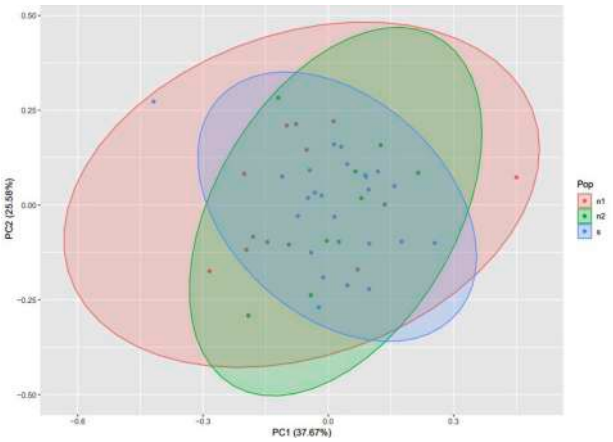

Figure 10. Multivariate analysis of variance based on the acoustic variables of advertisement calls from the N1, N2, and S lineages of populations under *Proceratophrys boiei*.

Proceratophrys boiei (Wied-Neuwied 1824)

Figs 11, 12

Ceratophrys boiei – Wied-Neuwied (1824). Type-locality: Rio de Janeiro and Bahia, Brazil. Restricted to Bahia by Hoogmoed (1990); further restricted to Rio de Janeiro by Prado and Pombal-Jr. (2008).
Stombus bojei – Gravenhorst (1829).
Ceratophris granosa – Cuvier (1829). Type-locality: not specified.
Rana (*Ceratophris*) *granosa* – Guérin-Méneville (1838).
Stombus granosus – Fitzinger (1861).
Ceratophrys bojei – Reinhardt and Lütken (1862).
Ceratophrys dorsata boiei – Gadow (1901).
Ceratophrys boiei – Miranda-Ribeiro (1920).
Stombus boiei – Miranda-Ribeiro (1920).
Stombus fryi – Miranda-Ribeiro (1920).
Stombus boiei – B. Lutz (1954).
Proceratophrys boiei – Lynch (1971).

Type-specimen. The species was described by Wied-Neuwied (1824) and redescribed by the same author in 1825, using lectotype RMNH 24109, from the state of Rio de Janeiro.

Lectotype. RMNH 24109 (SVL 53.1 mm), adult female (Fig. 12A). Type-locality: restricted to Rio de Janeiro, Brazil, by Prado and Pombal-Jr. (2008).

Diagnosis. *Proceratophrys boiei* is characterized by the following combination of characters: 1) medium size (SVL: 39.8–61.9 mm in males, n = 38; 40.0–74.3 mm in females, n = 18); 2) single palpebral appendage present; 3) ocular-dorsal ridges with constriction in the medial region of the back; 4) ventral spots vermicular or rounded (predominantly small or large circles); 5) advertisement call with 0.50–1.32 s, 32–71 pulses/call, 40.96–66.26 pulses/second, and dominant frequency of 602.93–818.26 Hz; 6) NOR develops on submetacentric pair 8.

Comparison with other species (characteristics of other species are in parentheses). The absence of a rostral appendage distinguishes *Proceratophrys boiei* 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* (rostral appendage present). The presence of palpebral appendages distinguishes *Proceratophrys boiei* from *P. ararype*, *P. bagnoi*, *P. branti*, *P. carranca*, *P. concavitympanum*, *P. cristiceps*, *P. cururu*, *P. dibernardoi*, *P. goyana*, *P. huntingtoni*, *P. korekore*, *P. minuta*, *P. moratoi*, *P. redacta*, *P. rotundipalpebra*, *P. salvatori*, *P. strussmannae*, *P. velhochico*, and *P. vielliardi* (palpebral appendages absent). The presence of a single palpebral appendage distinguishes *Proceratophrys boiei* from *P. rondonae* and *P. schirchi* (multi-cusped palpebral appendages). The absence of postocular swellings distinguishes *Proceratophrys boiei* from *P. avelinoi*, *P. bigibbosa*, *P. brauni*, and *P. palustris* (postocular swellings present). The pattern of vermicular or rounded spots, predominantly small or large circles on the ventral region of *P. boiei*, distinguishes it from *P. paviotti* (irregular spots on the ventral region). The advertisement call of *Proceratophrys boiei* has 32–71 pulses/call, which differentiates the species from *P. paviotti* (26–32 pulses/call) and *P. renalis* (13–30 pulses/call).

Additional characteristics of the lectotype. Prado and Pombal-Jr. (2008) provided the description and illustration of the lectotype, which features a short, triangular palpebral appendage; ocular-dorsal ridges with constriction in the presacral region, discontinuous, with short, rounded tubercles, fused or unfused; and a nearly rounded snout in dorsal view.

Osteology. HAP corresponds to 95% of the width of the premaxilla; DPPPm very deep, its length corresponds to more than 50% of the width of the premaxilla; TESPF can be smooth or smooth with three developed protrusions, distributed in a diagonal row under the orbit, the anterior one being the highest; PVFM can be very deep and evident, shallow, or not evident; SNBDV boomerang-shaped; TN irregular, with grooves and protuberances, these in greater number and more clustered in the posterodorsal region; HPdRN nasals slightly projected posterodorsally, their height does not exceed or slightly exceeds the height of the anterior region of the frontoparietals; CN nasals do not contact; CNFp nasals do not contact frontoparietals; TFp can be smooth, irregular, with grooves and protuberances distributed along the outer margin of the frontoparietals or excavated, with grooves and cavities distributed on the sides and throughout the posterior region of the frontoparietals; RFpDV flattened-concave, the flat region corresponding to the inner half of each frontoparietal and the concave region to the outer half; WFp frontoparietals wider medially; PlEFp absent; DPRFpC curved toward the adjacent frontoparietal, away from the margin of the orbit; LORS shorter than the length of the zygomatic branch of the squamosal (length of the otic branch corresponding to 65% of the zygomatic branch of the squamosal); CulPPph wider up to its middle third; EARCulPPph may or may not reach the palatines.

Variation. Palpebral appendages present, can be short (n = 20) or long (n = 35), with triangular (n = 53) or

pointed triangular (*n* = 2) shape; the snout shape in dorsal view can be rounded (*n* = 17), nearly rounded (*n* = 34), or semicircular (*n* = 4). The canthal crest can be slightly visible (*n* = 3), moderate (*n* = 10), or prominent (*n* = 42); it can be smooth (*n* = 43) or rough (*n* = 12). The frontoparietal crest can be slightly visible (*n* = 17), moderate (*n* = 4), or prominent (*n* = 34); it can be very (*n* = 7), slightly (*n* = 25), or not arched (*n* = 23); the posterior portion of the frontoparietals is curved toward the center of the head. The region between the frontoparietal crest can be shallow (*n* = 43) or deep (*n* = 12), with a predominance of shallow. The crest of the squamosal bones can be slightly (*n* = 49), moderate (*n* = 3), or very prominent (*n* = 3). In the region of the buccal commissure, one to four tubercles can be observed, with a conical (*n* = 49) or triangular (*n* = 6) shape, with variation in their size. The tympanum can be distinct (defined as a depression in the skin behind the eyes and below the crest of the squamosal bone) (*n* = 20) or indistinct (*n* = 35). The interocular ridge can be continuous (*n* = 2) or discontinuous (*n* = 53), slightly (*n* = 29) or markedly curved (*n* = 26). The ocular-dorsal ridge is always present, with constriction in the presacral region, can be continuous (*n* = 52) or discontinuous (*n* = 3), and the tubercles that constitute it can be short, rounded, fused, or unfused. The dorsal coloration in preservative varies between shades of brown, cream, and gray; regarding the ventral surface, males and females have vermicular or rounded spots (small or large circles), which can extend from the gular region to the limbs or be restricted to the abdominal region; males often have a dark or faded gular region. The inner metacarpal tubercle can be oval (*n* = 43) or elliptical (*n* = 12); and the outer ones can be separated (*n* = 16) or bifid (*n* = 39), with the medial oval (*n* = 46), elliptical (*n* = 4), or rounded (*n* = 5), and the distal can be oval (*n* = 40), elliptical (*n* = 13), or rounded (*n* = 2). The inner metatarsal tubercle can be elliptical (*n* = 34), curved elliptical and prominent (*n* = 14), or with a widened edge (*n* = 9). They may have one to two rows of tubercles on the forearm. The relative length of the fingers of the hands can follow six forms: relative length of the fingers (1) IV < II < I < III (*n* = 12), (2) II < IV < I < III (*n* = 15), (3) II < I < IV < III (*n* = 10), (4) I < IV < II < III (*n* = 4), (5) I < II < IV < III (*n* = 13), and (6) IV < I < II < III (*n* = 1); and of the feet: I < II < V < III < IV. The squamosal bones are fused, smooth or rough, with undulations or not. The frontoparietals can be smooth or rough, with strong expansion or not, juxtaposed with slight separation or very separated, with the anterior portion slightly open. The cultriform process of the parasphenoid is wide at the base and may or may not taper in the distal portion.

Advertisement call. The advertisement call of *Proceratophrys boiei* (Fig. 9A) consists of a multipulsed note, with an average duration of 0.973 ± 0.177 s (range: 0.50–1.32 s). The number of pulses per call is 51.206 ± 9.570 (range: 32–71 pulses/call), the pulse rate is 53.072 ± 6.077 pulses per second (range: 40.96–66.26 pulses/second), and the average dominant frequency is 717.424 ± 50.003 Hz (range: 602.93–818.26 Hz).

Tadpole. Described by Izecksohn et al. (1979) based on tadpoles collected at the municipality of Teresópolis, Rio de Janeiro state, Brazil. Individuals were deposited in the private collection of Eugenio Izecksohn (lots EI 5512 and EI 5513). The chondrocranium of the species tadpole was described by Dias et al. (2013a) based on individuals collected at the following locations: Brazil, state of Rio de Janeiro, municipality of Teresópolis—ZUFRJ 5099 (four tadpoles; stages 34–35) and state of Minas Gerais, municipality of Camanducaia, Monte Verde—DZSJRP 1104.2 (two tadpoles; stages 32 and 37, according to Gosner 1960).

Geographic distribution. *Proceratophrys boiei* occurs primarily in the Atlantic Forest biome, in the states of Rio de Janeiro, northern São Paulo, and southern Minas Gerais. It may also occur in transitional areas with the Cerrado biome (Fig. 1).

Proceratophrys fryi (Günther 1873)

Figs 11, 12

Proceratophrys fryi Günther (1873), revalidated status.
Ceratophrys fryi – Günther (1873).
Stombus fryi – Miranda-Ribeiro (1920).
Proceratophrys fryi – Lynch (1971).

Holotype. BMNH 1873.5.1.1 (SVL 68.0 mm), adult female (Fig. 12B), collected in Serra da Mantiqueira, Minas Gerais, Brazil, by A. Fry and donated to Günther (1873). There are no geographical coordinates or collection date available.

Diagnosis. *Proceratophrys fryi* is characterized by the following combination of characters: 1) medium size (SVL: 41.2–59.4 mm in males, *n* = 25; 41.3–71.2 mm in females, *n* = 20); 2) single palpebral appendage; 3) ocular-dorsal ridges with constriction in the medial region of the back; 4) ventral spots vermicular or rounded (predominantly small or large circles); 5) advertisement call lasting 0.58–1.68 s, with 28–97 pulses/call, 42.87–57.93 pulses/second, and dominant frequency of 646.00–904.40 Hz; 6) NOR develops on submetacentric pair 8.

Comparison with other species (characteristics of other species are in parentheses). The absence of a rostral appendage distinguishes *Proceratophrys fryi* 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* (rostral appendage present). The presence of palpebral appendages distinguishes *Proceratophrys fryi* from *P. ararype*, *P. bagnoi*, *P. branti*, *P. carranca*, *P. concavitympanum*, *P. cristiceps*, *P. cururu*, *P. dibernardoi*, *P. goyana*, *P. huntingtoni*, *P. korekore*, *P. minuta*, *P. moratoi*, *P. redacta*, *P. rotundipalpebra*, *P. salvatori*, *P. strussmannae*, *P. velhochico*, and *P. vielliardi* (palpebral appendages absent). The presence of a single palpebral appendage distinguishes *Proceratophrys fryi* from *P. rondonae* and *P. schirchi* (multi-cusped

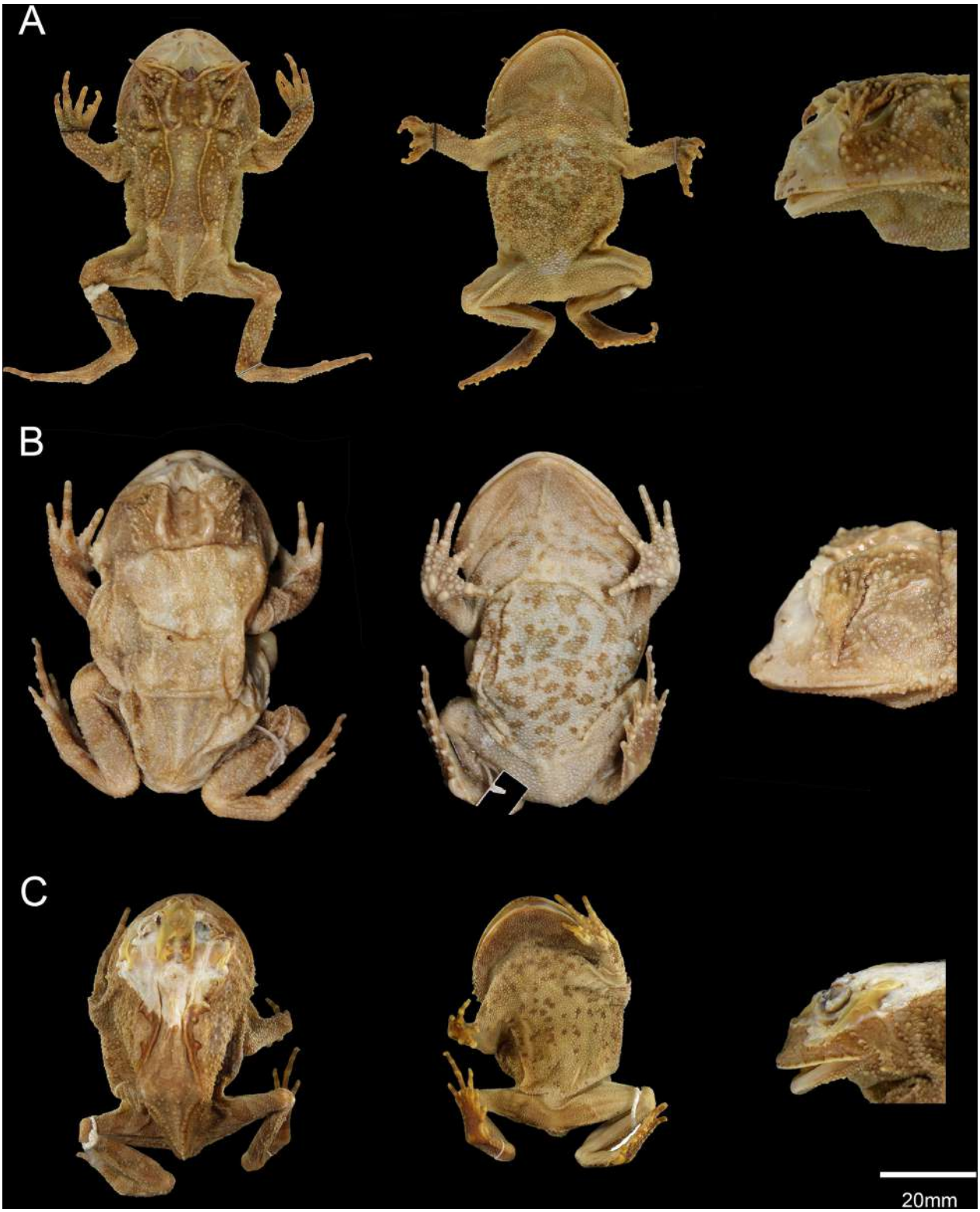

Figure 11. Dorsal and ventral views of (**A**) RMNH 24109, lectotype of *Proceratophrys boiei* (photo by Esther Dondorp, Nationaal Natuurhistorisch Museum, Leiden), (**B**) BMNH 1873.5.1.1, holotype of *P. fryi* (© The Trustees of the Natural History Museum, London), and (**C**) MCZ A-2254, holotype of *P. intermedia* (© MCZbase, Museum of Comparative Zoology, Harvard University).

palpebral appendage). The absence of postocular swellings distinguishes *Proceratophrys fryi* from *P. avelinoi*, *P. bigibbosa*, *P. brauni*, and *P. palustris* (postocular swellings present). The pattern of vermicular or rounded spots, predominantly small or large circles on the ventral region of *P. fryi*, distinguishes it from *P. paviotti* (irregular spots on the ventral region). The advertisement call of *Proceratophrys fryi* has 28–97 pulses/call, differentiating it from *P. paviotti* (0.012–0.015 pulses/s) and *P. renalis* (13–30 pulses/call), and has 42.87–57.93 pulses/s, differentiating

it from *P. renalis* (61.50–86.09 pulses/s). *Proceratophrys fryi* differs from *P. boiei* by having a larger mean SVL in males (51.5 mm and 50.0 mm, respectively) and genetic distances of 2.7% for 16S and 6.2% for *Cytb*.

In addition to the differences mentioned above based on analyses conducted in the present study, the tadpoles of *Proceratophrys fryi* (Pezzuti et al. 2021) differ from those of *P. boiei* (Izecksohn et al. 1979) by lacking the small fleshy projection on the medial margin of the nostrils, by having fewer submarginal papillae on the oral disc (usually 2–3 vs. 4 or more), by presenting a slightly more robust tail musculature with a more acutely pointed tip, and by lacking the pair of dorsal dark brown spots at the origin of the tail musculature reported for *P. boiei*. At the cytogenetic and molecular levels, the NOR develops on submetacentric pair 8. Furthermore, Bayesian and maximum likelihood reconstructions with three genes (16S, cytochrome *b*, and RAG-1) from populations throughout the distribution of *P. boiei* recovered three distinct lineages (Amaro et al. 2012). *Proceratophrys fryi* belongs to lineage N1.

Redescription of the holotype. [Based on the description by Prado and Pombal-Jr. (2008), and the analysis of holotype photos, Fig. 12B]. Palpebral appendages present, short, with pointed triangular shape; rostral appendage absent; snout shape in dorsal view rounded, and in lateral view, obtuse spatulate. The canthal crest is prominent and rough. The frontoparietal crest is prominent, very arched, and the posterior region is curved toward the center of the head. The region between the frontoparietals is deep. The crest of the squamosal bones is prominent. In the region of the buccal commissure, a tubercle can be observed, with a conical shape. Tympanum indistinct. The interocular ridge is continuous, markedly curved. The ocular-dorsal ridge is present, with constriction in the presacral region, discontinuous, and the tubercles that constitute it can be short, rounded, fused, or unfused. The skin texture is rough, with tubercles of different sizes, mainly conical and rounded. The inner metacarpal tubercle is elliptical, and the outer ones are separated, with the medial oval and the distal elliptical. The inner metatarsal tubercle has a widened edge, and the outer one is rounded. It has a row of tubercles on the forearm. Relative length of the fingers: II < IV < I < III; and toes: I < II < V < III < IV.

Additional characteristics of the holotype. Prado and Pombal-Jr. (2008) provided the description and illustration of the holotype, which has a short, triangular-pointed palpebral appendage; ocular-dorsal ridges with constriction in the presacral region, discontinuous, with short, rounded tubercles, fused or unfused; and a continuous and markedly curved interocular ridge.

Color in preservative of the holotype. The dorsal coloration is brown with parts in dark brown, with cream color on the snout; the ventral surface is cream, with vermicular and rounded spots (predominantly small or large circles), dark brown in color, extending from the gular region to the limbs; the gular region is cream.

Osteology. HAP is greater than the width of the premaxilla (height of the alar process corresponding to 103% of the width of the premaxilla); DPPPm very deep, its length corresponding to more than 50% of the width of the premaxilla; TESPF can be smooth or smooth, with three developed protrusions, distributed in a diagonal row under the orbit, the anterior one being the highest; PVFM can be very deep and evident or shallow; SNBDV boomerang-shaped; TN can be smooth or irregular, with grooves and protuberances, these in greater number and more clustered in the posterodorsal region; HPdRN nasals slightly projected posterodorsally, their height does not exceed, or slightly exceeds, the height of the anterior region of the frontoparietals; CN nasals do not contact; CNFp nasals in contact with frontoparietals; TFp is smooth, excavated, with grooves and cavities distributed on the sides and throughout the posterior region of the frontoparietals; RFpDV flattened-concave, the flat region corresponding to the inner half of each frontoparietal and the concave region to the outer half; WFp frontoparietals wider in their posterior half; PlEFp posterolateral extension absent; DPRFpC posterior region of the crest curved toward the adjacent frontoparietal, away from the margin of the orbit; LORS shorter than the length of the zygomatic branch of the squamosal (length of the otic branch corresponding to 74% of the zygomatic branch of the squamosal); CulPPph wider up to its middle third; EAR-CulPPph anterior region of the cultriform process does not reach the palatines.

Variation. Palpebral appendages can be short (n = 13) or long (n = 36) with a triangular shape (n = 35) or pointed triangular shape (n = 14); snout shape in dorsal view can be rounded (n = 25) or almost rounded (n = 24). The canthal crest can be barely visible (n = 12), moderate (n = 25), or pronounced (n = 12); it can be smooth (n = 27) or rough (n = 22). The frontoparietal crest can be barely visible (n = 12), moderate (n = 14), or pronounced (n = 23), with a predominance of pronounced; it can be very (n = 6), slightly (n = 26), or not arched (n = 17); the posterior portion of the frontoparietals can be not visible (n = 2) or curved toward the center of the head (n = 47). The region between the frontoparietal crests can be shallow (n = 28) or deep (n = 21). The crest of the squamosal bones can be barely (n = 32), moderately (n = 1), or very prominent (n = 16). In the region of the buccal commissure, one to three tubercles can be observed, with a conical shape (n = 26) or triangular (n = 1), with variation in their size. The tympanum can be distinct (defined as a depression in the skin behind the eyes and below the squamosal crest) (n = 7) or indistinct (n = 20). The interocular ridge can be continuous (n = 2) or discontinuous (n = 25), slightly (n = 11) or markedly curved (n = 14). The ocular-dorsal ridge is always present, with constriction in the presacral region, can be continuous (n = 41) or discontinuous (n = 8), and the tubercles that constitute it can be short or elongated, rounded, fused, or unfused. The dorsal coloration varies between shades of brown, cream, gray, and black; as for the ventral surface, males and females have vermicular or rounded spots (predominance of small or large circles), which can extend from the gular region to the limbs or be restricted to the abdominal region; males often have

a dark or faded gular region. The internal metacarpal tubercle can be oval (n = 45) or elliptical (n = 4), and the external ones can be separate (n = 36) or bifid (n = 13), with the medial oval (n = 39), elliptical (n = 7), or rounded (n = 3), and the distal oval (n = 31), elliptical (n = 17), or rounded (n = 1). The internal metatarsal tubercle can be elliptical (n = 35), curved and prominent elliptical (n = 9), or with a widened edge (n = 5), and the external one, rounded (n = 43) or oval (n = 6). They can have one to two rows of tubercles on the forearm. The relative length of the fingers can follow six forms: (1) IV < II < I < III (n = 26), (2) II < IV < I < III (n = 6), (3) II < I < IV < III (n = 5), (4) I < IV < II < III (n = 3), (5) I < II < IV < III (n = 5), and (6) IV < I < II < III (n = 4); and toes: I < II < V < III < IV. The squamosal bones are fused, smooth or rough, with undulations or not. The frontoparietals can be smooth or slightly rough, with slight or moderate expansion, fused or juxtaposed with slight separation, with the anterior portion very slightly or not open. The cultriform process of the parasphenoid is wide at the base and can be tapered or not at the distal portion.

Advertisement call. The advertisement call of *Proceratophrys fryi* (Fig. 9B) consists of a multipulsed note, with an average call duration of 0.831 ± 0.211 s (range: 0.58–1.68 s). The number of pulses per call is 41.011 ± 13.534 (range: 28–97 pulses/call), the pulse rate is 48.892 ± 4.113 pulses/second (range: 42.87–57.93 pulses/second), and the average dominant frequency is 770.316 ± 51.859 Hz (range: 646.00–904.40 Hz).

Tadpole. Described by Pezzuti et al. (2021) based on tadpoles collected at the municipalities of Congonhas and Nova Lima, Minas Gerais state, Brazil. Individuals were deposited in the Tadpole Collection of the Centro de Coleções Taxonômicas, Universidade Federal de Minas Gerais (UFMG).

Geographical distribution. *Proceratophrys fryi* occurs mainly in the Atlantic Forest biome, south of the Rio Doce region, in southern Espírito Santo and southeastern Minas Gerais. It may also be found in transitional areas with the Cerrado (Fig. 1).

Proceratophrys intermedia Barbour (1908)

Figs 11, 12

Proceratophrys intermedia Barbour (1908), revalidated status.
Ceratophrys intermedia – Barbour (1908).
Stombus boiei – Miranda-Ribeiro (1920).
Stombus intermedius – Miranda-Ribeiro (1920).
Stombus boiei – B. Lutz (1954).
Proceratophrys boiei – Lynch (1971).

Holotype. MCZ A-2254 (SVL 46.0 mm), adult male (Fig. 12C). Type locality: Santa Catarina, Brazil, Barbour (1908).

Diagnosis. *Proceratophrys intermedia* is characterized by the following combination of characters: 1) medium size compared to other species in the genus (SVL males 42.6–51.6 mm, n = 17; females 42.8–68.6 mm, n = 8); 2) single palpebral appendage; 3) ocular-dorsal ridges with constriction in the mid-dorsal region or absent; 4) ventral spots vermicular or rounded (predominantly small or large circles); 5) advertisement call with duration of 0.36–1.35 s (0.951 ± 0.121 s), 21–63 pulses/call (47.973 ± 6.374), 41.46–62.15 pulses/second (50.016 ± 4.572), and dominant frequency of 562.50–861.33 Hz (750.358 ± 67.658 Hz); 6) tadpole with a medial vent tube, absence of submarginal papillae on the oral disc, and a rounded tail tip; 7) reduced hypobranchial, IV ceratobranchial more developed, and expanded pars reuniens in the midline region; 8) NOR develops on subtelocentric pair 4.

Comparison with other species (characteristics of other species in parentheses). The absence of a rostral appendage distinguishes *Proceratophrys intermedia* 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* (rostral appendage present). The presence of palpebral appendages distinguishes *Proceratophrys intermedia* from *P. ararype*, *P. bagnoi*, *P. branti*, *P. carranca*, *P. concavitympanum*, *P. cristiceps*, *P. cururu*, *P. dibernardoi*, *P. goyana*, *P. huntingtoni*, *P. korekore*, *P. minuta*, *P. moratoi*, *P. redacta*, *P. rotundipalpebra*, *P. salvatori*, *P. strussmannae*, *P. velhochico*, and *P. vielliardi* (palpebral appendages absent). The presence of a single palpebral appendage distinguishes *Proceratophrys intermedia* from *P. rondonae* and *P. schirchi* (multicuspid palpebral appendage). The absence of postocular swellings distinguishes *Proceratophrys intermedia* from *P. avelinoi*, *P. bigibbosa*, *P. brauni*, and *P. palustris* (postocular swellings present). The pattern of vermicular or rounded spots, predominantly with small or large circles on the ventral region of *P. intermedia*, distinguishes it from *P. paviotti* (irregular spots on the ventral region). The advertisement call of *Proceratophrys intermedia* with 41.46–62.15 pulses/s distinguishes it from *P. paviotti* (0.012–0.015 pulses/s) and *P. renalis* (61.50–86.09 pulses/s). By having the smallest mean SVL in males (46.0 mm) and females (51.6 mm), *P. intermedia* differs from *P. boiei* (50.0 mm in males and 60.1 mm in females) and from *P. fryi* (51.5 mm in males and 60.3 mm in females). It further differs from *P. boiei* by genetic distances of 2.9% for 16S and 5.6% for *Cytb*, and from *P. fryi* by genetic distances of 2.8% for 16S and 7.3% for *Cytb*.

In addition to the differences mentioned above based on analyses conducted in the present study, the tadpoles of *Proceratophrys intermedia* (Rossa-Feres et al. 2024) differ from those of *P. fryi* (Pezzuti et al. 2021) and *P. boiei* (Izecksohn et al. 1979) by having a medial vent tube (vs. dextral in both species) and by lacking submarginal papillae on the oral disc (vs. present). In addition, *P. intermedia* exhibits larger dorsally positioned oval nostrils (vs. smaller in *P. fryi* and with a small fleshy projection on the medial margin in *P. boiei*) and a rounded tail tip (vs. acute in *P. fryi* and *P. boiei*). At Gosner stage 35, the tadpole of *P. intermedia* rev. can be distinguished from the tadpole of *P. boiei* by having reduced hypobranchial, IV ceratobranchial more developed, and expanded pars reuniens in the midline region (Dias et al. 2013a).

Figure 12. Live specimens of (**A**) *Proceratophrys boiei* from Reserva Biológica União, Rio das Ostras municipality, Rio de Janeiro state, and (**B**) from Serra do Japi, Jundiaí municipality; (**C, D**) *P. fryi* from Parque Estadual da Serra do Brigadeiro, Ervália municipality, Minas Gerais state; (**E**) *P. intermedia* from Ibirama municipality and (**F**) from Pedras Grandes municipality, Santa Catarina state, Brazil. Photos by Carlos Henrique de Oliveira Nogueira (**A**), Giulia Sorio (**B**), Diego José Santana (**C**), Sarah Mângia (**D**), and Luiz Ugioni (**E**, **F**).

At the molecular level, Bayesian and maximum likelihood reconstructions with three genes (16S, cytochrome *b*, and RAG-1) from populations throughout the distribution of *P. boiei* recovered three distinct lineages (Amaro et al. 2012). *Proceratophrys intermedia* belongs to lineage S. Furthermore, *P. intermedia* differs from *P. boiei* and *P. fryi* in developing the nucleolar organizer region (NOR) on subtelocentric pair 4, while northern species have it on submetacentric pair 8.

Redescription of the holotype. [Based on the description by Barbour (1908) and the analysis of the holotype photographs, Fig. 12C.] Palpebral appendages present, short, with a triangular shape; rostral appendage absent; snout shape semicircular in dorsal view and obtusely spatulate in lateral view. Canthal crest pronounced and smooth. Frontoparietal crest moderate, not arched, with the posterior region oriented toward the center of the head. The area between the frontoparietals is shallow. Squamosal bone crest is barely visible. Buccal commissure with two conical tubercles present, with the anterior being larger. Tympanum indistinct. Interocular region with no skin present, making it impossible to define its characteristics. Ocular-dorsal ridges present, with constriction in the presacral region, continuous, and the

tubercles that constitute them are short, fused, and elongated. Skin texture rugose, with tubercles of varying sizes, mainly conical and rounded. Metacarpal tubercles: the internal metacarpal tubercle is elliptical; external tubercles are separate, with the medial one oval and the distal one elliptical. Metatarsal tubercles: the internal one is curved, elliptical, and prominent; the external one is rounded. Forearm tubercles: two rows of tubercles are present. Relative length of fingers: I < II < IV < III; relative length of toes: I < II < V < III < IV.

Additional characteristics of the holotype. Barbour (1908) provided the description and illustration of the holotype, which has a short, triangular palpebral appendage; ocular-dorsal ridges with constriction in the presacral region, discontinuous with short, fused, and elongated tubercles; and a semicircular snout in dorsal view.

Color in preservative of the holotype. The dorsal coloration is light brown, with some brown spots along both lines of the ocular-dorsal ridges, as well as brown spots on the locomotor limbs; the snout has a cream color, with a black line dividing it from the rest of the body coloration; the ventral surface is cream-colored, with few rounded brown spots extending from the gular region to the belly; the palms of the hands and the soles of the feet are dark brown; the gular region is dark.

Osteology. HAP greater than the width of the premaxilla (height of the alar process corresponding to more than 90% of the width of the premaxilla); DPPPm very deep, its length corresponding to more than 50% of the width of the premaxilla; TESPF smooth; PVFM slightly or not evident; SNBDV boomerang-shaped; TN irregular, with grooves and protrusions, the latter being more numerous and clustered in the posterodorsal region; HPdRN nasals slightly projected posterodorsally, their height does not exceed or barely exceeds the height of the anterior region of the frontoparietals; CN nasals do not contact each other; CNFp nasals in contact with frontoparietals; TFp smooth, excavated, with grooves and cavities distributed on the sides and throughout the posterior region of the frontoparietals; RFpDV flat-concave, the flat region corresponding to the inner half of each frontoparietal and the concave region to the outer half; WFp frontoparietals wider medially; PlEFp posterolateral extension absent; DPRFpC posterior region of the crest curved toward the adjacent frontoparietal, away from the orbital margin; LORS shorter than the length of the zygomatic ramus of the squamosal (length of the otic ramus corresponding to 83% of the zygomatic ramus of the squamosal); CulPPph wider up to its middle third; EARCulPPph may or may not reach the palatines.

Variation. Palpebral appendages are present and can be short (n = 9) or long (n = 18), with a triangular shape (n = 25) or pointed triangular shape (n = 2), some exhibiting a white tip at the end; the snout shape in dorsal view can be semicircular (n = 1), rounded (n = 5), or nearly rounded (n = 22). The canthal crest can be pronounced (n = 6), moderate (n = 15), or barely visible (n = 6); it can be rough (n = 11) or smooth (n = 16). The frontoparietal crest can be barely visible (n = 20) or pronounced (n = 7); it may be slightly arched (n = 16) or not arched (n = 11); and the posterior portion may be either not visible (n = 3) or curved toward the center of the head (n = 24). The region between the frontoparietals is often shallow (n = 23), and in some individuals, it is deep (n = 4). The squamosal bone crest can be very (n = 3), moderately (n = 3), or slightly prominent (n = 21). In the region of the buccal commissure, one to three tubercles may be observed, with a conical shape (n = 12) or triangular shape (n = 8), which vary in size. The tympanum may be distinct (defined as a depression in the skin behind the eyes and below the squamosal crest) (n = 2) or indistinct (n = 19). The interocular ridge is discontinuous, slightly (n = 9) or markedly curved (n = 8). The ocular-dorsal ridge is always present, with or without constriction in the presacral region; it can be continuous (n = 19) or discontinuous (n = 8), and the tubercles that constitute it can be short, fused and elongated, or short, rounded, fused or unfused. Dorsal coloration in preservative varies between shades of brown, cream, and gray; regarding the ventral surface, males and females have vermicular or rounded spots (predominantly small or large circles), which can extend from the gular region to the limbs or be restricted to the abdominal region; males often have a dark or faded gular region. The internal metacarpal tubercle can be oval (n = 26) or elliptical (n = 1); the external ones can be separated (n = 14) or bifid (n = 13), with the medial one being oval (n = 20), elliptical (n = 2), or rounded (n = 5), and the distal one being oval (n = 21) or elliptical (n = 6). The internal metatarsal tubercle can be elliptical (n = 13), curved elliptical and prominent (n = 10), or have a widened border (n = 4); the external one can be rounded (n = 24) or oval (n = 3). They may exhibit one to two rows of tubercles on the forearm. Five variations were observed in the relative length of the fingers of the hands as follows: (1) IV < II < I < III (n = 13), (2) II < IV < I < III (n = 4), (3) II < I < IV < III (n = 4), (4) I < IV < II < III (n = 2), and (5) I < II < IV < III (n = 4); and of the feet: I < II < V < III < IV. The squamosal bones are fused, smooth or rough, with or without undulations. The frontoparietals can be smooth or slightly rough, with slight or moderate expansion, fused or juxtaposed with slight separation, with the anterior portion not open. The cultriform process of the parasphenoid is broad at the base and in the distal portion.

Advertisement call. The advertisement call of *Proceratophrys intermedia* (Fig. 9C) consists of a multipulsed note, with an average call duration of 0.951 ± 0.121 s (range: 0.36–1.35 s). The number of pulses per call is 47.973 ± 6.374 (range: 21–63 pulses/call), the pulse rate is 50.016 ± 4.572 pulses/second (range: 41.46–62.15 pulses/second), and the average dominant frequency is 750.358 ± 67.658 Hz (range: 562.50–861.33 Hz).

Tadpole. Described by Rossa-Feres et al. (2024) based on tadpoles collected at Serra e Gemido, São José dos Pinhais municipality, Paraná state, Brazil. Individuals were deposited at Departamento de Zoologia e Botânica, Universidade Estadual Paulista, Câmpus de

São José do Rio Preto (DZSJRP). The chondrocranium of the species was described by Dias et al. (2013a) based on tadpoles collected in the following localities: state of Paraná, municipality of Fazenda Rio Grande, Fazenda Gralha Azul—DZSJRP 819.3 (1 tadpole; stage 35), 830.2 (2 tadpoles; stages 35 and 38, according to Gosner 1960), and municipality of São José dos Pinhais, Serro e Gemido—DZSJRP 985.2 (1 tadpole; stage 37, according to Gosner 1960).

Geographic distribution. *Proceratophrys intermedia* occurs mainly in the Atlantic Forest biome, in the states of Santa Catarina and Paraná, and in the southern region of the state of São Paulo. It may also occur in transitional areas with the Cerrado (Fig. 1).

Discussion

Over the past two decades, the number of species described within the genus *Proceratophrys* has increased significantly (Kwet and Faivovich 2001; Prado and Pombal-Jr. 2008; Martins and Giaretta 2013; Dias et al. 2013a, 2013b; Mângia et al. 2014, 2018, 2022; Santana et al. 2021). These studies have contributed to taxonomic delimitation and a better understanding of the genus's evolutionary history by employing an integrative approach that combines morphological (external and osteological), molecular, and cytogenetic evidence.

Species delimitation is crucial, especially in cases such as *Proceratophrys boiei*, which for a long time was considered a widely distributed species in southeastern Brazil, occurring in the states of Espírito Santo, Minas Gerais, Paraná, Rio de Janeiro, Santa Catarina, and São Paulo (Prado and Pombal-Jr. 2008). However, more recent studies, such as Amaro et al. (2012), have revealed that *P. boiei* actually represents a complex of three distinct species: *P. boiei* and the two species revalidated in the present study, *P. intermedia* and *P. fryi*. These species, now recognized as having more restricted geographic distributions, highlight the importance of taxonomic reassessments to clarify biogeographic and diversification patterns.

In the present case, the recognition of these three species was not possible based solely on adult morphology or advertisement calls because these characters show substantial variation among individuals within the same species and also among lineages. Thus, the revalidation of *P. intermedia* and *P. fryi* was supported by a more complete set of evidence, including genetic distances, tadpole external morphology, and chondrocranial characters. Among the adult morphological characters examined, SVL was the only variable that contributed to distinguishing these taxa. *Proceratophrys intermedia* is the smallest of the three species, with the lowest mean SVL in both males and females, whereas *P. fryi* differs from *P. boiei* by having a larger mean SVL in males. These differences are accompanied by genetic divergence among species, with *p*-distances ranging from 2.7% to 2.9% for 16S and from 5.6% to 7.3% for *Cytb*. Together, these results support the recognition of *P. boiei*, *P. intermedia*, and *P. fryi* as distinct evolutionary lineages rather than as a single widespread species.

Similar taxonomic difficulties have been documented in other species complexes of *Proceratophrys*, in which species delimitation could not be based on a single external character but instead required the combined evaluation of multiple sources of evidence. In the *P. appendiculata* complex, lineages that had long been confused under the same name were better distinguished by the congruence of osteological, morphometric, and molecular data, even when genetic distances were relatively low (Dias et al. 2013a, 2013b). Likewise, in the Cerrado species from the *P. cristiceps* group, the complexity of external morphology and the difficulty of species identification based on isolated traits showed that diagnostic recognition often depends on the combination of several characters rather than on a single exclusive feature (Brandão et al. 2013). A similar situation was also documented in the *P. melanopogon* complex, in which a taxonomic revision demonstrated that what had been treated as a single widespread species comprised multiple distinct taxa (Mângia et al. 2014). Therefore, the case of *P. boiei*, *P. fryi*, and *P. intermedia* is consistent with a broader tendency in the genus: species limits are often obscured by morphological overlap, and more robust taxonomic decisions emerge only when morphology is interpreted together with molecular and other independent datasets.

The revalidation of *P. intermedia* and *P. fryi* carries significant implications not only for taxonomy but also for the conservation of these species, which should now be regarded as independent biological units. The recognition of more narrowly distributed taxa requires a reassessment of conservation strategies, as populations previously attributed to a widespread species may in fact be at greater risk due to habitat fragmentation and the presence of specific geographic barriers (Whittaker et al. 2005). In this context, it is possible that the observed barriers, especially those between the range limits of lineages, such as the Tietê River (between *P. intermedia* and *P. boiei*) and the Muriaé River (between *P. boiei* and *P. fryi*), are contributing to the maintenance of spatial isolation in these regions. Thus, rather than representing simple geographic discontinuities, these rivers may have acted as biogeographic barriers limiting gene flow and promoting lineage divergence, as has been suggested for other vertebrates in eastern Brazil (e.g., Grazziotin et al. 2006; Arruda et al. 2017; Pereira et al. 2022).

Further studies integrating population genetics, ecology, and biogeography could provide deeper insights into the historical processes that have shaped the current distribution of these species. More broadly, our results reinforce the importance of integrative taxonomy in groups with morphologically conservative species and highlight how taxonomic reassessments can substantially improve our understanding of diversity, biogeography, and conservation in Atlantic Forest amphibians.

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Appendix I

Examined specimens

Proceratophrys fryi - NORTH 1: ESPÍRITO SANTO: Ibitirama, Pedra Roxa (Caparaó): ZUEC-AMP 23621 (male); MINAS GERAIS: Alto Caparaó, Parque Nacional do Caparaó: ZUEC-AMP 23575 (female); Barão de Monte Alto: MZUFV 15767 (female), 15769 (male); Cataguases, Sinimbú: MZUFV 17591 (female), 18084 (female), 19620 (female), 19622 (male); Cataguases, Estação Ecológica de Água Limpa: MZUFV 13202 (male), 13204 (male); Cataguases, Horto – Estação Ecológica de Água Limpa: MZUFV 12202 (male); Cataguases, B. Tomé: MZUFV 6049 (male); Divino, Árvore Bonita: MZUFV 17073 (female), 17081 (female), 17111 (female), 17116 (female); Divino, Árvore Bonita: ZUFMS 5651 (female), 5666 (male), 5680 (male); Divino, Fortaleza: MZUFV 17092 (female); Ervália, Serra do Brigadeiro: ZUFMS-AMP 15672 (male), 15673 (male), 18904 (male), 18905 (male), 18906 (female), 18907 (female), 18908 (female), 18909 (female); Ipanema, PHC Areia Branca (AS4): MZUFV 8007 (male); Ipanema, Santa Constância: MZUFV 6828 (female); Miraí, APA Água Limpa (mata): MZUFV 13280 (female); Miraí, APA Água Limpa (borda): MZUFV 13304 (male), 13305 (female); Miraí, APA Água Limpa (brejo): MZUFV 13295 (female); Miraí, Área de Mata: MZUFV 13292 (male), 13293 (female); Ouro Preto, FLOE Uaimií: MZUFV 9661 (male); Ouro Preto, Tripuí: MZUFV 2334 (male); Ritápolis, Flona: MZUFV 19057 (male), 19058 (female), 19060 (female), 19062 (male), 19067 (male); Santa Bárbara do Monte Verde, Parque Estadual Serra Negra da Mantiqueira: ZUFMS-AMP 15671 (male), 18903 (female); Viçosa, Mata da Biologia: MZUFV 3621 (male), 5811 (male); Viçosa, Mata do Paraíso: MZUFV 7250 (female), 7133 (male).

Proceratophrys boiei - NORTH 2: SOUTH OF MINAS GERAIS: Cristina: CCLZU 1477 (female), 1478 (female), 1479 (male), 1605 (male), 1606 (female); Cristina, Brejinho: CCLZU 3140 (male); Cristina, Mata da Prefeitura: CCLZU 1598 (female), 1996 (male), 2002 (female), 2032 (male), 2033 (female), 2207 (female), 2208 (male), 2467 (female), 2955 (male); NORTH OF SÃO PAULO: Atibaia, Parque Municipal da Grota Funda: ZUEC-AMP 23974 (male), 23975 (male), 23976 (male); Bananal, Estação Ecológica Bananal: ZUEC-AMP 25196 (male), 25197 (female); Caçapava: CCLZU 407 (male), 408 (male), 409 (male), 410 (male), 411 (male), 412 (male), 413 (male), 414 (male), 415 (male), 758 (male); Campinas, Mata de Santa Genebra: ZUEC-AMP 18736 (female); São Francisco Xavier: ZUEC-AMP 24844 (male); São José dos Campos, Parque Municipal Augusto Ruschi: ZUEC-AMP 23307 (male); Santo Antônio do Pinhal: CCLZU 827 (male), 828 (male), 829 (male), 830 (male), 831 (male), 1109 (male), 1110 (female), 1295 (male), 1474 (female), 1475 (female); São Luíz do Paraitinga, Bairro Bom Retiro: ZUEC-AMP 19286 (female), 19296 (female); São Luiz do Paraitinga, Bairro Pantheon: ZUEC-AMP 19279 (male), 19280 (male), 19281 (male), 19277 (male), 19278 (male); São Luiz do Paraitinga, Fragment N° 1: ZUEC-AMP 22834 (male), 22835 (male); São Luiz do Paraitinga, Parque Estadual Serra do Mar, Núcleo de Santa Virgínia: ZUEC-AMP 23041 (female), 23042 (female); RIO DE JANEIRO: Nova Friburgo: ZUFMS-AMP 19392 (female), 19393 (male).

Proceratophrys intermedia - SOUTH: PARANÁ: Fazenda Rio Grande: MCP 10333 (female); Guaratuba: MCP 7634 (male), 7635 (female); SANTA CATARINA: Araquari, Itapocu: ZUFMS-AMP 19387 (male), 19388 (male); Blumenau, Testo Salto: ZUFMS-AMP 19428 (male); Florianópolis: MCP 6409 (male), 6355 (male); Jaraguá do Sul: MCP 8151 (male); Lauro Müller: MCP 8135 (male), 8136 (male), 12319 (male); Pedras Grandes: ZUFMS-AMP 19389 (female), 19390 (female); Pedras Grandes, Azambuja: ZUFMS-AMP 19386 (male), 19391 (male); São Bento do Sul: MCP 11137 (male), 11138 (male); SÃO PAULO: São Paulo, Parque Natural Jaceguava: ZUEC-AMP 20532 (male), 20533 (male); SOUTH OF SÃO PAULO: Apiaí: ZUEC-AMP 4754 (female), 4755 (female); Iporanga, Reserva Betary: ZUEC-AMP 20176 (male), 20177 (female); Ribeirão Grande: ZUEC-AMP 20552 (female).

Appendix II

Advertisement call recordings

Proceratophrys fryi - NORTH 1: ESPÍRITO SANTO: Ibitirama: FNJV_0033213; Santa Teresa: sound 2-1-xi-2012; MINAS GERAIS: Alto Caparaó: FNJV_0033266; Congonhas: CSN: Mascate01112008, 01112008 (2); Divino: JVAL_0274; Itabira: Ipoema: 11112009, 11112009 (2); Viçosa: FNJV_0031314.

Proceratophrys boiei - NORTH 2: MINAS GERAIS: CRISTINA: *P. boiei*; Munhoz: FNJV_0031310; RIO DE JANEIRO: Engenheiro Paulo de Frontin: FNJV_0044147; Rio de Janeiro: FNJV_0031906; Teresópolis: FNJV_0031317: SÃO PAULO: Salesópolis: FNJV_0033758; São José dos Campos: FNJV_0034249, 0044985, 004986.

Proceratophrys intermedia - SOUTH: PARANÁ: Morretes: FNJV_0031312; Palmeira: FNJV_0045258; Ponta Grossa: FNJV_0033569; RPPN Salto Morato: JVAL 0190; SANTA CATARINA: Araquari, Itapocu: ZUFMS-AMP 19387, 19388; Pedras Grandes: 1; Pedras Grandes: Azambuja: ZUFMS-AMP 19386, 19391; São Bonifácio, PE Tabuleiro: TASCAM_0376, 0377; SÃO PAULO: Ilha do Cardoso: FNJV_0012953, 0012954; Iporanga: FNJV_0040855; Ribeirão Grande: FNJV_0032520; Ribeirão Grande, Parque Estadual de Intervales: FT_00184, 00185 ind 2a; São Roque: FNJV_0040428.

Supplementary material 1

GenBank accession numbers

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Data type: docx

Explanation note: GenBank accession numbers for 16S and Cytb mitochondrial gene sequences of *Proceratophrys samples*.

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