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Revising the taxonomy of *Proceratophrys* Miranda-Ribeiro, 1920 (Anura: Odontophrynidae) from the Brazilian semiarid Caatinga: Morphology, calls and molecules support a single widespread species

Sarah Mângia[1] | Eliana Faria Oliveira[2] | Diego José Santana[2] | Ricardo Koroiva[3] | Fernando Paiva[2] | Adrian Antonio Garda[4]

[1]Departamento de Sistemática e Ecologia, Programa de Pós-Graduação em Ciências Biológicas (Zoologia), Universidade Federal da Paraíba, João Pessoa, Brazil

[2]Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, Cidade Universitária, Campo Grande, Brazil

[3]Laboratório de Citotaxonomia e Insetos Aquáticos, Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil

[4]Departamento de Botânica e Zoologia, Universidade Federal do Rio Grande do Norte, Natal, Brazil

Correspondence
Sarah Mângia, Departamento de Sistemática e Ecologia, Programa de Pós-Graduação em Ciências Biológicas (Zoologia), Universidade Federal da Paraíba, João Pessoa, Brazil.
Email: sarahmangia@yahoo.com.br

Present address
Sarah Mângia, Instituto de Biociências, Universidade Federal de Mato Grosso do Sul, Cidade Universitária, Campo Grande, Brazil

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Abstract

Recently, *Proceratophrys cristiceps* was redescribed along with the description of two species from the Caatinga biome: *P. aridus* and *P. caramaschii*. However, only a small fraction of the populations related to such species in Northeastern Brazil was examined, and most populations of central Caatinga were not contemplated in this analysis. Comparisons were also based exclusively on external morphology, precluding a more accurate delimitation of such taxa in the light of multiple characters. Such geographic paucity and reliance in only one data source caused the species status of most central Caatinga populations to be uncertain. Thus, the revision of *Proceratophrys* populations from the Caatinga biome is of utmost importance to establish a solid taxonomic background and to test the validity of the described species. Based on morphologic, morphometric, acoustic, and multilocus genetic data, we define the range of inter- and intrapopulation variation in the parameters we analyzed, establishing which ones are useful as diagnostic characters for *Proceratophrys* in the Caatinga. We found no evidence supporting *P. aridus* and *P. caramaschii* as distinct species and thus place them as junior synonyms of *P. cristiceps*. Our results reinforce the importance of using multiple lines of evidence to avoid taxonomic instability.

KEYWORDS

acoustic, molecular, morphology, *Proceratophrys aridus*, *Proceratophrys caramaschii*, *Proceratophrys cristiceps*, synonymization

1 | INTRODUCTION

Taxonomy is a critical discipline for scientists aiming to explore and understand biodiversity (Mace, 2004). To properly unveil the world's diversity, we must rely on using integrative taxonomic approaches, where multiple lines of evidence are considered (combining several lines of evidence) (Padial, Miralles, De la Riva, & Vences, 2010). Taxonomic revisions based solely on one type of characteristic frequently lack resolution in species diagnosis that can lead to incoherence in biogeographic, phylogenetic, and evolutionary analyses (Bickford et al., 2007). In fact, such simplistic taxonomic studies must be treated with caution, especially nowadays when integrative

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taxonomy studies are easier to conduct and strongly recommended (Padial et al., 2010). To better comprehend and describe biological diversity more rigorous species delimitation (SD) using integrative taxonomy is crucial, rendering a more stable nomenclature by both describing (Brusquetti, Jansen, Barrio-Amorós, Segalla, & Haddad, 2014; Kergoat et al., 2015) and synonymizing (Brusquetti et al., 2014; Petrusek et al., 2008; Steiner et al., 2006) species.

The genus *Proceratophrys* Miranda-Ribeiro, 1920, has been historically arranged in morphological groups (Cruz, Nunes, & Juncá, 2012; Prado & Pombal, 2008), which were later shown to lack phylogenetic support (Amaro, Pavan, & Rodrigues, 2009; Dias, Amaro, Carvalho-e-Silva, & Rodrigues, 2013; Mângia et al., 2018; Teixeira-Jr., Amaro, Recoder, Vechio, & Rodrigues, 2012). The *Proceratophrys cristiceps* complex currently includes 16 species and is characterized by the absence of rostral and palpebral appendages or postocular elevations (Cruz et al., 2012; details on species, authority and habitat are provided in Table S1).

Proceratophrys caramaschii Cruz et al., 2012 and *P. aridus* Cruz et al., 2012 were described from populations initially considered as *P. cristiceps* (Cruz et al., 2012). Hence, Cruz et al. (2012) redescribed *P. cristiceps* and limited its distribution to costal Northeastern Brazil. The authors also suggested that individuals occurring in the Espinhaço Mountain Range must be treated as *P. cururu* Eterovick & Sazima, 1998, while those occurring in the Cerrado biome should be considered *P. goyana* (Miranda-Ribeiro, 1937). However, Cruz et al. (2012) did not examine, comparatively, individuals of *P. cristiceps*, *P. cururu*, and *P. goyana* nor from populations encompassing all their ranges. Indeed, most populations from the core of the Caatinga were not included in their analyses. Furthermore, as pointed by Teixeira-Jr. et al. (2012), the morphological similarity of large-size species from the *P. cristiceps* complex makes the group prone to misidentifications. Therefore, recent species descriptions, dubious identifications from several localities, and the lack of comprehensive taxonomic assessments across all species' distributions reinforce the need for broader taxonomic revisions.

The revision of *Proceratophrys* populations from the Caatinga biome is paramount to establish a solid taxonomic background and to test the validity of the described species. To shed light on these issues, in this study our goals were to (a) revise the taxonomic status of all *Proceratophrys* populations from the Caatinga biome, covering a significant portion of its geographic distribution, based on morphologic, morphometric, acoustic, and multilocus genetic data; (b) identify the inter- and intrapopulation variations of the parameters analyzed in order to select which characters can be used as diagnostic and which are polymorphic; and (c) evaluate the genetic structure of *Proceratophrys* populations from Caatinga biome.

2 | MATERIAL AND METHODS

We analyzed the morphology of 358 specimens of *P. aridus*, *P. caramaschii*, and *P. cristiceps*, and other 298 of other species of the genus (Appendix 1). Regarding the bioacoustics analysis, we evaluated a total of 404 advertisement calls of *P. caramaschii* and *P. cristiceps*. We also sequenced one mitochondrial and three nuclear genes for 110 individuals of the three species and used sequences for other 22 species of the genus available in GenBank (Table S2, Table S3).

2.1 | Taxon sampling and material examined

We focused on examining individuals from lowland areas in the Caatinga biome, which include *P. aridus*, *P. caramaschii*, and *P. cristiceps* housed in 11 herpetological collections from Brazil (Appendix 1). We attributed individuals to one of the three species based on geographic distribution (proximity to the type locality) and available literature. For *P. aridus*, we considered the type locality (Milagres municipality, Ceará State—Cruz et al., 2012) and some nearby locations (Missão Nova, Barbalha and Farias Brito municipalities, Ceará State, approximately 20 km, 30 km and 90 km from the type locality, respectively). For *P. caramaschii*, we considered the type locality (Mucuripe neighborhood, Fortaleza municipality, Ceará State—Cruz et al., 2012), the species occurrence expansion reported in the literature (Ubajara municipality, Ceará State—Nunes, Loebamann, Cruz, & Haddad, 2015), and the nearest place from the type locality we got tissues and recording samples (Aquiraz municipality, Ceará State, 20 km from the type locality) (Table S2). We also examined representative specimens of other *Proceratophrys* species (Appendix 1).

2.2 | Morphologic and morphometric assessment

We analyzed morphological and morphometric data from 33 preserved specimens of *P. aridus* (12 males, 21 females), 31 of *P. caramaschii* (27 males, 4 females), and 294 of *P. cristiceps* (160 males, 134 females) (Appendix 1). We followed the terminology for external morphology of Prado and Pombal (2008), Brandão, Caramaschii, Vaz-Silva, and Campos (2013), and Mângia, Santana, Cruz, and Feio (2014), and the terminology for morphometric measurements from Mângia et al. (2014). Abbreviations used for the measurements of adult specimens are SVL (snout-vent length), HL (head length), HW (head width), DICS (distance from the interocular crest to the tip of snout), IND (inter-narial distance), END (eye–nostril distance), ED (eye diameter), UEW (upper eyelid width), IOD (inter-orbital distance), THL (thigh length), TL (tibia length), FL (foot + tarsus length), and FHL (forearm and hand length). We described coloration patterns in life using photographs because in specimens in preservative the colors are faded.

To morphologically discriminate among species and identify which variables contributed the most to their separation, we used a machine learning approach based on a random forest of decision trees (Breiman, 2001). The random forest algorithm implemented in the R package randomForest (Liaw & Wiener, 2002) generates random classification trees by using bootstrap samples from the original dataset to grow unpruned classification trees (usually 1,000). Next, these trees are used to generate classifiers choosing the best splits based on a random sample of predictors. At last, the algorithm uses

these predictors aggregated to classify new data based on a majority rule. At each bootstrap step, it predicts the data not present in the bootstrap sample ("out of the bag" samples, or OOB) and aggregates these results at the end to generate an error estimate of the classification (for more detail, see Liaw & Wiener, 2002). The analysis also generates a measure of importance for each variable and a measure of the internal structure of the data. Variable importance is estimated based on the effect of permuting a variable while leaving others unchanged on prediction error. Our dataset was inspected for univariate and multivariate outliers. A few values (DCOF for three individuals and DIN and DO for one individual each) were substituted by NA values and imputed using missForest package in R (Stekhoven, 2011). No multivariate outlier was detected.

2.3 | Acoustic analyses

We analyzed calls of *P. cristiceps* from 12 localities (Table 1), which were recorded using a Marantz PMD 660 digital recorder coupled with a Sennheiser ME 66 directional microphone. We deposited recordings in the Arquivos Sonoros da Universidade Federal do Rio Grande do Norte (ASUFRN) and in the Fonoteca Mapinguari da Universidade Federal de Mato Grosso do Sul (MAP-V). We also analyzed calls from several populations loaned from scientific collections (see Table 1). We obtained and analyzed calls from Aquiraz municipality, Ceará State, which we considered to belong to *P. caramaschii* due to the proximity to the type locality Mucuripe (20km away from Mucuripe municipality, Ceará State).

We analyzed calls with Raven Pro 1.5 for Mac (Cornell Lab of Ornithology) and constructed audio spectrograms in R software using the package *seewave* (Sueur, Aubin, & Simonis, 2008; R Development Core Team) with the following parameters: FFT window width = 256, Frame = 100, Overlap = 75, and flat-top filter. We also analyzed the same recordings used to describe the advertisement call of *P. caramaschii* from Ubajara, Ceará State (Nunes et al., 2015). We were unable to obtain calls of *P. aridus*. We analyzed acoustic parameters commonly employed in *Proceratophrys* taxonomy papers (e.g., Mângia et al., 2018; Mângia et al., 2014; Mângia, Santana, & Feio, 2010; Santana et al., 2011; Santana, São-Pedro, Bernarde, & Feio, 2010): call duration, pulse number per call, pulse number per second, and dominant frequency. Call terminology follows Köhler et al. (2017). For acoustic comparisons, we used published records of the advertisement call of *P. cristiceps* (Nunes & Juncá, 2006) and *P. caramaschii* (Nunes et al., 2015).

2.4 | Sequencing, genetic diversity, and haplotype networks

We obtained a total of three samples of *P. aridus* from three localities, 13 of *P. caramaschii* from three localities, and 94 of *P. cristiceps* from 23 localities in the Caatinga biome and adjacent areas (Figure 1, Table S3). We extracted whole genomic DNA from muscle or liver samples using the phenol-chloroform protocol (Sambrooks, Fritsch, & Maniatis, 1989). We used polymerase chain reaction (PCR) to amplify four loci (Table S4). PCR products were delivered to Macrogen (Seoul, Korea) for sequencing. First, we sequenced all individuals for the mitochondrial gene (mtDNA) 16S ribosomal RNA (*16S*), used as barcode for amphibians (Vences, Nagy, Sonet, & Verheyen, 2012). We also amplified and sequenced three nuclear genes (nuDNA): beta-crystallin (*CRYBA*), proopiomelanocortin precursor (*POMC*), and rhodopsin (*RHO*). For all reactions, the PCR cycling program used was 94°C for 2 min, followed by 35 cycles of 94°C for 45 s, 48–58.7°C (48°C to *16S*, 57°C to *CRYBA*, 58.7°C to *POMC*, and 58°C to *RHO*) for 45 s, 72°C for 1 min, and concluding with a 5 min extension at 72°C. PCR conditions for amplification consisted of 1 × buffer, dNTP at 0.2 mM, each primer at 0.2 μM, MgCl2 at 2 mM, 1U Taq polymerase, and 2 μl of template DNA, in a total reaction volume of 25 μl. For nuDNA genes, we sequenced a subset of individuals (42 for *CRYBA*, 49 for *POMC*, and 35 for *RHO*), which we chose to represent a wide geographic range within the Caatinga, following the approach of previous studies (Oliveira et al., 2015; Ruane, Bryson, Pyron, & Burbrink, 2014).

We checked and edited sequences by aligning forward and reverse reads in Geneious 9.1.2 with MUSCLE algorithm using default parameters (Edgar, 2004). We found gaps in *16S*, and to avoid possible bias, we removed them using Gblocks (Castresana, 2000; Talavera & Castresana, 2007), available as a web server (http://molevol.cmima.csic.es/castresana/Gblocks_server.html). To determine the most probable pair of alleles for nuDNA genes, we used the PHASE algorithm (Stephens, Smith, & Donnelly, 2001) implemented in the DnaSP 5.10 software (Librado & Rozas, 2009) using default options, except for the output probability threshold (we considered only samples with probability of pairs of alleles in heterozygosis higher than 80%). We deposited all sequences in GenBank (Table S5).

We calculated haplotype number (*h*), haplotype diversity (*Hd*), and nucleotide diversity (π) for each molecular marker using DnaSP 5.10 (Librado & Rozas, 2009). In order to explore the relationship among haplotypes, we estimated haplotype networks for mtDNA and nuDNA (phased) genes in Haploviewer (Salzburger, Ewing, & Von Haeseler, 2011) using Bayesian gene trees constructed in BEAST v.1.8 (Drummond, Suchard, Xie, & Rambaut, 2012). We identified each species using different colors in the haplotype network (gray: *P. aridus*; black: *P. caramaschii*; white: *P. cristiceps*). To generate Bayesian gene trees, we selected the model of nucleotide substitution for each gene based on the Bayesian information criterion (BIC) with jModelTest (Darriba, Taboada, Doallo, & Posada, 2012). The best-fit models were HKY for *16S* and *POMC*, F81 for *CRYBA*, and K80 + I for *RHO*. Then, we performed a run with 5×10^7 generations, sampling every 1,000 steps using a Birth-Death prior tree. We checked for stationarity by visually inspecting trace plots and ensuring that all values for effective sample size were above 200 in Tracer v1.5 (Rambaut & Drummond, 2007). We discarded the first 20% of sampled genealogies as burn-in and inferred the most credible clade with TreeAnnotator. To visualize the posterior probabilities values on the nodes, we used FigTree 1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/). In addition, we

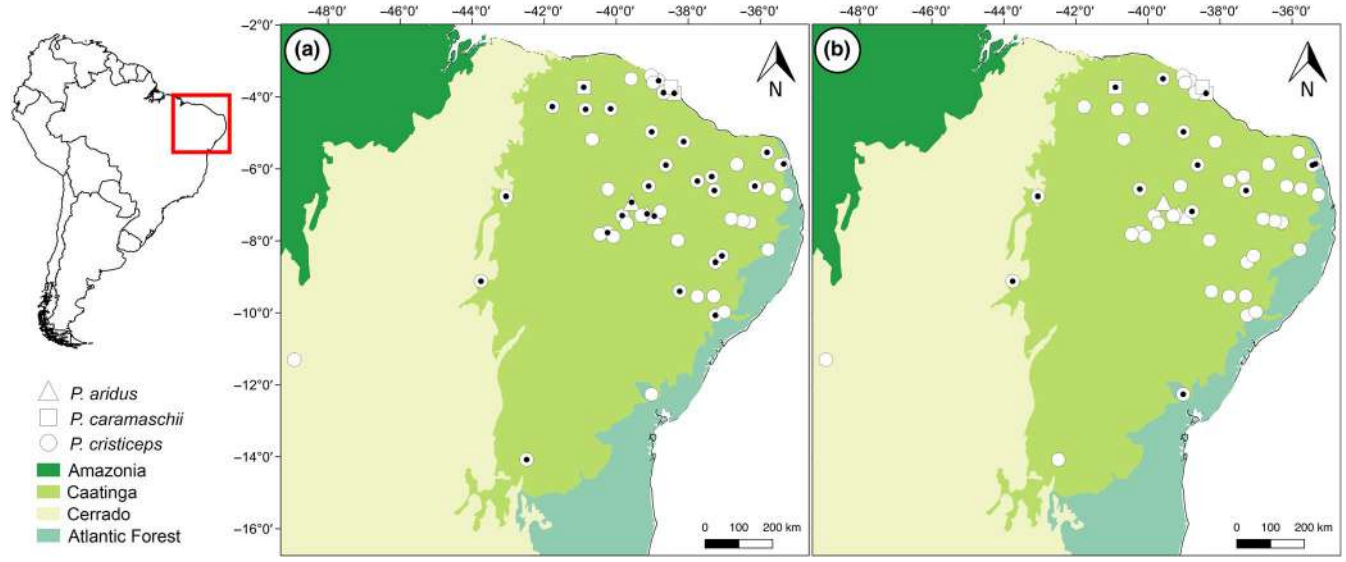

FIGURE 1 (a) Sample localities for morphological (circles, triangles and squares) and molecular (dotted) data. (b) Sample localities for morphological (circles, triangles and squares) and acoustic (dotted) data. Detailed locality information is shown in Table S2

constructed gene trees with RAxML (v 8.2.12) using GTR GAMMA model and 1,000 bootstrap replicates (Alignments S1–S4). We tested the convergence of the bootstrap in the RAxML using a posteriori bootstrapping analysis. For all gene trees, including Bayesian and RAxML, we used one out group for each gene (Table S5).

2.5 | Population assignment and genetic distances

To investigate the population structure among and within the studied species, we performed an exploratory analysis using GENELAND 4.0.3 (Guillot, Estoup, Mortier, & Cosson, 2005; Guillot, Mortier, & Estoup, 2005) implemented in R platform (R Core Team, 2014). This analysis evaluates the presence of population structure in a group of geo-referenced haplotypic data by inferring and locating genetic discontinuities. We used two different haplotype data sets, one with just nuDNA data of the three species and another with both mtDNA and nuDNA data. The most probable number of population units (*k*) was determined by a Markov Chain Monte Carlo (MCMC) method, with 10 repetitions (5×10^6 iterations in each) of *k* from 1 to 5. We used these values of *k* to explore initially the dataset and posteriorly we ran the analysis using higher values (i.e., *k* from 1 to 10). However, since our results remained the same, we kept *k* varying from 1 to 5 for the sake of simplicity.

We calculated genetic distances (uncorrected p-values) for all genes in Mega v 6.06 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013), using default options and including 20 sequences from other *Proceratophrys* species available in GenBank (Table S5).

2.6 | Species delimitation

We assessed the taxonomic status of *Proceratophrys* species included in the phylogenetic analysis applying three species delimitation (SD) methods: Automatic Barcode Gap Discovery (ABGD) (Pulliandre, Lambert, Brouillet, & Achaz, 2012), Poisson Tree Process (PTP, Zhang, Kapli, Pavlidis, & Stamatakis, 2013), and a Bayesian implementation of the Poisson Tree Process (bPTP). We used a concatenated alignment of *16S* and *RHO* genes, removing duplicate haplotypes from the alignment using DnaSP 5.10.01 (Librado & Rozas, 2009). We used these two genes to perform the analysis because they were the only sequences of other species of *Proceratophrys* available in GenBank. Haplotype codes (SD) used in this step are indicated in Table S5.

We performed ABGD analyses online (http://wwwabi.snv.jussieu.fr/public/abgd/), using three distance metrics (JC, K2P, and p-distance) with parameters set to default values. The result was the same regardless of the model of evolution employed. For the PTP analysis, we constructed a tree with RAxML (v 8.2.12) using GTRGAMMA model and 1,000 bootstrap replicates. We tested the convergence of the bootstrap in the RAxML using a posteriori bootstrapping analysis. We conducted PTP and bPTP analyses in the PTP web servers (http://species.h-its.org/) using default settings. At last, we built a Bayesian tree using MrBayes 3.2.6 with 30 million generations and a burn-in value of 10% to represent the phylogenetic relationships among haplotypes.

3 | RESULTS

3.1 | Morphology and coloration assessment

After observations of the coloration of *P. aridus*, *P. caramaschii*, and *P. cristiceps* populations, we noticed a wide polychromatism in all *Proceratophrys* populations along the Caatinga biome (Figure 2). The dorsal background color can be cream, brown, or reddish, with scattered dark spots. In some individuals, the region delimited by the

TABLE 1 Acoustic parameters of the advertisement calls of *Proceratophrys cristiceps* and *Proceratophrys caramaschii*. Localities are followed by Brazilian State Acronyms. BA-Bahia, CE-Ceará, PI-Piauí, RN-Rio Grande do Norte

Locality/acoustic parameters	Recording collection label	Date/air temperature (At)	Duration (s)	Pulse/call	Pulse/sec	Dominant frequency (Hz)
P. cristiceps						
Macaíba, RN (1 male, 20 calls)	ASUFRN034	12 May 2011 (At not available)	0.553 ± 0.06 0.418–0.619	51 ± 5.47 40–59	93.5 ± 1.55 90.4–95.8	1,033.6
Macaíba, RN (1 male, 14 calls)	ASUFRN044	17 June 2011 (At 24.8°C)	0.592 ± 0.03 0.550–0.633	55 ± 2.27 51–58	92.3 ± 1.40 90–94.6	1,033.6
ESEC Seridó, RN (10 calls)	MAP-V0289	9 May 2013 (24.2°C)	0.556 ± 0.04 0.506–0.633	53.5 ± 3.74 48–60	96.4 ± 1.81 93.9–98.8	1,125
Aiuaba, CE (13 calls)	URCA7396	6 February 2014 (At not available)	0.706 ± 0.12 0.450–0.896	61 ± 9. 67 38–73	84.4 ± 1.80 81.9–81.5	1,125 ± 82.22 937.5–1,125
Barro, CE (27 calls)	URCA7920	23 February 2014 (At not available)	0.509 ± 0.08 0.354–0.638	47 ± 6.96 33–57	91.9 ± 1.31 88.7–93.8	937.5
Ipú, CE (12 calls)	URCA9203	8 April 2014 (25.5°C)	0.559 ± 0.04 0.499–0.619	50.5 ± 2.84 46–55	90.3 ± 1.25 88.5–92.2	937.5
Itapipoca, CE (10 calls)	MAP-V0290	20 March 2014 (24.0°C)	0.570 ± 0.08 0.477–0.728	54.4 ± 6.81 46–68	95.7 ± 1.86 93.4–97.8	1,125
Jaguaribe, CE (1 male, 10 calls)	MAP-V0291	12 April 2014 (At not available)	0.609 ± 0.08 0.447–0.704	59.5 ± 7.57 45–68	98.0 ± 1.92 96.6–101.9	1,125 ± 79.06 937.5–1,125
Jaguaribe, CE (1 male, 3 calls)	MAP-V0292	12 April 2014 (At not available)	0.650 ± 0.05 0.557–0.652	63 ± 5.20 54–63	96.9 ± 0.18 96.6–96.9	1,125
Jaguaribe, CE (1 male, 4 calls)	MAP-V0293	12 April 2014 (At not available)	0.578 ± 0.06 0.531–0.645	60.5 ± 4.99 56–66	104.3 ± 1.88 102.3–106.3	1,125
Quixadá, CE (9 calls)	MAP-V0305	18 April 2015 (At not available)	0.902 ± 0.14 0.623–1.011	77 ± 11.30 55–85	86.3 ± 1.61 88.3–84.1	861.3
Floriano, PI (1 male, 20 calls)	AN0659	28 November 2013 (At 29.4°C)	0.537 ± 0.05 0.443–0.630	52.5 ± 4.79 44–62	98.2 ± 4.12 81.9–101.5	1,033.6
Floriano, PI (1 male, 18 calls)	AN0663	28 November 2013 (At 29.4°C)	0.633 ± 0.04 0.532–0.694	62 ± 4.23 52–68	97.7 ± 1.27 94.9–99.1	1,033.6
Floriano, PI (1 male, 14 calls)	AN0662	28 November 2013 (At 29.4°C)	0.892 ± 0.04 0.814–0.955	90.5 ± 7.92 82–113	102.7 ± 6.06 100.1–124.0	1,033.6 ± 46.05 861.3–1,033.6
PARNA Serra das Confusões, PI (1 male, 9 calls)	MAP-V0294	22 October 2014 (At not available)	0.740 ± 0.04 0.653–0.782	53 ± 2.55 47–56	71.6 ± 0.32 71.4–72.4	1,033.6
PARNA Serra das Confusões, PI (1 male, 31 calls)	MAP-V0295	22 October 2014 (At not available)	0.703 ± 0.05 0.643–0.901	52 ± 4.01 48–66	74.3 ± 1.02 73.5–76.9	1,033.6
PARNA Serra das Confusões, PI (1 male, 37 calls)	MAP-V0296	22 October 2014 (At not available)	0.650 ± 0.02 0.599–0.707	49 ± 2.40 45–58	74.2 ± 2.63 73.3–89.2	1,033.6
PARNA Serra das Confusões, PI (1 male, 24 calls)	MAP-V0297	22 October 2014 (At not available)	0.680 ± 0.06 0.591–0.781	52.5 ± 3.92 47–61	77.9 ± 1.31 75.9–80.5	1,033.6 ± 48.6 861.3–1,033.6
PARNA Serra das Confusões, PI (1 male, 40 calls)	MAP-V0298	22 October 2014 (At not available)	0.686 ± 0.07 0.570–0.811	51.5 ± 4.23 44–59	73.5 ± 1.40 71.6–77.2	1,033.6
Feira de Santana, BA (1 male, 9 calls)	MAP-V0299	8 June 2011 (At 29.9°C)	0.561 ± 0.04 0.506–0.628	48 ± 4.25 43–55	84.7 ± 2.63 83.0–92.1	1,033.6 ± 76.0 861.3–1,033.6
Feira de Santana, BA (1 male, 7 calls)	MAP-V0300	29 November 2004 (At 26°C)	0.668 ± 0.09 0.520–0.800	59 ± 7.28 46–69	87.1 ± 0.90 86.2–88.5	861.3
Feira de Santana, BA[a] (29 calls)	—	—	0.660 ± 0.05 0.520–0.790	57.5 ± 6.02 46–69	89.5 ± 1.20 87.4–91.5	940 ± 0.02 900–990

(Continues)

TABLE 1 (Continued)

Locality/acoustic parameters	Recording collection label	Date/air temperature (At)	Duration (s)	Pulse/call	Pulse/sec	Dominant frequency (Hz)
P. caramaschii						
Aquiraz, CE (22 calls)	MAP-V0301	23 March 2015 (At not available)	0.492 ± 0.09 0.364–0.742	42 ± 7.09 33–60	87.7 ± 2.30 80.7–91.9	861.3 ± 79.76 861.3–1,033.6
Ubajara, CE (1 male, 25 calls)	MAP-V0303	22 January 2008 (At not available)	0.456 ± 0.03 0.400–0.581	35.9 ± 3.08 32–46	78.8 ± 0.89 76.0–80.6	861.3
Ubajara, CE (1 male, 16 calls)	MAP-V0304	28 January 2008 (At not available)	0.674 ± 0.04 0.609–0.737	54.2 ± 3.27 49–59	80.5 ± 0.83 78.7–82.1	861.3
Planalto do Ibiapaba, Ubajara/Viçosa do Ceará, both in CE[b]	—	Between February 2007 and January 2008	0.570 ± 11.0 0.410–0.740	45 ± 9.19 33–59	80.0 ± 0.86 78.6–81.2	860–1,030

[a]Nunes and Juncá (2006)

[b]Nunes et al. (2015).

ocular-dorsal ridge of warts is light cream or yellowish. The number of morphotypes varies among populations, and we could not assign one morphotype to any specific population or species.

We also classified morphological variation among populations of *Proceratophrys* from across the Caatinga biome and among individuals from the same population. The snout can be (1) rounded or (2) triangular (Figure 3). The ocular-dorsal ridge of warts can be (1) continuous, extending from the edge of the eyelid to the sacral region, (2) interrupted in the pre-sacral constriction, or (3) absent (Figure 4). The interocular ridge can be formed by (1) one or (2) two rows of warts (observed only in two individuals of *P. cristiceps*, the holotype and one individual from Piripiri municipality, Piauí State) (Figure 5). The inner part of metacarpal tubercle can be (a) smaller or (b) equal to the outer one (Figure 6). The ventral region coloration can be (a) cream without blotches or (b) cream with brown spots or blotches (males usually present darkish gular region) (Figure 7). Based on these results, males and females of the three species do not differ with respect to morphological characters. However, the gular region in males is usually darker and more prominent than in females due to the presence of vocal sac. Males also present two vocal slits in the inner part of the mouth, each one on both sides of the maxilla, extending from the insertion of the tongue to close to the jaw joint.

At last, all morphometric characters measured for *P. aridus* and *P. caramaschii* overlapped with those from *P. cristiceps* (Table 2). Our random forest classification results indicated large overlap between specimens in the MDS plot (Figure 8a). Furthermore, the confusion matrix shows no fit of the data to classify correctly *P. caramaschii* (100% error) and moderate errors to identify *P. aridus* (Figure 8b). The variable that most contributed to the separation between species was ED (Figure 8c).

3.2 | Advertisement calls

The advertisement calls of individuals of *P. cristiceps* from the 12 different populations are composed of a single pulsed note (Figure 9a). The values of the acoustic parameters are similar among populations (Table 1). Based on the calls of all populations analyzed (including data from literature), the advertisement call of *P. cristiceps* presents a duration of 0.379–1.011 s (0.557 ± 0.11), with 36–85 pulses/note (52 ± 8.71), an emission rate of 69.1–105.3 pulses/s (92.4 ± 5.92), and dominant frequency of 861–1125 Hz (1,033.6 ± 97.14).

The advertisement calls of *P. caramaschii* from Aquiraz and Ubajara municipalities, Ceará State, are composed of one multipulsed note (Table 1, Figure 9b). The calls present a duration of 0.364–0.742 s (0.482 ± 0.11), with 32–60 pulses/note (41 ± 8.74), emission rate of 76.0–91.9 pulses/s (80.5 ± 4.19), and dominant frequency of 861–1033.6 Hz (861.3 ± 51.36).

3.3 | Molecular data

We obtained a final nuDNA dataset of 238 bp for *CRYBA*, 446 bp for *POMC*, and 329 bp for *RHO*. The mtDNA *16S* preserved around 78% (433 bp) of its original size after gap exclusion in Gblock. Both Bayesian and Maximum Likelihood gene trees (Figures S1 and S2) grouped *P. aridus*, *P. caramaschii*, and *P. cristiceps* in the same clade. Uncorrected mtDNA p-distances exhibited very low genetic differences (around 0.19%) among *P. aridus*, *P. caramaschii*, and *P. cristiceps* (Table S6).

The 110 sequences of barcode *16S* mtDNA of the three studied species resulted in 18 haplotypes in a star-shaped network, presenting no geographic structure and extremely low levels of genetic diversity (Table 3, Figure 10). The central haplotype H1 is the most frequent, containing 84 individuals and occurring in 27 localities (Table S3). The three nuDNA genes also showed low genetic diversity (Table 3), however, marginally higher than *16S* mtDNA. The nuDNA genes also presented one haplotype more frequent (Table 3, Figure 10, Table S3). All haplotypes of *P. aridus* and *P. caramaschii* are shared with *P. cristiceps*. Using two different haplotype datasets, GENELAND detected only one population ($k = 1$; Figure S3), grouping *P. aridus*, *P. caramaschii*, and *P. cristiceps*.

Our Bayesian concatenated tree (*16S* and *RHO*) recovered the same topology of the gene trees, grouping *P. aridus*, *P. caramaschii*, and *P. cristiceps* as a single lineage with no structure regarding geography or potential species. In addition, ABGD, PTP, and bPTP methods delimited this lineage as a single species. These approaches agree with the morphology used to diagnose species (Figure 11).

4 | DISCUSSION

Disputes surrounding the use of single or multiple lines of evidences in systematics have been ongoing for centuries, but the subject seemed settled in the early 1980s (Mayr, 1982). However, the rapid accumulation of molecular data in the upcoming decades reignited the debate, triggering calls for systematic research based on integrative approaches (Dayrat, 2005; Padial et al., 2010). Accordingly, several studies have shown the importance of using multiple evidences to achieve more reliable results in systematics since then (e.g., Padial et al., 2010; Valdecasas, Williams, & Wheeler, 2008; Will, Mishler, & Wheeler, 2005). The methodological advantage of using a diverse set of characters in systematics, especially for SD, is now again a matter of common sense.

This ideal scenario, however, is still far from reach for most taxonomic groups, especially understudied Neotropical animals such as frogs. For instance, among the 41 species of the genus *Proceratophrys*, few have been described using multiple lines of evidence, and only three studies used molecular data complementarily for species identification or description (Dias et al., 2013; Mângia et al., 2018; Teixeira-Jr. et al., 2012). In the present study, we highlight how the use of integrative taxonomy is critical for widespread, understudied groups such as Neotropical amphibians. The results from our effort to study the genus across the Caatinga biome under an integrative taxonomy framework is contributing to taxonomic and nomenclatural stability by describing new taxa (Mângia et al., 2018) and, in the present study, synonymizing other species. As shown next, extensive and representative geographic assessment using multiple lines of evidence is key to correctly interpret characters in light of reciprocal illumination among different, complementing data sources.

Until recently, morphological differences were the main source of characters used in amphibian taxonomy (Bickford et al., 2007). Still, many taxonomic revisions and species descriptions did not adequately described within-species geographic variation, concentrating on the comparisons among few individuals from a handful of localities. In the Neotropics, this has been somewhat common, given that many areas and entire biomes are still severely under-sampled. However, uneven geographic coverage associated with the use of morphological characters alone can easily lead taxonomists to consider populational differences as evidences for multiple species. *Proceratophrys* species in the Caatinga were recently described based on external morphology and coloration patterns of individuals from few populations across the biome (Cruz et al., 2012). A full appreciation of coloration and external morphology across the Caatinga, however, indicates that such characters do not support the previously described species.

Based on the samples collected by ourselves and data from the literature, it is impracticable to use dorsal coloration patterns as diagnostic characters for *Proceratophrys* species from the Caatinga. Coloration has been vastly used in taxonomy, especially in visually guided animals such as birds (McKay et al., 2014). Most species of frogs, however, are nocturnal, and many of these have cryptic colorations. Most importantly, color polymorphism is widely known for frogs (Hoffman & Blouin, 2000), especially tropical cryptically colored species. The use of coloration in such species' taxonomy is challenging, as clearly exemplified by African treefrogs of the genus *Hyperolius* (Laurent, 1965; Lötters et al., 2004; Wieczorek, Channing, & Drewes, 1998). Indeed, *Proceratophrys* species have polymorphic cryptic colorations (Nunes et al., 2015; Vieira, Arzabe, Hernandéz, & Vieira, 2008).

Likewise, morphological characters used to diagnose *P. aridus*, *P. caramaschii*, and *P. cristiceps* as three different species did not withstand our thorough comparisons using multiple populations and individuals across the distribution. These characters include snout shape, number of interocular ridge of warts, and size of metacarpal tubercles, which were shown to vary drastically within *P. cristiceps* (see detailed comparisons in the Appendix 2). Morphometrically, differences were also tenuous (*P. aridus*) or inexistent (*P. caramaschii*), albeit the number of individuals available for comparisons was very reduced. Nevertheless, allied to the lack of significant differences in advertisement calls, mitochondrial and nuclear DNA (see next), and other external morphology characters, the lack of support from morphometric characters further endorses the synonymization of these three species.

Proceratophrys aridus, *P. caramaschii*, and *P. cristiceps* are genetically indistinguishable considering uncorrected mtDNA *p*-distances (Table S6). According to our population assignment results, all individuals correspond to a single population widely distributed across the Caatinga including individuals of *P. aridus*, *P. caramaschii*, and *P. cristiceps*, endorsing that these three taxa are actually one widespread species. We also observed that the most common mtDNA haplotype is found all over the species distribution. Geomorphological barriers such as rivers (e.g., Kaefer, Tsuji-Nishikido, Mota, Farias, & Lima, 2013; Oliveira, Martinez, et al., 2018; Pellegrino et al., 2005) and mountain ranges (e.g., Firkowski, Bornschein, Ribeiro, & Pie, 2016; Oliveira, Gehara, et al., 2018; Shepard & Burbrink, 2008) are usually responsible for patterns of geographic structure in taxa resulting from speciation events. Climatic oscillations or climatic gradients may also act at different latitudes, altitudes, and ecotone zones, directly affecting the genetic structure of some widely distributed species and species pairs (e.g., *Boana albopunctata*, Prado, Haddad, & Zamudio, 2012; *Ameivula ocellifera*, Oliveira et al., 2015; *Polychrus acutirostris*, Fonseca et al., 2018; *Lygodactylus klugei*, Lanna et al., 2018). For the Caatinga, the largest continuous block of Seasonally dry Tropical Forests (Werneck, Costa, Colli, Prado, & Sites, 2011), the São Francisco River has been shown to act as a soft barrier

to gene flow for some species (Faria, Nascimento, de Oliveira, & Bonvicino, 2013; Nascimento et al., 2013; Oliveira, Martinez, et al., 2018; Werneck, Leite, Geurgas, & Rodrigues, 2015), while the Espinhaço Mountain Range (Garda et al., 2017) and the middle São Francisco Dunes region (Mesquita, Costa, Garda, & Delfim, 2017; Passoni, Benozzati, & Rodrigues, 2008; Siedchlag, Benozzati, Passoni, & Rodrigues, 2010) are known centers of endemism, harboring a large number of endemics of the Caatinga herpetofauna. However, none of these landscape features and processes seem to have affected the genetic structure of *Proceratophrys* from lowland areas of Caatinga. This is a common feature for many species in the biome which show low genetic structure and evidences for recent population size expansions at the end of the Pleistocene (Gehara et al., 2017). Despite its widespread occurrence, we recovered one single population with no geographic genetic structure.

5 | TAXONOMIC IMPLICATIONS

5.1 | Synonymization of *Proceratophrys aridus* Cruz et al., 2012

Cruz et al. (2012) described *Proceratophrys aridus* based on 56 specimens, all collected in Milagres municipality, Ceará State, Brazil. From the analysis of a topotype of *P. aridus* (AAGARDA 11910), the holotype (MNRJ 55782), the type series (MNRJ 55349, 55778–55781, 55783–55822, 75156, 75157, 75158–75168), and our thorough evaluation of *P. cristiceps* across its distribution, no character we evaluated distinguishes *P. aridus* from *P. cristiceps*. We observed all the diagnostic characters proposed for *P. aridus* in individuals of *P. cristiceps* along its distribution in the Caatinga and adjacent areas. Thus, all characters used to diagnose *P. aridus* are individual variation within populations of *P. cristiceps* along its distribution (as previously discussed). Once we

FIGURE 2 Inter- and intrapopulational chromatic variation in *Proceratophrys* from Caatinga. (a–e) Individuals from Parque Nacional Serra das Confusões, Piauí State (*P. cristiceps*). (f–i) individuals from Jaguaribe municipality, Ceará State (*P. cristiceps*). (j–m) individuals from Macaíba municipality, Rio Grande do Norte State (*P. cristiceps*). (n) individual from Aquiraz municipality, Ceará State (*P. caramaschii* new synonymy). (o) individuals from Milagres municipality, Ceará State (*P. aridus* new synonymy). (p, q) individuals from Parque Nacional de Ubajara, Ceará State (*P. caramaschii* new synonymy). (r) Paraipaba, Ceará State (*P. cristiceps*). Photographs: D.J. Santana (f–m); I. Joventino (n, o, r); S. Mângia (a–e, p–q)

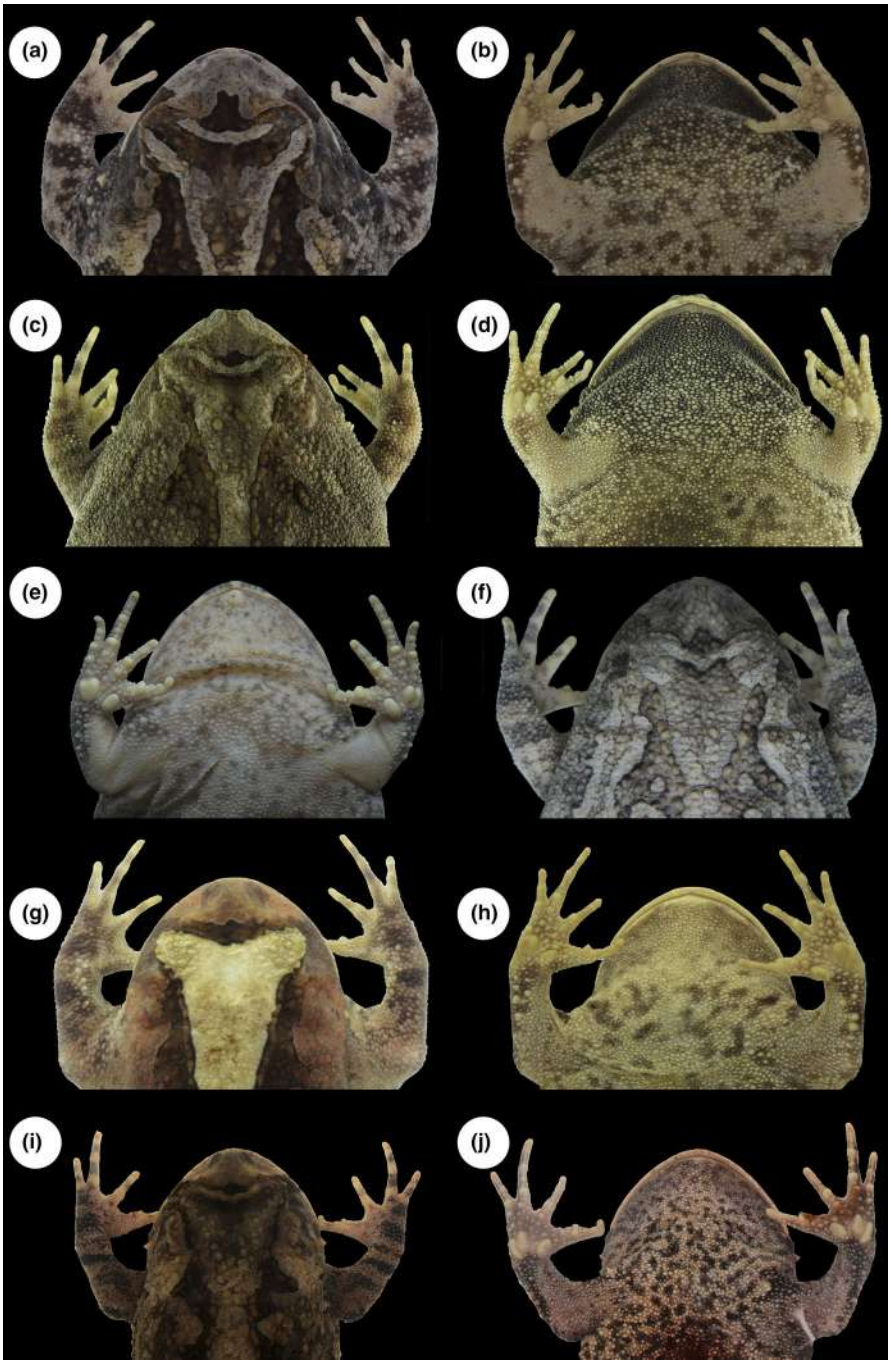

FIGURE 3 Snout shape in *Proceratophrys* from Caatinga. (1) triangular: (a–b) *P. cristiceps*, AAGARDA 10453, Itapipoca municipality, Ceará State; (c–d) *P. caramaschii* new synonymy, PRC 003, Aquiraz municipality, Ceará State; (e, f) *P. aridus* new synonymy, MNRJ 55785, Milagres municipality, Ceará State. (2) rounded: (g, h) *P. cristiceps*, AAGARDA 7824, Parque Nacional de Catimbau, Pernambuco State; (i, j) *P. caramaschii* new synonymy, AAGARDA 10961, Parque Nacional de Ubajara, Ceará State; (k, l) *P. aridus* new synonymy, AAGARDA 11.910, Milagres municipality, Ceará State

found no characteristics supporting these taxa as two different species and genetic distances between them are very low (0.5% in *16S* mtDNA barcoding), we consider *Proceratophrys aridus* Cruz et al., 2012 as a junior synonym of *P. cristiceps* (Müller 1884 "1883").

5.2 | Synonymization of *Proceratophrys caramaschii* Cruz et al., 2012

Cruz et al. (2012) described *Proceratophrys caramaschii* based on 30 individuals, all collected with the holotype in Mucuripe, Fortaleza municipality, Ceará State, Brazil. The type series was collected in 1945 by A.L. Carvalho and, since then, the area has been drastically modified. We visited the type locality of *P. caramaschii* in April 2015 and found a degraded creek inside an urban square within the city, surrounded by buildings. In spite of searching even within green areas around the type locality (such as urban parks), we were unable to find individuals of *P. caramaschii* or suitable natural habitats for the species. The nearest site we successfully recorded individuals of *Proceratophrys* was in Aquiraz municipality, approximately 20 km south from the type locality, where we collected specimens, tissues, and recorded advertisement calls.

Brandão et al. (2013) used the type series and individuals from Piripiri municipality, Piauí State, which they called *P. caramaschii*, to

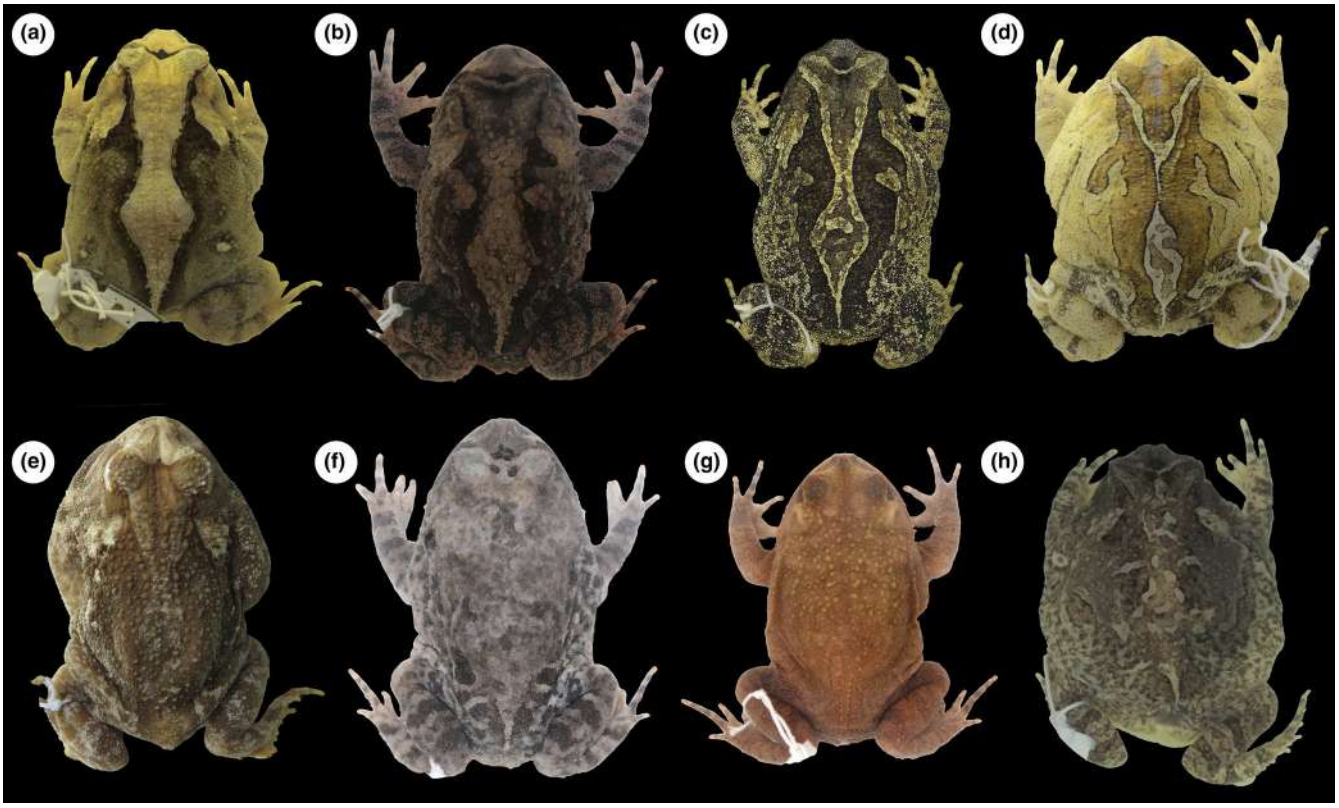

FIGURE 4 Ocular-dorsal ridge of warts in *Proceratophrys* from Caatinga. (1) continuous extending from the edge of the eyelid to the sacral region: (a) *P. cristiceps*, CHUFPB 12126, Poço Redondo municipality, Sergipe State; (b) *P. caramaschii* new synonymy, AAGARDA 10961, Parque Nacional de Ubajara, Ceará State; (c) *P. aridus* new synonymy, AAGARDA 11910, Milagres municipality, Ceará State. (2) interrupted in the presacral constriction: (d) *P. cristiceps*, CHUFPB 10345, Piripiri municipality, Piauí State; (e) *P. caramaschii* new synonymy, MNRJ 16597, Mucuripe municipality, Ceará State; (3) absent: (f) *P. cristiceps*, AAGARDA 10286, Jaguaribe municipality, Ceará State; (g) *P. caramaschii* new synonymy, AAGARDA 10983, Parque Nacional de Ubajara, Ceará State; (h) *P. aridus* new synonymy, MNRJ 55779, Milagres municipality, Ceará State

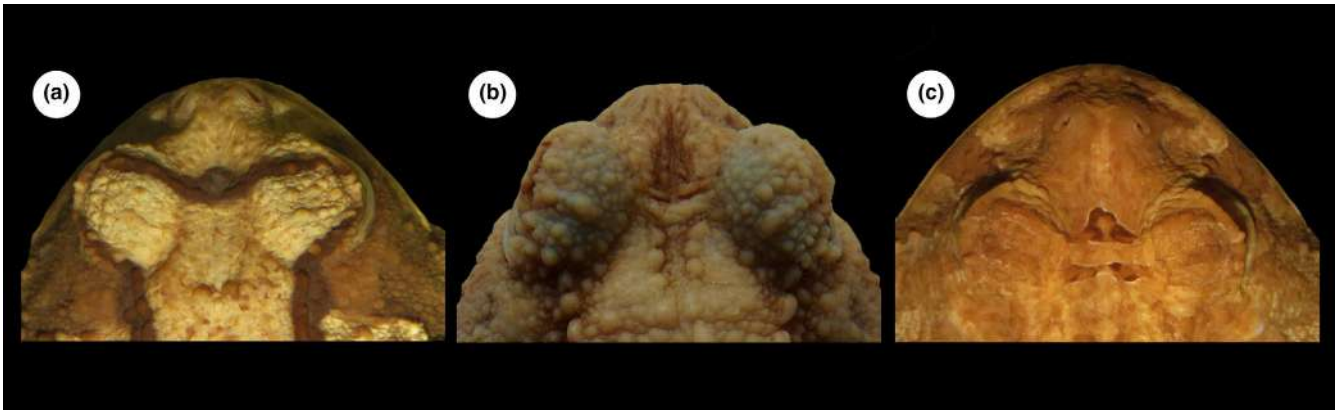

FIGURE 5 Interocular ridge of warts in *Proceratophrys* from Caatinga. (1) one row: (a) *P. caramaschii* new synonymy, MNRJ 1419, Mucuripe municipality, Ceará State. (2) two rows: (b) *P. cristiceps* holotype, NHMB 1503; (c) *P. caramaschii* new synonymy, MNRJ 16487, Mucuripe municipality, Ceará State

compare with three new species of *Proceratophrys* from Cerrado. However, the authors did not formally extend the distribution of *P. caramaschii*. Nunes et al. (2015) extended the geographic distribution of *P. caramaschii* to Ubajara, Ceará State, approximately 300 km far from its type locality, and described the advertisement call from individuals of this same place. The authors also indicated the occurrence of this species in Piauí State (500 km far from the type locality).

To verify the diagnose of *P. caramaschii* from the original description (Cruz et al., 2012), we analyzed the morphology of specimens from Piripiri municipality, Piauí State, along with specimens from Ubajara, Itapipoca, and Aquiraz municipalities, and other localities near to the

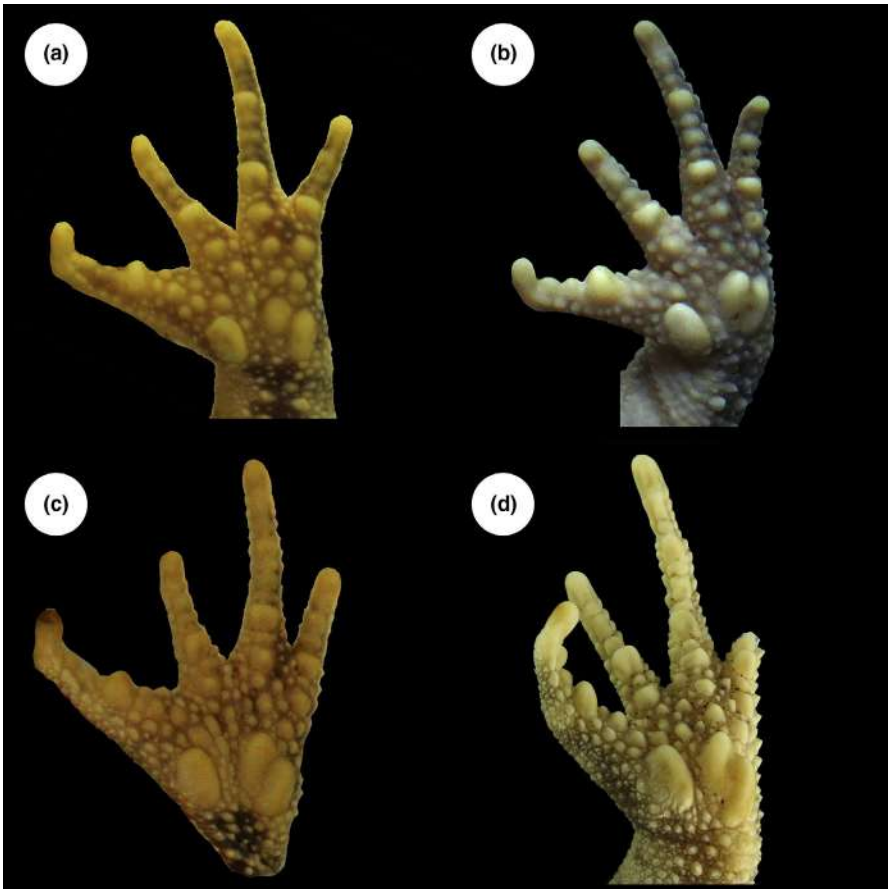

FIGURE 6 Inner part of metacarpal tubercle in *Proceratophrys* from Caatinga. (1) smaller than the outer: (a) *P. cristiceps*, CHUFPB 10344, Piripiri municipality, Piauí, State; (b) *P. aridus* new synonymy, MNRJ 55785, Milagres municipality, Ceará State. (2) similar size of the outer: (c) *P. cristiceps*, CHUFPB 10146, Piripiri municipality, Piauí, State; (d) *P. caramaschii* new synonymy, PRC 002, Aquiraz municipality, Ceará State

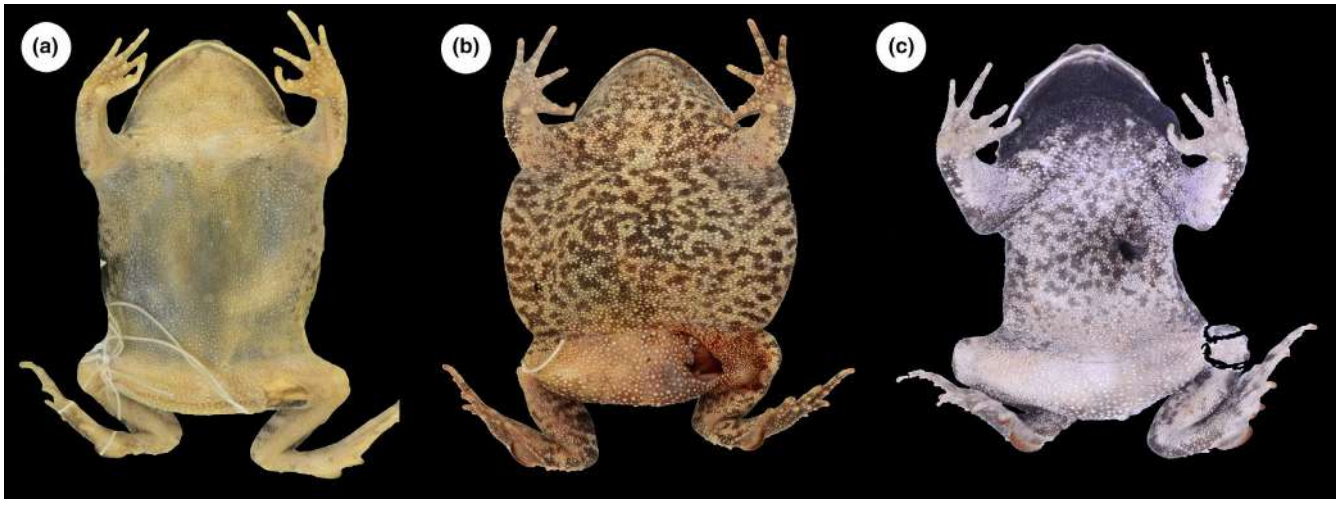

FIGURE 7 Ventral region coloration in *Proceratophrys* from Caatinga (1) cream without blotches: (a) *P. cristiceps*, URCA 5773, Paracuru municipality, Ceará State. (2) cream with brown spots or blotches: (b) *P. caramaschii* new synonymy, AAGARDA 10796, Parque Nacional de Ubajara, Ceará State. (3) males present darkish gular region: (c) *P. cristriceps*, AAGARDA 9809, João Câmara municipality, Rio Grande do Norte State

type locality, in Ceará State, as well as the holotype (MNRJ 16592) and the type series (MNRJ 1419–1420, 1680, 16470–16484, 16487–16489, 16591, 16593–16600). As shown in the Results, the diagnostic characteristics used to describe *P. caramaschii* are within the inter- and intrapopulational variation in these characters for *P. cristiceps*.

Nunes et al. (2015) described the advertisement call of *P. caramaschii* based on recordings from Planalto do Ibiapaba, Ceará State. The authors separate the calls of this species from *P. cristiceps* calls (Nunes & Juncá, 2006, calls from Feira de Santana, Bahia State) due to the lower number of pulses per second (75.7–81.8 pulses/s; 87.4–91.9 in *P. cristiceps*) and by the dominant frequency (860 or 1,030 Hz; 900–990 Hz in *P. cristiceps*). As shown previously, all acoustic parameters overlap when we include calls from different localities (Table 1). Therefore, there are no differences between the calls of *P. caramaschii* and *P. cristiceps*.

TABLE 2 Measurements of specimens of *Proceratophrys aridus* new synonymy, *P. caramaschii* new synonymy, and *P. cristiceps*

	P. cristiceps		*P. aridus* new synonym		*P. caramaschii* new synonym	
	Males	Females	Males	Females	Males	Females
	(*n* = 160)	(*n* = 134)	(*n* = 12)	(*n* = 21)	(*n* = 27)	(*n* = 4)
SLV	34.8 ± 4.4	51.3 ± 6.6	35.9 ± 5.7	35.5 ± 4.3	50.1 ± 4.7	58 ± 9.1
	33.1–53.6	35.1–64.5	26.5–46.2	25.8–42.4	41.6–57.2	47.9–67.1
HW	18.3 ± 1.8	21.6 ± 2.9	14.6 ± 2.0	14.4 ± 2.0	21.9 ± 2.4	24.9 ± 4.3
	14.9–22.6	14.7–27.6	11.6–17.8	11.6–18.1	18.1–25.8	21.1–29.2
HL	12.6 ± 1.1	14.7 ± 1.9	10.6 ± 1.4	10.5 ± 1.4	14.8 ± 1.5	16.9 ± 2.8
	10.4–16.3	10.5–19.0	8.4–12.7	8.1–12.9	12.3–17.0	14.1–19.6
DICS	8.9 ± 1.3	10.3 ± 1.3	7.6 ± 0.9	7.5 ± 1.1	10.4 ± 1.0	12.4 ± 1.8
	1.8–11.2	7.0–13.7	6.1–9.1	5.7–9.5	8.4–12.2	10.5–14.7
IND	2.6 ± 0.5	2.9 ± 1.0	2.4 ± 0.4	2.4 ± 0.3	3.3 ± 0.4	3.5 ± 0.6
	1.5–4.0	1.8–12.3	1.9–3.2	1.9–3.1	2.6–3.9	2.8–4.3
END	3.7 ± 0.4	4.2 ± 0.6	3.2 ± 0.5	3.2 ± 0.4	4.2 ± 0.5	5.0 ± 0.6
	2.8–5.0	3.1–6.6	2.5–4.1	2.4–3.9	3.5–5.3	4.4–5.6
ED	4.7 ± 0.7	5.0 ± 0.8	3.0 ± 0.4	3.1 ± 0.4	5.3 ± 0.7	5.5 ± 1.0
	3.4–7.4	3.3–7.4	2.6–3.0	2.4–3.8	3.7–6.4	4.4–6.5
UEW	5.2 ± 0.6	5.7 ± 0.9	3.8 ± 0.6	3.8 ± 0.4	5.9 ± 0.7	6.1 ± 0.4
	3.9–6.7	0.4–7.4	3.0–4.8	3.1–4.5	4.7–6.8	5.6–6.6
IOD	2.9 ± 0.6	3.0 ± 2.0	3.3 ± 0.6	3.1 ± 0.6	3.7 ± 0.6	4.7 ± 1.1
	1.8–5.0	1.9–19.5	2.7–4.3	2.2–4.4	2.5–5.4	4.0–6.3
THL	17.8 ± 2.2	19.8 ± 3.2	13.5 ± 2.0	13.0 ± 2.3	19.3 ± 2.5	21.6 ± 3.2
	12.8–22.9	13.8–28.1	10.8–17.1	8.9–17.0	13.5–22.7	18.7–25.3

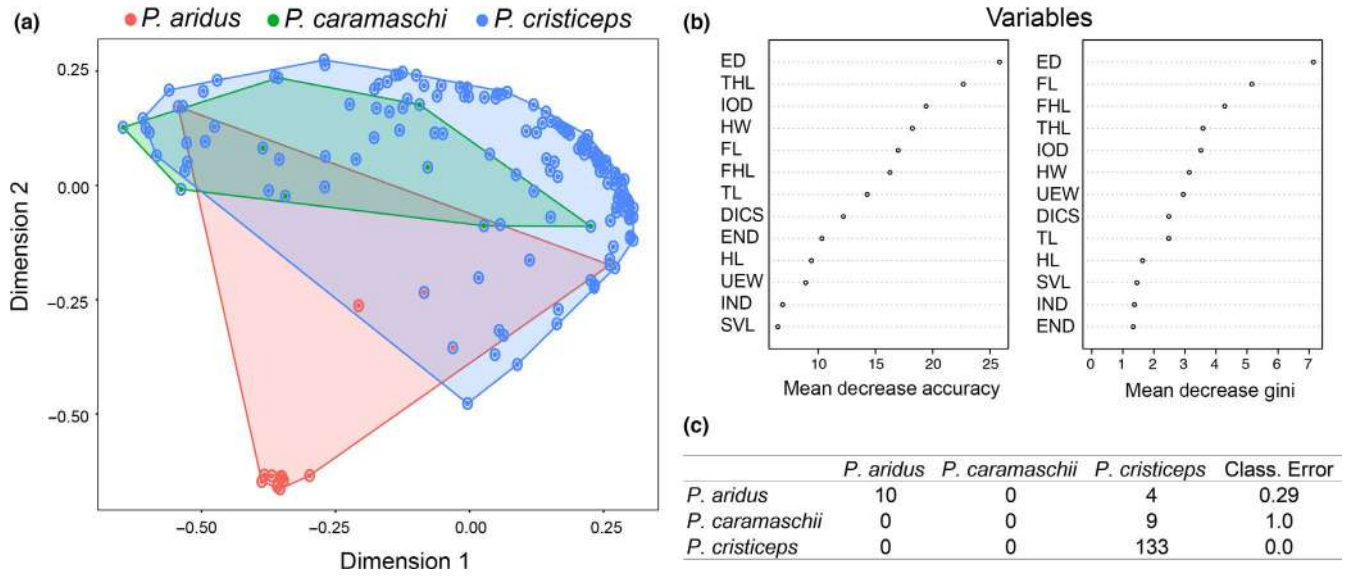

FIGURE 8 Results for the random forest classification based on morphometric variables for *Proceratophrys aridus*, *Proceratophrys caramaschii*, and *Proceratophrys cristiceps*. (a) Plot of the first and second multidimensional scaling axis of the proximity matrix. (b) Dotcharts of variable importance scores. (c) Confusion matrix showing the classification error of the individuals based on the analysis

Lastly, genetic distances between *P. caramaschii* and *P. cristiceps* are very low (0.19% in *16S* mtDNA barcoding) and our analyses of population assignment detected only one population, grouping all three species (Figure S3). Thus, based on this integrative approach, we consider *Proceratophrys caramaschii* Cruz et al., 2012 as a junior synonym of *P. cristiceps* (Müller 1884 "1883").

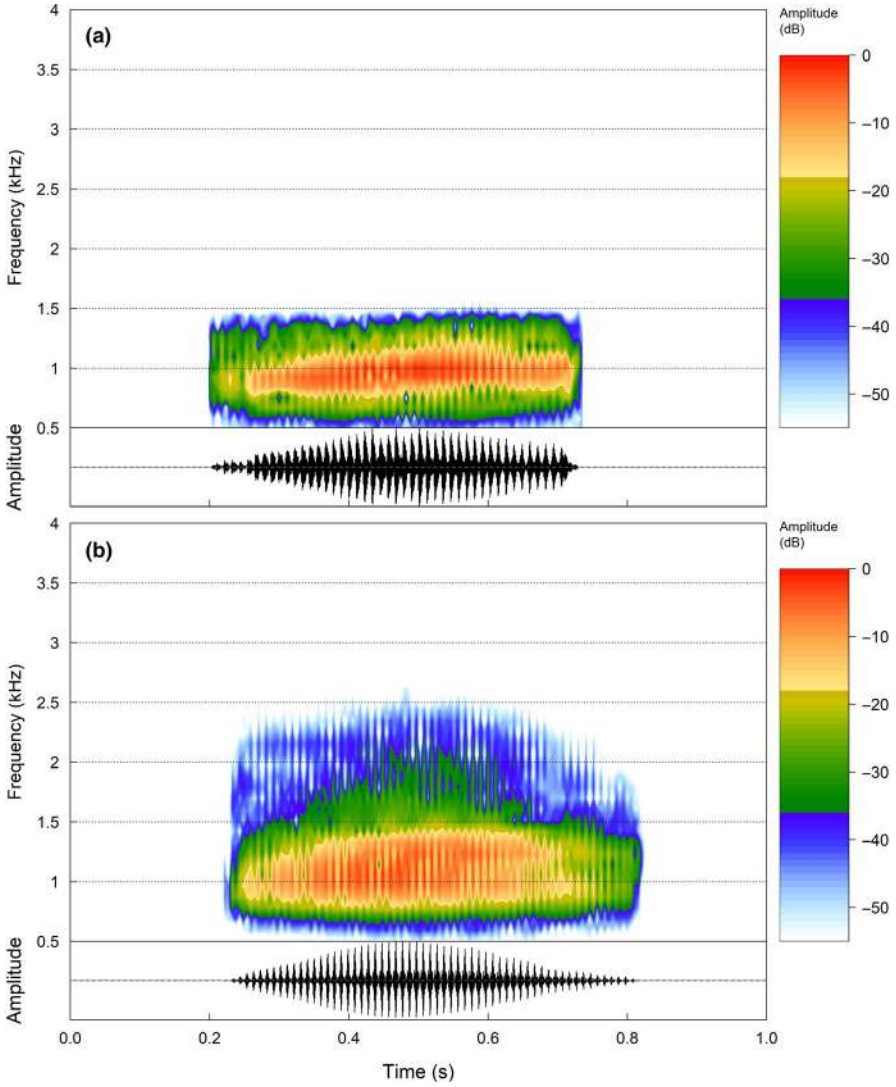

FIGURE 9 Advertisement call of (a) *Proceratophrys cristiceps* (AAGARDA 10176, Jaguaribe municipality, Ceará State) and (b) *Proceratophrys caramaschii*, new synonymy (Parque Nacional de Ubajara, Ceará State)

TABLE 3 Genetic statistics for each locus sequenced for *Proceratophrys aridus* new synonymy, *P. caramaschii* new synonymy, and *P. cristiceps* from the Caatinga biome in Northeastern Brazil

Locus	*L* (bp)	*N*	*S*	*H*	*Hd*	π
16S	485	110	19	19	0.392	0.00112
CRYBA	238	84[a]	5	7	0.706	0.00420
POMC	454	98[a]	6	10	0.708	0.00256
RHO	351	70[a]	5	6	0.565	0.00247

Abbreviations: *H*, number of haplotypes; *Hd*, haplotype diversity; *L*, length in base pairs; *N*, sample size; *S*, number of polymorphic sites; π, nucleotide diversity.

[a]Phased sequence.

5.3 | Redescription of *P. cristiceps* (Müller, 1884 "1883")

Ceratophrys cristiceps Müller, 1883

Stombus cristiceps Miranda-Ribeiro, 1920

Proceratophrys cristiceps Lynch, 1971

Proceratophrys aridus Cruz et al., 2012, S. Am. J. Herpetol., 7:118. Holotype: MNRJ 55782, by original designation. Type locality: "Minador farm, municipality of Milagres (38° 56′ W and 07° 18′ S, 334 m a.s.l.; SAD69 datum), state of Ceará, northeastern Brazil". New synonymy.

Proceratophrys caramaschii Cruz et al., 2012, S. Am. J. Herpetol., 7:117. Holotype: MNRJ 16592, by original designation. Type locality: "Mucuripe, municipality of Fortaleza (03° 43′ S and 38° 29′ W, 334 m at sea level; WGS84 datum), state of Ceará, northeastern Brazil". New synonymy.

Holotype: NHMB 1503, adult female, collected in Brazil, no coordinates, no collector and no date of collecting.

Diagnosis: P. cristiceps is diagnosed by the following combination of characters: (1) medium size (33.1–53.6 mm in adult males, *n* = 199; 35.1–64.5 mm in adult females, *n* = 159); (2) eyelid tubercles fused, short, and round (formulae L 2, 3/5; R 2, 3/5); (3) contact point between the ocular-dorsal ridge of warts and the external eyelid margin tubercles in the posterior third of portion of the eyelid; (4) tubercles in the forearm organized in two rows (one row with enlarged, pointed, and individualized tubercles, and another with short

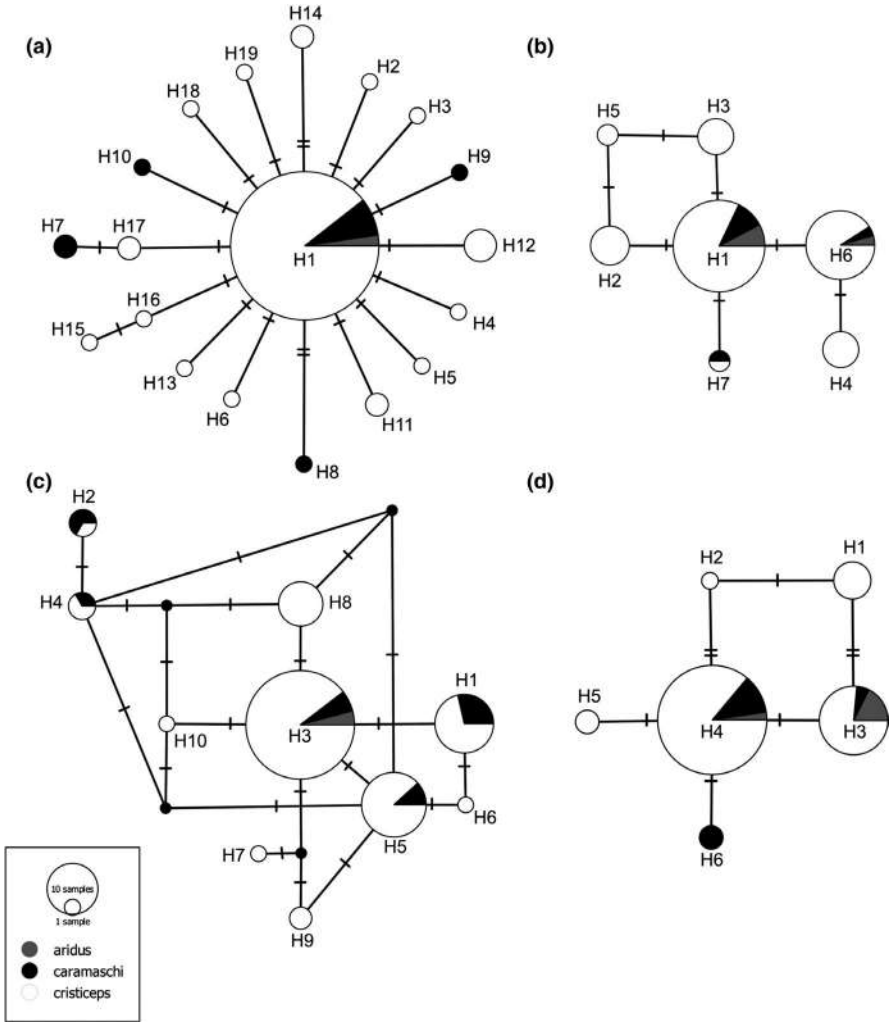

FIGURE 10 Haplotype networks showing the lack of genetic structure among *Proceratophrys aridus* new synonymy (gray), *Proceratophrys caramaschii* new synonymy (black), and *Proceratophrys cristiceps* (white) in the Caatinga biome, Northeastern Brazil, according to each gene. (a) mtDNA *16S*. (b) nuDNA *CRYBA*. (c) nuDNA *POMC*. (d) nuDNA *RHO*

and fused tubercles); (5) ventral region cream with light-brown dots on the gular region and chest; and (6) advertisement call with 0.364–1.011 s in duration (0.548 ± 0.11), 32–85 pulses/note (51 ± 9.75), 69.1–105.3 pulses/s (89.6 ± 7.05), and dominant frequencies of 861.3–1125 Hz (937.5 ± 105.30).

Comparisons with other species (data for other species within parentheses): P. cristiceps is promptly distinguished from *P. appendiculata* (Günther, 1873), *P. belzebul* Dias et al., 2013, *P. boiei* (Wied-Neuwied, 1824), *P. gladius* Mângia et al., 2014, *P. itamari* Mângia et al., 2014, *P. izecksohni* Dias et al., 2013, *P. laticeps* Izecksohn & Peixoto, 1981, *P. mantiqueira* Mângia et al., 2014, *P. melanopogon* (Miranda-Ribeiro, 1937), *P. moehringi* Weygoldt & Peixoto, 1985, *P. paviotii* Cruz, Prado, & Izecksohn, 2005, *P. phyllostomus* Izecksohn, Cruz, & Peixoto, 1999, *P. pombali* Mângia et al., 2014, *P. renalis* (Miranda-Ribeiro, 1920), *P. rondonae* Prado & Pombal, 2008, *P. sanctaritae* Cruz & Napoli, 2010, *P. subguttata* Izecksohn, Cruz, & Peixoto, 1999, and *P. tupinamba* Prado & Pombal, 2008, by the absence of single, uni-cuspidate palpebral and rostral appendages (present in all these species, single, short and multi-cuspidate in *P. rondonae*). *Proceratophrys cristiceps* can also be distinguished from *P. appendiculata*, *P. gladius*, *P. itamari*, *P. izecksohni*, *P. laticeps*, *P. mantiqueira*, *P. melanopogon*, *P. moheringi*, *P. phyllostomus*, *P. pombali*, *P. sanctaritae*, *P. subguttata*, and *P. tupinamba* by lacking a rostral appendage (present in these species). From *P. avelinoi*, *P. bigibbosa*, *P. brauni*, and *P. palustris*, *P. cristiceps* differs by lacking postocular swellings (present).

Proceratophrys cristiceps presents eyelid tubercles fused, short, and rounded (fused with small points in *P. goyana*, *P. strussmannae*, *P. carranca*, *P. branti*, and *P. concavitympanum*; small, rounded, and not fused in *P. cururu* and *P. rotundipalpebra*; slightly fused without appendage in *P. huntingtoni*, *P. vielliardi*, and *P. moratoi*; conical and pointed in *P. bagnoi*; enlarged, pointed and with the largest central tubercle more projected than lateral ones in *P. minuta*; small and rounded in *P. redacta*; multiple short and pointed expansions in *P. schirchi*). *Proceratophrys cristiceps* differs from *P. bagnoi*, *P. concavitympanum*, *P. dibernardoi*, and *P. goyana* by having tubercles on the forearm organized in two rows, one row with enlarged, not fused and pointed tubercles, and the other with short, fused tubercles (two rows in *P. bagnoi*, *P. concavitympanum*, and *P. dibernardoi*; tubercles not organized in rows in *P. goyana*). From *P. ararype*, *P. cristiceps* differs by the cream-colored ventral region with light-brown dots on the gular region and chest (dark-brown mottling on the gular region, chest, and belly in *P. ararype*). *Proceratophrys cristiceps* is similar in size (medium size, SVL 33.1–53.6 mm in adult males, 35.1–64.5 mm

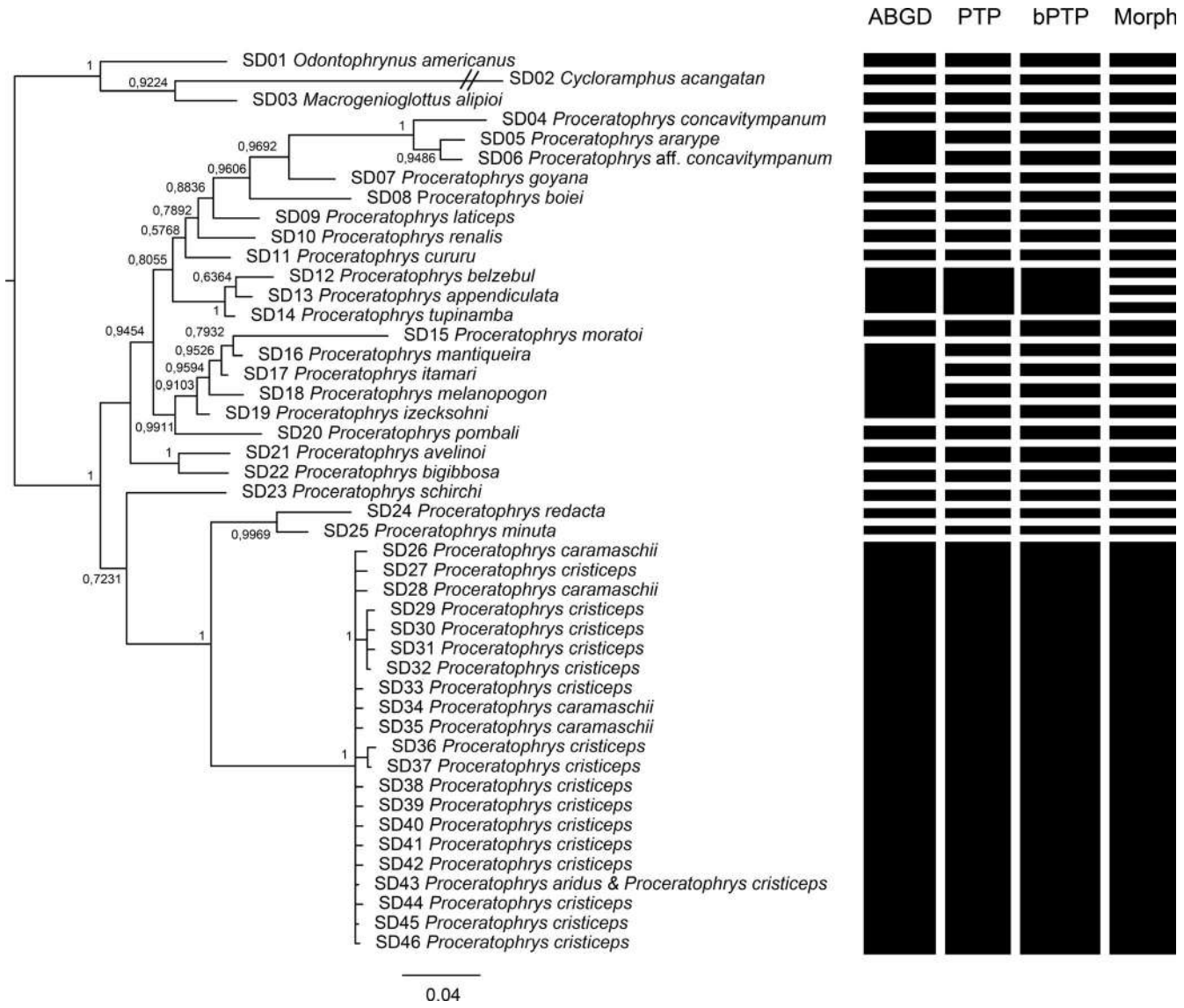

FIGURE 11 Concatenated tree (*16S* and *RHO*) recovered by Bayesian analysis in MrBayes. Posterior probabilities are given near the nodes. Black bars represent each species delimited by the following methods: ABGD (automatic barcoding gap discovery), PTP (poisson tree process), bPTP (Bayesian implementation of the poisson tree process), and Morph (Morphologic)

in adult females) to congeners from the *P. cristiceps* group, except for males of *P. dibernardoi* and *P. moratoi* that are smaller (SVL 28.8–34.6 mm and 25.8–31.0 mm, respectively). *Proceratophrys cristiceps* differs from some species of the *P. cristiceps* group in advertisement call (first values in parentheses correspond to *P. cristiceps*): longer duration (0.364–1.011s; 0.045–0.195 in *P. goyana*, 0.200–0.320 in *P. huntingtoni*, 0.050–0.200 in *P. rotundipalpebra*, 0.146–0.335 in *P. moratoi*, and mean of 0.059 in *P. vielliardi*), higher number of pulses per call (32–85; 5–21 in *P. carranca*, 19–25 in P. *huntingtoni*, 5–19 in *P. rotundipalpebra*, 12–26 in *P. moratoi*, and 3–20 in *P. vielliardi*), lower number of pulses per second (69.1–105.3; 109.9–111.1 in *P. carranca*, and 100.0–119.3 in *P. concavitympanum*), higher number of pulses per second (69.1–105.3; 45 in *P. cururu*, and 7–18 in *P. goyana*), and lower dominant frequency (861.3–1125; 1153–1594 in *P. moratoi*).

Tadpole: Described by Vieira, Vieira, and Gomes-Santana (2007) based on tadpoles collected at Estação Experimental de São João do Cariri, in São João do Cariri municipality (7°29′34″S, 36°41′53″W), Paraíba State, Brazil. Individuals were deposited at the Museu Nacional do Rio de Janeiro (MNRJ 41840) and Coleção Herpetológica do Departamento de Sistemática e Ecologia, Universidade Federal da Paraíba (CHUFPB 4315).

Advertisement call: Nunes and Juncá (2006) described the advertisement call of *Proceratophrys cristiceps* based on calls of two individuals from Serra de São José, Feira de Santana municipality, Bahia State, Brazil. In the present work, we present a redescription of the advertisement call of *P. cristiceps* based on calls from 14 other localities. The advertisement call of *P. cristiceps* presents a duration of 0.354–0.955 s, with 32–113 pulses/note, an emission rate of 71.4–124 pulses/s, and dominant frequency of 860–1205.9 Hz (see Table 1 for details).

Variation: We discuss variation in the present study in the topics "Morphological and morphometric assessment" and "Advertisement

call." Descriptive statistics of morphometric variables from adults are presented in Table 2, while call characteristics are detailed in Table 1.

Distribution: Proceratophrys cristiceps occurs in the Caatinga biome and adjacent areas (Cerrado biome to the west and Atlantic Forest biome to the east) (Figure 1).

5.4 | *Proceratophrys cristiceps* type locality

Von F. Müller, in 1883, when cataloging the amphibians and reptiles from the Naturhistorisches Museum, Basel, Switzerland, described *Ceratophrys cristiceps* (= *P. cristiceps*) based on an adult female. The author briefly characterized the species and determined its type locality as "Brasilien" (Brazil). Miranda-Ribeiro (1920) allocated *Ceratophrys cristiceps* in the genus *Stombus* due to the absence of external cranial ossification, and Nieden (1923) returned to the genus *Ceratophrys* and provided some data on morphology and coloration, while Forcart (1946) appointed the number NHMB1503 as the holotype. Finally, Lynch (1971) reallocated *Ceratophrys cristiceps* to *Proceratophrys*.

Proceratophrys cristiceps was described without a specific type locality, and there is no other information about the holotype in the Naturhistorisches Museum (Urs Wüest, *pers. comm.*). Thus, we are not able to follow the steps of the collector to attempt to identify where the individual was collected. We tried to associate one population of *P. cristiceps* from all over its distribution to the morphotype of the holotype to define the type locality. However, based on just one female individual (the holotype), and because *P. cristiceps* presents a large morphological variation (see above), we cannot relate the holotype to a specific population. Therefore, we define the type locality of *P. cristiceps* to the Caatinga biome and adjacent areas, in all its geographic distribution (Article 76, Recommendation 76A.1.4.—ICNZ, 1999) (Figure 1).

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ORCID

Sarah Mângia https://orcid.org/0000-0003-0038-123X
Diego José Santana https://orcid.org/0000-0002-8789-3061
Adrian Antonio Garda https://orcid.org/0000-0002-1178-1207

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Bayesian gene trees. (a) *16S*; (b) *CRYBA*; (c) *POMC*; and (d) *RHO*.

Figure S2. Maximum likelihood gene trees. (a) *16S*; (b) *CRYBA*; (c) *POMC*; and (d) *RHO*.

Figure S3. Plot of the number of populations simulated from the posterior distribution obtained with GENELAND for *Proceratophrys aridus* new synonymy, *P. caramaschii* new synonymy and *P. cristiceps* in the Caatinga biome, Northeastern Brazil.

Table S1. *Proceratophrys cristiceps* species group: authority and habitat.

Table S2. Samples of *Proceratophrys aridus*, *P. caramaschii*, and *P. cristiceps* used in this study. For each species is presented locality, state, coordinates and the material analyzed (morphology, call and/or tissue).

Table S3. Samples of *Proceratophrys aridus*, *P. caramaschii*, and *P. cristiceps* used in this study. For each sample locality, state, and mtDNA and nuDNA haplotypes are presented (see Figure 9). Number 2 between parentheses indicates homozygous individuals.

Table S4. Information on primers used in the present study.

Table S5. Specimens used in the molecular analyses of this study, including GenBank numbers for mitochondrial *16S* and nuclear *CRYBA*, *POMC* and *RHO* sequences. SD = haplotype code used in "Species delimitation" analysis.

Table S6. Uncorrected *p*-distances of *16S* mitochondrial gene for the genus *Proceratophrys*.

Alignment S1. Alignment used to construct the mitochondrial *16S* gene tree.

Alignment S2. Alignment used to construct the nuclear *CRYBA* gene tree.

Alignment S3. Alignment used to construct the nuclear *POMC* gene tree.

Alignment S4. Alignment used to construct the nuclear *RHO* gene tree.

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APPENDIX 1
SPECIMENS EXAMINED

We examined specimens housed in the following institutions: Coleção Herpetológica da Universidade Federal da Paraíba (UFPB-CHUFPB); Núcleo Regional de Ofidiologia da Universidade Federal do Ceará (NUROF-UFC); Coleção Herpetológica da Universidade Federal do Piauí (CHUFPI); Coleção Herpetológica da Universidade Federal de Pernambuco (CHUFPE); Coleção Herpetológica da Universidade Federal de Alagoas (CHUFAL); Museu de Zoologia da Universidade Federal da Bahia (MZUFBA); 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, Rio de Janeiro, Universidade Federal do Rio de Janeiro (MNRJ); Coleção de Herpetologia da Universidade Regional do Cariri (URCA-H); and Coleção Zoológica da Universidade Federal de Mato Grosso do Sul (ZUFMS).

Proceratophrys appendiculata

BRAZIL: SÃO PAULO: Cunha: CFBH 10751–10752; Mococa: CFBH 12150, 12709; São Luiz do Paraitinga: CFBH 5820, 6489, 8062, 9887; Santa Virgínia: CFBH 16614; Ubatuba: CFBH 4324, 5414, 5660, 8410–8411.

Proceratophrys ararype

BRAZIL: CEARÁ: Crato: CHUFPE 156 (holotype), CHUFPE 160–161, CHUFPE 152, CHUFPE 226–227, URCA83-84, URCA-H 1030, URCA-H 1031–34 (paratopotypes), AAGARDA 2736, AAGARDA 2741, URCA-H 114, URCA-H 4028–29 (paratypes).

Proceratophrys aridus **new synonym**

BRAZIL: CEARÁ: Milagres: MNRJ 55349, 55778–822, 75156–68 (paratopotypes); URCA-H 106, 142–43; AAGARDA 11910 (topotype).

Proceratophrys avelinoi

BRAZIL: PARANÁ: Toledo: ZUFMS-AMP 994.

Proceratophrys boiei

BRAZIL: MINAS GERAIS: Divino: ZUFMS-AMP 5651, 5666, 5680.

Proceratophrys branti

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

Proceratophrys caramaschii **new synonym**

BRAZIL: CEARÁ: Fortaleza: Mucuripe: MNRJ 1419–20, 1680, 16470–84, 16487–89, 16591–600 (paratopotypes). Ubajara, Parque Nacional de Ubajara: AAGARDA 10672, 10695, 10698–99, 10703, 10707–09, 10782, 10796, 10907, 10909, 10911–14, 10961, 10974, 10981, 10983.

Proceratophrys concavitympanum

BRAZIL: MATO GROSSO: Alta Floresta: ZUEC 21201. Apiacás: UFMT 7906. Aripuanã: UFMT 11697, 11699; MZUFV 9552, 9554–56. Colniza: UFMT 6808. Juína: UFMT 6996, 7825. Paranaíta: UFMT 7534, 7963, 9882, 9990, 10038, 10041, 10046, 10054, 10067, 10109; CFBH 20675–77; UFMS LA03, LA34, LA35, LA43, LA69; ZUEC 14505–06, 16011–15, 16719, 21201. Vila Bela da Santíssima Trindade: UFMT 4105. PARÁ: Canaã dos Carajás: MNRJ 90328. Parauapebas: MNRJ 90327; PUC-MG 10561, 11206. Jacareacanga: ZUEC 14505–06. Vitória do Xingú: UFMG 18351–57. RONDÔNIA: Espigão do Oeste: CFBH 5136; MZUFV 10477 (topotype). Ministro Andreaza: CFBH 19818. TOCANTINS: Araguaína: UFMS Z36-37. Porto Nacional: UFMS 1146–47. Wanderlândia: CFBH 28521.

Proceratophrys cristiceps

BRAZIL: NHMB1503 (holotype; photo). ALAGOAS: Olho D'água do Casado: UFAL 8168–70. Piranhas: UFBA 8–9, 43. Traipu: Serra da Mão: UFAL 8968, 9035–36, 9043, 9196, 9510, 9656. BAHIA: Caetité: UFMG 5851. Paulo Afonso: UFPB 12114, 12119, 12122–23, 12128. CEARÁ: Aiuaba: AAGARDA 5111, 5132; URCA-H 7366, 7385, 7393, 7396, 7408, 7416, 7418. Barbalha: URCA-H 4293, 4571. Baturité: UFC3722. Crateús: URCA-H 4744. Crato: AAGARDA 2735, 2737–40. General Sampaio: UFC 5351. Itapipoca: AAGARDA 9817, 10453–55. Ipu: UFPB 6117–19, 6121, 6123, 6125. Jaguaribe: AAGARDA 10176–79, 10286, 10398–402. Pacajus: UFC 4562. Paracuru: URCA-H 5773–74. Pentecoste: UFC 5001, 5018–19, 5193. São Gonçalo do Amarante: URCA-H 5669, 5775, 5860. Santa Quitéria: UFPB 10651, 10753–58. Serra das Almas: UFC 32, 131, 213, 224, 3319, 3464, 3467–68, 3470. Serra de Ibiapaba: UFPB 6117–26. Várzea da Conceição: UFPB 9661, 9665, 9667. PARAÍBA: Araruna: UFPB 8427, 8438, 8447, 8451, 8453, 8456, 8465, 8467, 8469, 8487. Boa vista: UFPB 1573–81. Cabaceiras: UFPB 6691–94, 11271, 11274. São José dos Cordeiros: UFPB 5866. PERNAMBUCO: Arcoverde: UFPB 9678–82, 9684, 9686–88, 9692, 9701. Betânia: UFC 3331. Bezerros: UFPB 7098. Exu: URCA 1462–63; UFPB 7214–17. Nascente: UFPB 9670. Ouricuri: URCA 2988–89. Buíque, Parque Nacional do Catimbau: AAGARDA 7706–12, 7747, 7760–61, 7765, 7799, 7802, 7804–05, 7824, 7886, 7975, 8056, 8362, 8417, 8435, 8437–40, 8450, 8463. Serra Talhada: UFPB 9656, 9659, 9660. Trindade: UFPB 974, 9673–77. PIAUÍ: Floriano: UFPI 214–16, 222, 236. Piripiri: UFPB 10340, 10342–46. RIO GRANDE DO NORTE: Serra Negra do Norte, Estação Ecológica do Seridó: AAGARDA 5447, 5528, 5583, 5689, 6061, 6790. João Câmara: AAGARDA 8913–15, 9806–11; URCA 422, 427, 483–85, 487–88, 493, 498, 501. Macaíba, Escola Agrícola de Jundiaí: AAGARDA 1013–14, 1019–20, 1753–71, 1773, 1776, 1778, 1786–91, 1935, 2495–96, 2583, 3757, 5447, 5528, 5554, 5583, 5689, 6061, 6790, 8866–71, 8913–15, 9806–11. SERGIPE: Poço Redondo: UFPB 12120–21, 12125–27.

Proceratophrys dibernardoi

BRAZIL: MATO GROSSO DO SUL: Campo Grande: ZUFMS-AMP 3320–3321, 3527, 3540.

Proceratophrys gladius

BRAZIL: SÃO PAULO: São José do Barreiro, Campo de Fruticultura: MZUSP 96345 (holotype), MZUSP 96336–39, 96341–96342, 96347, MNRJ 82577–82579 (paratopotypes).

Proceratophrys goyana
BRAZIL: TOCANTINS: Arraias: ZUFMS-AMP 12550–12554.

Proceratophrys itamari
BRAZIL: SÃO PAULO: Campos do Jordão, Cidade Azul: MZUSP 14931–14932, 14934; Eugênio Lefevre: MZUSP 11330, 14905. MINAS GERAIS: Passa Quatro: MNRJ 41872.

Proceratophrys laticeps
BRAZIL: ESPÍRITO SANTO: Aracruz: CFBH 4185; Cariacica: CFBH 23609, 23611, 23613–23615; Santa Teresa: MNRJ 30905, 30885–30887, 56018–56020; Sooretama, Paraju: CFBH 14925, 14936, 14944.

Proceratophrys mantiqueira
BRAZIL: MINAS GERAIS: Aiuruoca: MZUSP 142433; Conceição de Ibitipoca: CHUFMG 534–536, MZUFV 6859, 9044; Ervália, Parque Estadual Serra do Brigadeiro: MZUFV 4303 (cleared and stained), 6795 (cleared and stained), 6813 (cleared and stained), 6389, 6390, 6391 (cleared and stained), 6711 (cleared and stained), 6712, 6858, 7333–7334). RIO DE JANEIRO: Itatiaia: CCLZU 2883, 2885–2886, CFBH 5774, MZUSP 4137, 76407–76408, 7754, 4119–4120, ZUEC 13353–13354; Piquete, região do Pico dos Marins: CCLZU 2869–2870, 2873, 2887, 2895–2906, 2967–2968, 2970–2975.

Proceratophrys melanopogon
BRAZIL: RIO DE JANEIRO: Macaé de Cima: MNRJ 34020, 40714, 64229; Nova Friburgo: MNRJ 51363; Santa Maria Madalena, Parque Estadual do Desengano: MNRJ 41874, 41937, 54017; Visconde de Mauá: MZUSP 68953. SÃO PAULO: Alto da Serra, Paranapiacaba: MNRJ 293, 5283–5284; Barra do Una: MZUSP 139116; Bertioga, Parque das Neblinas: CHUFMG 529–533, MZUSP 135740–135742; Campo de Fruticultura da Bocaina: DZSJRP 12085–12086, MZUSP 31357, 53036–53043, 53045–53046, 53048–53051; Estação Biológica de Boracéia: MZUSP 950, 3508, 4000, 23385, 31352, 31358, 68994, 68997, 69216, 137463–137465; Franca: MZUSP 612; Mongaguá: ZUEC 3897 (photo); Paranapiacaba: CFBH 867, ZUEC 6895; Peruíbe: CFBH 24010; Salesópolis: MZUSP 70431; São Paulo: MZUSP 931, 1035; Santa Virgínia: ZUEC 12082; São Luiz do Paraitinga: CFBH 12295, 16288, 16289 (cleared and stained); São José do Barreiro: ZUEC 6807–6808; São José dos Campos: CCLZU 658, 835–838; São Sebastião, Parque Estadual da Serra do Mar: MZUSP 135333–135334.

Proceratophrys minuta
BRAZIL: BAHIA: Miguel Calmon, Parque Estadual das Sete Passagens: UFBA 6229–30, 6716–20, 6722, 6725–26 (paratypes).

Proceratophrys pombali
BRAZIL: SÃO PAULO: Itanhaém, Parque Estadual da Serra do Mar: CFBH 15982 (holotype), CFBH 15983 (paratopotype); MZUSP 69286, 148085, 148114 (paratypes).

Proceratophrys redacta
BRAZIL: BAHIA: Morro do Chapéu: PUC-MG 7910–11, 7913; UFMG 6049–57.

Proceratophrys schirchi
BRAZIL: MINAS GERAIS: Santa Maria do Salto: PUC-MG 402.

Proceratophrys strussmannae
BRAZIL: MATO GROSSO: Araputanga: UFMT 7879; Vale de São Domingos: UFMT 1834, 1836, 7882, 7885, 8319, 8320, 8377, 8380 (paratypes); Vila Bela da Santíssima Trindade: UFMT 4105.

Proceratophrys subguttata
BRAZIL: SANTA CATARINA: Anitápolis: CFBH 20268; Humboldt: MNRJ 290 (paratype); Joinville: MNRJ 2293 (paratype); São Bento do Sul: CFBH 4435.

APPENDIX 2

Taxonomic background—Evaluation of the Diagnostic characters of *P. aridus* and *P. caramaschii* based on comparisons across the Caatinga and neighboring Biomes

We compared some diagnostic characteristics available in the literature with the morphological analysis of 358 specimens encompassing the three species. Cruz et al. (2012) used the following characters to separate *P. aridus* from *P. cristiceps*: (1) wider head (HL/HW 86%–96% in *P. aridus* and 77%–84% in *P. cristiceps*)—we measured the *P. aridus* type series and the values of the relation HL/HW overlap with *P. cristiceps* (67%–83% in *P. aridus* and 56%–77% in *P. cristiceps*); (2) triangular snout in dorsal view (nearly rounded in *P. cristiceps*)—in the present work we defined two shapes of snout to *P. cristiceps* all over its distribution (rounded or triangular). We also observed individuals of the type series of *P. aridus* presenting snout rounded on dorsal and ventral views; (3) skin texture of small granules (several warts in *P. cristiceps*)—although the type series of *P. aridus* presents small granules on the dorsal skin, we observed individuals of *P. cristiceps* with the same pattern; and (4) presence of one interocular transverse crest of tubercles in *P. aridus* (two crests in *P. cristiceps*)—we observed the presence of two interocular transverse crests of tubercles only on two individuals of *P. cristiceps* (the holotype and one individual from Piripiri, Piauí State), and in some individuals of *P. caramaschii*.

Cruz et al. (2012) distinguished *P. caramaschii* from *P. cristiceps* by the following combination of characters: (1) absence of tubercles on the snout and top of the head (present in *P. cristiceps*)—we have not observed tubercles on the snout and top of the head in individuals of *P. aridus*, *P. caramaschii*, and *P. cristiceps*; (2) presence of one interocular transverse crest of tubercles (two in *P. cristiceps*)—we observed the presence of two interocular transverse crests of tubercles only on two individuals of *P. cristiceps* (the holotype and one individual from Piripiri municipality, Piauí State), and in some individuals of *P. caramaschii*; (3) presence of pronounced frontoparietal crest with depression between them (frontoparietal smooth in *P. cristiceps*)—this crest varies from pronounced to indistinct on *P. cristiceps* and *P. caramaschii*, and because it is difficult to define which crest is very or poorly pronounced, we did not use this character; (4) the inner part of metacarpal tubercle bigger than the outer (the inner part smaller than the outer in *P. cristiceps*)—we identify here that the inner part of metacarpal tubercle of *P. cristiceps* can be smaller or similar

size to the outer. Individuals of *P. caramaschii* type series present the same pattern of *P. cristiceps*, and the inner part of metacarpal tubercle is never bigger than the outer. Also, only four individuals from all *P. cristiceps*' distribution present the inner part smaller than the outer; and (5) few bigger blotches on venter (larger and variable scattered small blotches in *P. cristiceps*)—in the present study we identified two patterns of ventral region coloration of *P. cristiceps*: cream without blotches or cream with brown spots and/or blotches in various sizes and shapes. Therefore, the diagnostic characteristics used to describe *P. caramaschii* are within the inter- and intrapopulational variation in these characters for *P. cristiceps*.