

SARAH MÂNGIA BARROS

SISTEMÁTICA E BIOGEOGRAFIA DO COMPLEXO DE ESPÉCIES *PROCERATOPHRYS*
CRISTICEPS (ANURA, ODONTOPHRYNIDAE)



João Pessoa
Paraíba – Brasil
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SISTEMÁTICA E BIOGEOGRAFIA DO COMPLEXO DE ESPÉCIES *PROCERATOPHRYS*
CRISTICEPS (ANURA, ODONTOPHRYNIDAE)

Tese apresentada à Universidade Federal da Paraíba, como parte das exigências do Programa de Pós-Graduação em Ciências Biológicas (Zoologia), para obtenção do título de *Doctor Scientiae*.

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1 **Ata da 101ª Apresentação e Banca de Defesa**
2 **de Doutorado de Sarah Mângia Barros**
3

4 Ao(s) treze dias do mês de março de dois mil e dezessete, às 14:00 horas, no(a) Sala do PPGCB,
5 da Universidade Federal da Paraíba, reuniram-se, em caráter de solenidade pública, membros da
6 banca examinadora para avaliar a tese de doutorado de **Sarah Mângia Barros**, candidato(a) ao
7 grau de Doutor em Ciências Biológicas. A banca foi composta pelos seguintes
8 professores/pesquisadores: **Dr. Adrian Antonio Garda (orientador), Dr. Pedro Cordeiro**
9 **Estrela de Andrade Pinto (titular), Dr. Márcio Bernardino da Silva (titular), Dr. Sérgio Maia**
10 **Queiroz Lima (titular) e Dr. Pedro Murilo Sales Nunes (titular)**. Compareceram à solenidade,
11 além do(a) candidato(a) e membros da banca examinadora, alunos e professores do PPGCB. Dando
12 início à sessão, a coordenação fez a abertura dos trabalhos, apresentando o(a) discente e os
13 membros da banca. Foi passada a palavra para o(a) orientador(a), para que assumisse a posição de
14 presidente da sessão. A partir de então, o(a) presidente, após declarar o objeto da solenidade,
15 concedeu a palavra a **Sarah Mângia Barros**, para que dissertasse, oral e sucintamente, a respeito
16 de seu trabalho intitulado "**Sistemática e Biogeografia do complexo de espécies *Proceratophrys***
17 ***cristiceps* (Müller, 1983) (Anura, Odontophrynidae)**". Passando então a discorrer sobre o aludido
18 tema, dentro do prazo legal, o(a) candidato(a) foi a seguir arguido(a) pelos examinadores na forma
19 regimental. Em seguida, passou a Comissão, em caráter secreto, a proceder à avaliação e
20 julgamento do trabalho, concluindo por atribuir-lhe o conceito Aprouado.
21 Perante a aprovação, declarou-se o(a) candidato(a) legalmente habilitado(a) a receber o grau de
22 **Doutor em Ciências Biológicas**, área de concentração **Zoologia**. Nada mais havendo a tratar eu,
23 **Dr. Adrian Antonio Garda**, como presidente, lavrei a presente ata que, lida e aprovada, assino
24 juntamente com os demais membros da banca examinadora.

25
26 João Pessoa, 13/03/2017.

27 Adrian A. Garda
28 Dr. Adrian Antonio Garda (orientador)

29
30 Sérgio Maia Queiroz Lima
31 Dr. Sérgio Maia Queiroz Lima (titular)

32 Pedro Cordeiro Estrela de Andrade Pinto
33 Dr. Pedro Cordeiro Estrela de Andrade Pinto (titular)

34 Pedro M. Sales Nunes
35 Dr. Pedro Murilo Sales Nunes (titular)

36 Márcio Bernardino da Silva
37 Dr. Márcio Bernardino da Silva (titular)

38
39 Ciente do Resultado:

40 Sarah Mângia Barros
41 Sarah Mângia Barros

“Nada a temer
Senão o correr da luta
Nada a fazer
Se não esquecer o medo
Abrir o peito à força
Numa procura
Fugir às armadilhas da mata escura

Longe se vai sonhando demais
Mas onde se chega assim
Vou descobrir o que me faz sentir
Eu, caçador de mim”

Caçador de mim – Milton Nascimento

Agradecimentos

Fiz questão de fechar os olhos e relembrar todos os momentos (ora felizes, ora desesperadores) que passei durante os quatro anos de Doutorado. Eu aprendi que não se faz ciência sozinha. Fazer parcerias torna o trabalho menos árduo e gera resultados mais completos e de melhor qualidade. Eu aprendi que é possível recarregar as energias com apenas uma palavra/companhia de um bom amigo ou familiar. E que sem estes colaboradores, tanto profissionais da área, quanto pessoas queridas que buscavam amenizar essa minha caminhada, eu não conseguiria alcançar os resultados que apresento aqui nesta tese.

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Resumo

BARROS, Sarah Mângia, *D. Sc.*, Universidade Federal da Paraíba, Março de 2017. **Sistemática e biogeografia do complexo de espécies *Proceratophrys cristiceps* (Anura, Odontophrynidae)**. Orientador: Adrian Antonio Garda.

A Caatinga é um bioma no nordeste do Brasil caracterizado por uma vegetação decídua, xerófito e espinhosa, com cactos, arbustos e pequenas árvores além de precipitação esporádica. Atualmente, este domínio abriga 56 espécies de anfíbios, porém, novas espécies típicas da Caatinga são continuamente descritas, incluindo espécies restritas aos Brejos de altitude, o que indica que a riqueza da região ainda está subestimada. O gênero *Proceratophrys* compreende 40 espécies divididas em grupos e complexos morfológicos sem corroboração filogenética. Com base em estudos recentes, definimos o complexo *P. cristiceps* constituído por seis espécies: *P. cristiceps*, *P. minuta*, *P. redacta*, *P. schirchi*, *P. caramaschii* e *P. aridus*. O presente estudo teve como objetivo avaliar a taxonomia, biogeografia e evolução das espécies relacionadas ao complexo *P. cristiceps*, combinando características morfológicas, morfométricas, acústicas e análises moleculares *multilocus*, com inferência bayesiana. Nossos resultados, baseados nessas análises integrativas, mostraram que *P. aridus*, *P. caramaschii* e *P. cristiceps* pertencem a uma mesma linhagem e não apresentam distinção morfológica e acústica. Dessa forma, nós sinonimizamos as duas primeiras espécies com *P. cristiceps*. Também encontramos uma população proveniente da Chapada do Araripe, previamente chamada de *P. cristiceps*, relacionada com a espécie amazônica *P. concavitympanum*, que aqui descrevemos como uma espécie nova para o gênero. Esta nova espécie se encaixa na categoria Ameaçada da IUCN, uma vez que ocorre em uma pequena área (ca. 3.100 km²) severamente impactada pelo desmatamento e canalização de riachos locais para agricultura. Nós também revisamos as populações de *Proceratophrys* que ocorrem em áreas de altitude na Chapada Diamantina e descrevemos duas novas espécies com base em caracteres da morfologia, morfometria e no relacionamento filogenético (usando um marcador nuclear e um mitocondrial). Verificamos que cada linhagem na Chapada Diamantina corresponde a diferentes ilhas de altitude, separadas por vales com temperaturas mais quentes, o que poderia ter agido como barreira entre estas populações, propiciando diversificação alopátrica entre elas. Portanto, nós consideramos cada linhagem como uma espécie distinta, exceto a população do município de Pindobaçu, que requer a análise de mais exemplares e a utilização de mais marcadores moleculares para definir com mais precisão seu *status* taxonômico. Por fim, nós conduzimos análises filogeográficas *multiloci*, para avaliar a diversidade genética, estruturação geográfica e demografia histórica de *P. cristiceps*. Nossos resultados mostraram *P. cristiceps* constituído por apenas uma população amplamente distribuída na Caatinga, com baixa diversidade genética para todos os genes avaliados (16S mtDNA, *CRYb*, *POMC* e *rhodopsina nuDNAs*). Nossas análises para avaliar a demografia histórica de *P. cristiceps* revelaram que a espécie sofreu uma recente expansão, coincidindo com as flutuações climáticas do Pleistoceno.

Palavras-chave: Anfíbios, Taxonomia integrativa, Filogeografia, Molecular, Diversificação.

Abstract

BARROS, Sarah Mângia, *D. Sc.*, Universidade Federal da Paraíba, March de 2017. **Systematic and biogeography of the species complex *Proceratophrys cristiceps* (Anura, Odontophrynidae)**. Adviser: Adrian Antonio Garda.

The Caatinga is a biome in northeastern Brazil characterized by deciduous xerophytic and thorny vegetation, and by a severe water deficit caused by intense and unpredictable dry seasons. Currently, the Caatinga harbors 56 species of amphibians. However, new species from this domain are being continuously described, including taxa restricted to rainforest natural enclaves (called “*Brejos de altitude*”), indicating that the richness of the region is still underestimated. The *Proceratophrys* genus is composed by 40 species, organized into morphological groups or complexes without phylogenetic support. Based on recent studies, we defined the complex *P. cristiceps* as the group including six species: *P. cristiceps*, *P. minuta*, *P. redacta*, *P. schirchi*, *P. caramaschii* and *P. aridus*. Herein, we evaluated the taxonomy, biogeography, and evolution of the species related to the *P. cristiceps* complex, combining morphologic, morphometric and acoustic characteristics, and molecular multilocus analyses. Our results, based on these integrative analyses, show *P. aridus*, *P. caramaschii* and *P. cristiceps* as belonging to the same lineage, without morphologic or acoustic distinction. Thus, we place the first two species as junior synonyms of *P. cristiceps*. We also found one population from Chapada do Araripe, previously called *P. cristiceps*, related to the Amazonian species *P. concavitympanum*, that we described as a new species. We considered this new species as Endangered based on IUCN criteria, once it occurs in a small area (ca. 3.100 km²), severely impacted by deforestation and canalization of local streams for agriculture and recreational use. Next, we revised *Proceratophrys* populations from higher elevations in the Chapada Diamantina and described two new species based on morphology and phylogenetic relationships (we used one mitochondrial and one nuclear marker). We found that each lineage from the Chapada Diamantina corresponds to a specific mountain block, each separated by warmer and more xeric valleys, which may act as barriers to gene flow, promoting allopatric diversification. Therefore, we considered each one as distinct species, except the Pindobaçu population, which requires further analyses (more individuals and molecular markers) to define more precisely its taxonomic status. Finally, we conducted a *multilocus* phylogeographic analysis to evaluate the genetic diversity, geographic structure and demography history of *P. cristiceps*. We recovered *P. cristiceps* as one single population widely distributed in the Caatinga, with low levels of haplotype diversity for 16S mtDNA and *CRYb*, *POMC*, and rhodopsin nuDNAs. Our results indicate that *P. cristiceps* effective population size varied, with a rapid and recent expansion throughout the upper Pleistocene.

Key-words: Amphibian, Integrativa taxonomy, Phylogeography, Molecular, Diversification.

Introdução Geral

A Caatinga é historicamente conhecida como um domínio de paisagem homogênea e com baixa diversidade (Vanzolini, 1963). Contudo, estas características são adotadas por quem não conhece a riqueza e a importância da “Mata Branca”. Talvez seja por isso que a maioria dos estudos envolvendo a diversidade Neotropical estejam focados em taxa restritos à ambientes florestados (Furley e Metcalfe, 2007). Cobrindo 850.000 km² no nordeste do Brasil, a Caatinga é caracterizada principalmente por uma vegetação decídua xerófila e espinhosa, com cactos, arbustos e pequenas árvores. A temperatura média anual varia entre 27 a 29°C e a precipitação é esporádica (Ab’Saber, 1998). Contudo, existem áreas na Caatinga que contrastam com essa vegetação árida, chamadas de “paisagem de exceção” (Ab’Saber, 2003). O exemplo mais conspícuo de exceção na Caatinga são os fragmentos de florestas úmidas localizados a 600 metros de altitude ou em maiores elevações. Tais fragmentos são chamados de Brejos de Altitude e persistem em áreas montanhosas no nordeste do Brasil como as Chapadas de Baturité, Borborema, Ibiapaba e Araripe, abrigando várias espécies típicas de florestas tropicais, as quais são incapazes de habitar as regiões áridas da Caatinga adjacente.

Atualmente, a Caatinga abriga 56 espécies de anfíbios (Albuquerque et al., 2012). Porém, novas espécies típicas da Caatinga são continuamente descritas (Pombal-Jr et al., 2012; Magalhães et al., 2014), incluindo espécies restritas aos Brejos de altitude (Napoli et al., 2011; Teixeira-Jr et al., 2012; Roberto et al., 2014), o que indica que a riqueza do bioma ainda está subestimada. Com efeito, trabalhos em andamento onde todas as espécies de Brejos de Altitude e regiões de transição com a Floresta Atlântica são incluídas indicam que a riqueza de anfíbios do Bioma é de ao menos 98 espécies (Garda, comunicação pessoal).

Embora recentes pesquisas moleculares tenham revelado os padrões e processos que diversificaram a fauna de anfíbios da América do Sul (Amaro et al., 2013), estudos focados

apenas em espécies típicas da Caatinga são raros (Thomé et al., 2016; Oliveira et al., 2015; Werneck et al., 2015; São-Pedro et al., manuscrito em preparação). É importante ressaltar que elucidar as questões taxonômicas é parte primordial na busca por respostas às questões ecológicas e evolutivas (Bortolus, 2008; Padial et al., 2010). Neste contexto, no intuito de reconhecer as espécies, considerando-as como linhagens independentes (de Queiroz, 2007), e ao mesmo tempo inferir os processos responsáveis pela origem e manutenção da biodiversidade, o uso de abordagens integrativas é cada vez mais exigido e recomendado, uma vez que diversas fontes de caracteres podem resolver o status taxonômico e evolutivo dos organismos.

O gênero *Proceratophrys* Miranda-Ribeiro, 1920

O gênero *Proceratophrys* compreende 40 espécies com ocorrência no Brasil, Argentina e Paraguai (Dias et al., 2013, Brandão et al., 2013, Martins e Giaretta 2013; Mângia et al. 2014). Nos últimos 15 anos, foram descritas 27 espécies (Eterovick e Sazima 1998; Izecksohn et al., 1999; Giaretta et al., 2000; Kwet e Faivovich 2001; Cruz et al., 2005; Prado e Pombal 2008; Cruz e Napoli 2010, Ávila et al., 2011, Martins e Giaretta 2011, Napoli et al., 2011, Teixeira-Jr. et al., 2012; Ávila et al., 2012; Brandão et al., 2013, Dias et al., 2013; Godinho et al., 2013; Martins e Giaretta 2013; Mângia et al., 2014), além da inclusão da espécie antes conhecida como *Odontophrynus moratoi* no gênero *Proceratophrys* (Amaro et al., 2009). Essa alta taxa de descrição de espécies sugere que a riqueza do gênero esteja subestimada (Teixeira-Jr. et al., 2012).

As espécies do gênero *Proceratophrys* estão agrupadas em complexos e grupos apenas com base em similaridades morfológicas (Prado e Pombal 2008; Cruz et al., 2012), sem corroboração filogenética até o momento (Amaro et al., 2009; Dias et al., 2013). O grupo *P. cristiceps* reúne as espécies sem apêndices palpebrais, rostrais ou elevações pós-oculares: *P.*

cristiceps (Muller, 1883), *P. goyana* (Miranda-Ribeiro, 1937), *P. cururu* Eterovick e Sazima, 1998, *P. concavitympanum* Giaretta, Bernarde e Kokubum, 2000, *P. moratoi* (Jim e Caramaschi, 1980), *P. strussmannae* Ávila, Kaeashita-Ribeiro e Morais, 2011, *P. vielliardi* Martins e Giaretta, 2011, *P. caramaschii*, *P. aridus* Cruz, Nunes e Juncá, 2012, *P. redacta* Teixeira-Jr., Amaro, Recoder, Dal-Vechio e Rodrigues, 2012. *P. huntingtoni* Ávila, Pansonato e Strussmann, 2012, *P. carranca* Godinho, Moura, Lacerda e Feio, 2013, *P. rotundipalpebra* Martins & Giaretta, 2013, *P. bagnoi* Brandão, Caramaschii, Vaz-Silva e Campos, 2013, *P. brandi* Brandão, Caramaschii, Vaz-Silva e Campos, 2013 e *P. dibernardoi* Brandão, Caramaschii, Vaz-Silva e Campos, 2013.

Incertezas taxonômicas e filogenéticas no grupo *cristiceps*

Diversos trabalhos descrevendo novas espécies do gênero *Proceratophrys* utilizam a denominação de “grupo *P. cristiceps*” (e.g. Cruz et al. 2005; Cruz e Napoli 2010; Napoli et al., 2011; Cruz et al., 2012; Ávila et al., 2012; Brandão et al., 2013; Dias et al., 2013; Godinho et al., 2013), citando *sensu* Giaretta et al. (2000). A proposição deste grupo foi feita pelo compartilhamento de apenas uma característica morfológica (ausência de apêndices palpebrais e elevações pós-oculares) e com base no padrão de distribuição das espécies: as espécies *cristiceps*, *cururu* e *goyana* ocupam ambientes de área aberta e sazonalmente secos e a espécie *P. concavitympanum*, descrita no trabalho, pode ter alcançado a Floresta Amazônica a partir de formações vegetacionais de Cerrado e Caatinga. Posteriormente, foram inseridos no grupo *P. moratoi*, que também ocorre em áreas de Cerrado (Santana et al., 2010), corroborando a proposta de Giaretta et al. (2000), *P. strussmannae*, *P. vielliardi*, *P. huntingtoni*, *P. carranca*, *P. rotundipalpebra*, *P. bagnoi*, *P. brandi* e *P. dibernardoi* em áreas de Cerrado (Ávila et al., 2011; Martins e Giaretta 2011; Ávila et al., 2012; Brandão et al., 2013; Godinho et al., 2013; Martins e

Giaretta, 2013), *P. caramaschii*, *P. aridus* e *P. redacta* com ocorrência na Caatinga (Cruz et al., 2012; Teixeira-Jr. et al., 2012).

Recentemente, Cruz et al. (2012) redescreveram *P. cristiceps* e descreveram duas novas espécies relacionadas, *P. caramaschii* e *P. aridus*. Neste mesmo trabalho, restringiram a distribuição de *P. cristiceps* aos ambientes costeiros e sugeriram que os indivíduos que ocorrem no Espinhaço sejam considerados *P. cururu*, enquanto as espécies que ocorrem no Cerrado devem ser consideradas *P. goyana*. Contudo, os autores não examinaram, comparativamente, exemplares das espécies citadas de toda a área de distribuição e os indivíduos que ocorrem na Caatinga ficaram sem um nome específico. Segundo Teixeira-Jr et al. (2012), estas são identificações errôneas, e as recentes descrições de novas espécies indicam que o gênero provavelmente é mais diverso, destacando a necessidade de uma revisão taxonômica de várias espécies.

Nos trabalhos de Amaro et al. (2009), Teixeira-Jr. et al. (2012) e Dias et al. (2013) foram apresentadas “topologias” que demonstram claramente que o grupo *cristiceps* é parafilético. As espécies do “grupo *cristiceps*” estão em diferentes ramos das árvores apresentadas por estes autores, indicando que os caracteres morfológicos adotados atualmente para separação dos grupos fenéticos não suportam essa hipótese. Mesmo para o gênero *Proceratophrys*, a presença/ausência de apêndices palpebrais e rostrais não é uma sinapomorfia para nenhum grupo, uma vez que estas características aparecem em diferentes clados.

Dessa forma, a partir das informações citadas acima e dos dados apresentados por Amaro et al. (2009), Teixeira-Jr. et al. (2012) e Dias et al. (2013), não é correto a utilização de “grupo *cristiceps*” à espécies como *P. goyana*, *P. cururu*, *P. concavitympanum* e *P. moratoi*. Sendo assim, para fins deste trabalho, nós estudamos apenas as espécies relacionadas ao clado o qual pertence *P. cristiceps*. Portanto, a partir deste momento, chamarei de complexo *P. cristiceps* o

conjunto formado pelas seguintes espécies: *P. cristiceps*, *P. minuta*, *P. redacta* e *P. schirchi*.

Também foram estudadas as espécies *P. caramaschii* e *P. aridus*, as quais foram descritas a partir de exemplares identificados *a priori* como *P. cristiceps*. Além disso, utilizamos como grupos externos para as análises filogeográficas as espécies de *Proceratophrys* não relacionadas ao complexo *P. cristiceps*.

Principais resultados

No primeiro artigo realizamos uma revisão das populações de *Proceratophrys* com ocorrência na Caatinga, incluindo *P. aridus*, *P. caramaschii* e *P. cristiceps*. Examinamos a morfologia e morfometria de um total de 358 exemplares, cantos de anúncio provenientes de 14 localidades, e sequenciamos amostras de fígado e músculo de 115 indivíduos. Nós não encontramos características que diferenciem estas três espécies como linhagens distintas.

Portanto, nós colocamos *P. aridus* e *P. caramaschii* como sinônimos juniores de *P. cristiceps*. Os nossos resultados indicam que trabalhos de taxonomia alfa devem ser realizados com cuidado, e que precisamos ser cautelosos com as diversas linhas de evidência, para prevenir decisões taxonômicas errôneas.

No segundo artigo, com base em dados de morfologia, canto e marcadores moleculares descrevemos uma nova espécie de *Proceratophrys* para a Chapada do Araripe. Por meio de marcadores nucleares e mitocondriais, nós mostramos que esta nova espécie está relacionada com uma espécie amazônica, *P. concavitympanum*, e que provavelmente sua distribuição é relictual, resultante de flutuações climáticas do Pleistoceno. A nova espécie se encaixa na categoria Ameaçada da IUCN, uma vez que ocorre em uma pequena área (ca. 3.100 km²) severamente impactada pelo desmatamento e canalização de riachos locais para agricultura.

No terceiro artigo nós revisamos as populações de *Proceratophrys* que ocorrem em áreas de altitude na Chapada Diamantina, e descrevemos duas novas espécies. Nós analisamos a morfologia, morfometria e o relacionamento filogenético, com base em um marcador nuclear e um mitocondrial, entre as populações. Verificamos que cada linhagem corresponde a diferentes ilhas de altitude, separadas por vales com temperaturas mais quentes que podem ter agido como barreiras entre estas populações. Portanto, nós consideramos cada linhagem como uma espécie distinta, exceto a população do município de Pindobaçu, que requer a análise de mais exemplares e a utilização de mais marcadores moleculares para definir com mais precisão seu status taxonômico.

No quarto artigo nós conduzimos análises filogeográficas multilocus para avaliar a diversidade genética, estruturação geográfica, e demografia histórica de *P. cristiceps*. Nossos resultados mostraram *P. cristiceps* constituído por apenas uma população amplamente distribuída na Caatinga, com baixa diversidade genética para todos os genes avaliados (16S mtDNA, *CRYb*, *POMC* e *rhodopsina* nuDNAs). Nossas análises para avaliar a demografia histórica de *P. cristiceps* revelaram que a espécie sofreu uma recente expansão, coincidindo com as flutuações climáticas do Pleistoceno.

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Artigo I – Mângia, S., Santana, D.J., Sant’Anna, A.C., Garda, A.A. Taxonomic revision of *Proceratophrys cristiceps* (Müller, 1883) (Amphibia: Anura: Odontophrynidae) from the Brazilian Caatinga.

Taxonomic revision of *Proceratophrys cristiceps* (Müller, 1883) (Amphibia: Anura: Odontophrynidae) from the Brazilian Caatinga

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Abstract: Recently, *Proceratophrys cristiceps* was redescribed, along with the description of two new species from the Caatinga domain: *P. caramaschii* and *P. aridus*. However, the authors did not examine individuals from across their ranges, including the populations from Caatinga domain. Thus, the revision of *Proceratophrys* populations from the Caatinga is paramount to establish a solid taxonomic background and to test the validity of the described species. Based on morphologic, morphometric, acoustic, and 16S mtDNA barcoding analysis, we define the range of inter and intrapopulation variation in the parameters analyzed, establishing which ones are useful as diagnostics characters. 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 the use of several lines of evidence to avoid taxonomic instability.

Key words: Morphology, acoustic, molecular, integrative taxonomy, synonymization.

Introduction

Taxonomic revisions frequently lack enough resolution in species diagnosis, which can lead to incoherence in biogeographic, phylogenetic, and evolutionary analyses. Indeed, taxonomic studies that use solely one base of data (such as morphology) must be treated with caution, especially nowadays where integrative taxonomy studies are easier to conduct and have been strongly recommended (e.g. Padial et al. 2010).

Proceratophrys Miranda-Ribeiro, 1920 has been historically arranged in morphological groups (Prado and Pombal 2008; Cruz et al. 2012) without phylogenetic support (Amaro et al. 2009; Teixeira-Jr. et al. 2012; Dias et al. 2013). *Proceratophrys cristiceps* complex includes 15 species and is characterized by the absence of rostral and palpebral appendages or post-ocular elevations (Cruz et al. 2012). It was suggested based mostly on geographic distribution: *P. cristiceps* (Müller 1883), *P. cururu* Eterovick and Sazima, 1998, and *P. goyana* (Miranda-Ribeiro 1937) from open areas seasonally dry, and *P. concavitympanum* in the Amazon region (Giaretta et al. 2000). Later, *P. moratoi* (Jim and Caramaschi 1980), from Cerrado areas, was placed in the “*P. cristiceps* group” (Santana et al. 2010), corroborating Giaretta's et al. (2000) proposal.

All recently-described species for the group are distributed in open areas of the Cerrado and Caatinga biomes. *Proceratophrys strussmannae* Ávila, Kawashita-Ribeiro and Morais, 2011, *P. vielliardi* Martins and Giaretta, 2011, *P. huntingtoni* Ávila, Pansonato and Strüssmann, 2012, *P. carranca* Godinho, Moura, Lacerda and Feio, 2013, *P. rotundipalpebra* Martins and Giaretta, 2013, *P. bagnoi* Brandão, Caramaschi, Vaz-Silva and Campos, 2013, *P. branti* Brandão, Caramaschi, Vaz-Silva and Campos, 2013, and *P. dibernardo* Brandão, Caramaschi, Vaz-Silva and Campos, 2013 also occur in the Cerrado domain (Ávila et al. 2011; Martins and Giaretta 2011; Ávila et al. 2012; Brandão et al. 2013; Godinho et al. 2013; Martins and Giaretta 2013).

Proceratophrys caramaschii Cruz, Nunes and Juncá, 2012, *P. aridus* Cruz, Nunes and Juncá, 2012, and *P. redacta* Teixeira, Amaro, Recoder, Vechio and Rodrigues, 2012 are distributed in the Caatinga domain (Cruz et al. 2012; Teixeira-Jr. et al. 2012).

Recently, Cruz et al. (2012) redescribed *P. cristiceps* and described two new species from the Caatinga semi-arid region of Brazil: *P. caramaschii* and *P. aridus*. Cruz et al. (2012) limited the distribution of *P. cristiceps* to coastal Northeastern Brazil and suggested that individuals occurring in the Espinhaço Mountain Range must be treated as *P. cururu*, while those occurring in the Cerrado domain should be considered *P. goyana*. However, Cruz et al. (2012) did not examine, comparatively, individuals of all cited species or from populations encompassing all their ranges. Indeed, most populations from Caatinga domain were not included. Furthermore, as pointed by Teixeira et al. (2012), the morphological similarity of large-size species from the *P. cristiceps* complex makes the group prone to misidentifications. Hence, recent species descriptions, dubious identifications from several localities, and the lack of appropriate taxonomic appraisals across all species distributions indicate that the group is likely species-rich, highlighting the need for broader taxonomic revisions.

Therefore, the revision of *Proceratophrys* populations from the Caatinga 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: 1) revise the taxonomic status of all *Proceratophrys* populations from the Caatinga domain, covering a significant portion of its geographic distribution, based on morphologic, morphometric, acoustic, and 16S mtDNA barcoding analyses, 2) identify the inter and intrapopulation variations of the parameters analyzed, to select which characters can be used as diagnostic and which are polymorphic, and 3) to set out a type locality for *P. cristiceps*.

Material and Methods

Specimens examined are housed in the following institutions: UFRN-CHBEZ (Coleção Herpetológica do Departamento de Botânica, Ecologia e Zoologia, Universidade Federal do Rio Grande do Norte), NUROF-UFC (Núcleo Regional de Ofidiologia da Universidade Federal do Ceará), CHUFPI (Coleção Herpetológica da Universidade Federal do Piauí), CHUFPE (Coleção Herpetológica da Universidade Federal de Pernambuco), CHUFAL (Coleção Herpetológica da Universidade Federal de Alagoas), MZUFBA (Museu de Zoologia da Universidade Federal da Bahia), MZFS (Museu de Zoologia da Universidade Estadual de Feira de Santana), CHUFMG (Coleção Herpetológica da Universidade Federal de Minas Gerais), MCNAM (Museu de Ciências Naturais, Pontifícia Universidade Católica de Minas Gerais); MNRJ (Museu Nacional, Rio de Janeiro, Universidade Federal do Rio de Janeiro), URCA-H (Coleção de Herpetologia da Universidade Regional do Cariri).

Morphologic and morphometric assessment

Morphological and morphometric data from 294 preserved specimens of *Proceratophrys cristiceps* (160 males, 134 females), 33 of *P. aridus* (12 males, 21 females), and 31 of *P. caramaschii* (27 males, 4 females) (Appendix I) were analyzed. We follow the terminology for external morphology of Prado and Pombal (2008), Brandão et al. (2013), and Mângia et al. (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 (internarial distance), END (eye-nostril distance), ED (eye diameter), UEW (upper eyelid width), IOD (interorbital distance), THL (thigh length), TL (tibia length), FL (foot + tarsus length), and FHL (forearm and hand length). We focused on examining individuals from the

lowlands areas in the Caatinga domain in this study, including *P. aridus*, *P. caramaschii*, and *P. cristiceps*. To verify the coloration pattern we used only photographs of individuals in life, because specimens in preservative lack important coloration characters present only in life.

Acoustic analysis

We analyzed calls from 14 localities (Table 1). We recorded calls of two individuals from Macaíba municipality, Rio Grande do Norte State, one on May 12, 2011 (air temperature not available) and one Jun 17, 2010 (air temperature 24.8°C); one individual from ESEC Seridó, Serra Negra do Norte municipality, Rio Grande do Norte State on May 2013 (air temperature 24.2°C); one individual from Itapipoca municipality, Ceará State on March 20, 2014 (air temperature 24.0°C); three individuals from Jaguaribe municipality, Ceará State on April 12, 2014 (air temperatures not available), one individual from Quixadá municipality, Ceará State on April 18, 2015 (air temperature not available); five individuals from PARNA Confusões, Caracol municipality, Piauí State on October 22, 2014 (air temperature not available). All recordings were conducted using a Marantz PMD 660 digital coupled with a Sennheiser ME 66 directional microphone. Recordings are deposited in the Arquivos Sonoros da Universidade Federal do Rio Grande do Norte (ASUFRN). We also analyzed calls from several populations loaned from scientific collections (see Table 1).

We analyzed calls with Raven Pro 1.5 for Windows (Cornell Lab of Ornithology) and constructed audio spectrograms in R software using the package “seewave” (Sueur et al. 2008; R Development Core Team) with the following parameters: FFT window width = 256, Frame = 100, Overlap = 75, and flat top filter. We analyzed usual acoustic parameters for *Proceratophrys* taxonomy (e.g. Mângia et al. 2010; Santana et al. 2010; Santana et al. 2011; Mângia et al. 2014): call duration, pulse number per call, pulse number per second and dominant frequency. Call

terminologies followed Duellman and Trueb (1994). For acoustic comparisons, we used published records of the advertisement call of *P. cristiceps* (Nunes and Juncá, 2006) and *P. caramaschii* (Nunes et al. 2016).

Genetic distances and phylogenetic analysis

We extracted genomic DNA from muscle or liver using the phenol-chloroform protocol (Sam Brooks et al., 1989). PCR amplification and sequencing with the primers 16Sa (5–CGC CTG TTT ATC AAA AAC AT–3) and 16Sb (5–CCG GTC TGA ACT CAG ATC ACG T–3) of Palumbi et al. (2002) were performed to amplify a section of the mitochondrial 16S ribosomal RNA gene. PCR conditions followed those described by Costa et al. (2016). We purified PCR products using Ethanol/Sodium Acetate protocols and sequenced them in ABI 3730 XL DNA Analyzer (Applied Biosystems). We edited sequences by aligning forward and reverse reads using the Geneious 9.1.2 program and deposited final sequences in GenBank (access numbers not available yet).

We aligned sequences we generated with sequences from other *Proceratophrys* species available in GenBank in Geneious Pro v9.1.2 using the MUSCLE algorithm with default parameters (Edgar 2004). The final sequence length used for the phylogenetic analysis was 450 bp. The best model of nucleotide substitution for the alignment was GTR+I+G, determined with jModeltest (Posada 2008), using the Bayesian Information Criterion (BIC). We performed a Bayesian phylogenetic analysis using MrBayes v3.2 software (Ronquist et al. 2012). We ran 30 million generations, sampling every 1,000 steps. 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 and Drummond 2007). Genetic distances (uncorrected p-values) were calculated using Mega v 6.06 (Tamura et al. 2013).

Results and Discussion

Morphologic and morphometric assessment

After observations of the coloration of *P. cristiceps*, *P. aridus* and *P. caramaschii* populations, we noticed a wide polychromatism in all *Proceratophrys* populations along the Caatinga domain (Fig. 1). The number of morphotypes varied among populations, and we could not assign one morphotype to any specific population.

Some studies previously showed polychromatism in *P. cristiceps* (Vieira et al. 2008; Nunes et al. 2016). Vieira et al. (2008) recovered two sympatric basic color patterns in *P. cristiceps* throughout their range in the Caatinga domain: a "common" morphotype (brown to gray) and a "yellow" morphotype (or an approximate tone). Nunes et al. (2016) described the following color variation to *P. caramaschii*: "dorsal color pattern is marbled with several shades of brown on a cream background. The cream back-ground and the light brown tones may have a reddish hue. The mid-dorsal region, internal to the limits of a spear-shaped ornamentation, may have the same dorsal pattern, a lighter pattern, or a uniform pattern without drawings. If there is no spear-shaped ornamentation, the mid-dorsal pattern corresponds to the dorsolateral pattern. The rostral region can have a lighter smooth coloration, like a mask."

Studies on chromatism generally describe such variations as characteristics of distinct species or treat them in a context related to taxonomic identification (Kwet and Faivovich 2001; Garcia et al. 2007; Kwet 2008). Conversely, other studies use these characters to distinguish populations according to the areas in which they occur (Feio et al. 2006; Marcelino et al. 2009; Vieira et al. 2008). However, based on the samples collected by ourselves and the data from the literature, using dorsal coloration patterns as diagnostic characters for Caatinga *Proceratophrys* populations is impossible.

We also quantified morphological variation among populations of *P. cristiceps* from across the Caatinga biome and among individuals from the same population. The snout can be (1) rounded or (2) triangular (Fig. 2). 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 (Fig. 3). The interocular ridge can be formed by (1) one or (2) two rows of warts (observed only on two individuals of *P. cristiceps* - the holotype and one individual from Piripiri municipality, Piauí State, and four individuals of *P. caramaschii*, Fig. 4). The inner part of metacarpal tubercle can be (1) smaller or (2) equal to the outer one (Fig. 5). The ventral region coloration can be (1) cream without blotches or (2) cream with brown spots or blotches (males usually present darkish gular region) (Fig. 6).

Some morphological characteristics, such as snout shape, number of interocular ridge of warts, and size of metacarpal tubercles, are used to diagnose *P. aridus*, *P. caramaschii* and *P. cristiceps* as three different species (Cruz et al. 2012). However, the large interspecific variation involving these diagnostic morphological characteristics makes it impossible the recognition of these populations as three distinct species.

Males and females do not differ with respect to morphological characters. However, the gular region in males is 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.

Advertisement call variation

The advertisement calls of individuals from the 14 different populations are composed by one single multipulsed note (Fig. 7). The values of the acoustic parameters have similar values among the populations (Table 1). Based on the calls of all populations analyzed (including data

from literature), the advertisement call of *Proceratophrys cristiceps* presents a duration of 0.354 – 0.955 s, with 32 – 113 pulses/note, a emission rate of 71.4 – 124 pulses/s, and dominant frequency of 860 – 1205.9 Hz.

The call of *P. caramaschii* was described by Nunes et al. (2016). We reanalyzed the same recordings used in the description, all from Planalto de Ibiapaba, Ceará State. We found that the advertisement calls are indistinguishable between *P. cristiceps* and *P. caramaschii*, and there are no differences when we compare calls of populations from the entire distribution of *P. cristiceps* in the Caatinga domain (Table 1). Because these two species are morphologically similar (see above) and were recovered as belonging to the same lineage in the tree topology (see below), the similarity of their calls was expected.

Molecular phylogeny

Our 16S mtDNA gene tree (Fig. 8) shows *Proceratophrys aridus*, *P. caramaschii*, and *P. cristiceps* in the same lineage with high support value (1.00 pp). Aside from a small clade within *P. cristiceps*, the tree shows absolutely no structure among the three species. This same marker, when compared across other recognized species of *Proceratophrys*, presents moderate to high *p*-distances (Table 2). Remarkably, these three species are virtually identical considering uncorrected *p*-distances (Table 2). This is not a surprise, considering the results of an ongoing phylogeographic study on *P. cristiceps* that corroborates the low diversity for this marker and a clear signal of population expansion within the Caatinga (Mangia, in prep.).

Taxonomic Implications

Synonymization of *Proceratophrys aridus* Cruz, Nunes and Juncá, 2012

Cruz et al. (2012) described *Proceratophrys aridus* based on 56 specimens, all collected in Milagres municipality, Ceará State, Brazil. The following diagnostic characters were used to separate it 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 define 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*.

From the analysis of the topotype of *P. aridus* (AAGARDA 11910), the holotype (MNRJ 55782) and the type series (MNRJ 55349, 55778–55781, 55783–55822, 75156, 75157, 75158–75168), there are no characters that allow a differentiation between *P. aridus* from *P. cristiceps*. We observed all the diagnostic characters of *P. aridus* in individuals of *P. cristiceps* along the entire 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 showed in the topic variation). Furthermore, all morphometric characters analyzed in *P. aridus* overlap with those in *P. cristiceps* (Table 3).

Once we found no characteristics supporting these taxa as two different species, and there is no genetic distance (0.00% in 16S mtDNA barcoding) between them (occurring in the same

clade) (see above), we consider *Proceratophrys aridus* Cruz, Nunes & Juncá, 2012 as a junior synonym of *Proceratophrys cristiceps* (Müller 1883).

Synonymization of *Proceratophrys caramaschii* Cruz, Nunes & Juncá, 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 modified drastically. We went in April 2015 to the type locality of *P. caramaschii* (Mucuripe) to collect new individuals, sample tissues and to record vocalizations. However, nowadays the locality had become an urban square within the city, surrounded by buildings (Fig. 9). Thus, we found no individuals of *P. caramaschii* or suitable natural habitats for harbor individuals of the species. We searched for the species even in green areas near the type locality (e.g. urban parks), but without success. The nearest place we have recorded individuals of *Proceratophrys* was in Aquiraz municipality, approximately 20 km away from the type locality, where we gathered recordings and specimens (Appendix).

Brandão et al. (2013) use the type series and individuals from Piripiri municipality, Piauí State, which they called as *P. caramaschii*, to compare with three new species of *Proceratophrys* from Cerrado. However, the authors did not formally extend the distribution of *P. caramaschii*. Nunes et al. (2016) extended the geographic distribution of *P. caramaschii* in approximately 300 km far from its type locality to Ubajara, Ceará State, and described the advertisement call from this same place. The authors also indicate the occurrence of this species in the Piauí State (500 km far from the type locality).

We analyzed the morphology of specimens from Piripiri municipality, Piauí State, Ubajara, Itapipoca, Aquiraz municipalities, and other localities close to the type locality, in Ceará State, the holotype (MNRJ 16592) and the type series (MNRJ 1419-1420, 1680, 16470-16484, 16487-16489, 16591, 16593-16600), in order to verify the diagnose of *P. caramaschii* from the original description (Cruz et al. 2012). The authors 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. cristiceps*, *P. aridus* and *P. caramaschii*; (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; and (5) few bigger blotches on venter (larger and variable scattered small blotches in *P. cristiceps*) – In the present study we identify 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. As shown in the topic “variation”, the diagnostic characteristics used to describe *P. caramaschii* are within the inter and

intrapopulational variation of these characters for *P. cristiceps*. At last, all the morphometric characters analyzed in *P. caramaschii* overlap with those in *P. cristiceps* (Table 3).

Nunes et al. (2016) 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 and Juncá, 2006, calls from Feira de Santana, Bahia State) because of 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 1030 Hz; 900 – 990 Hz in *P. cristiceps*). As shown in the topic “variation” all the acoustic parameters overlap when we observed calls from different localities (Table 1). Therefore, there are no differences between the calls of *P. caramaschii* and *P. cristiceps*.

Lastly, in the tree topology of the genus (Fig. 8) presented herein, *P. caramaschii* and *P. cristiceps* were recovered embedded in the same clade and there is no genetic distance between them (0.00% in 16S mtDNA barcoding). Thus, we considered *P. caramaschii* Cruz, Nunes and Juncá, 2012 as a junior synonym of *Proceratophrys cristiceps* (Müller 1883).

Redescription of *Proceratophrys cristiceps* (Müller, 1883)

Ceratophrys cristiceps Müller, 1883

Stombus cristiceps Miranda-Ribeiro, 1920

Proceratophrys cristiceps Lynch, 1971

Proceratophrys aridus Cruz, Nunes, and Juncá, 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, Nunes, and Juncá, 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.—*Proceratophrys cristiceps* is diagnosed by the following combination of characters: (1) medium size (33.1–53.6 mm in adult males, 35.1–64.5 mm in adult females); (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 not fused, enlarged and pointed tubercles, and other with fused and short tubercles) (5) advertisement call with duration of 0.354 – 0.955 s, 32 – 113 pulses/note, 71.4 – 124 pulses/s, and dominant frequency of 860 – 1205.9 Hz.

Comparisons with other species (data for species in comparison are given in parenthesis).—

Proceratophrys cristiceps is promptly distinguished from *P. appendiculata*, *P. belzebul*, *P. boiei*, *P. gladius*, *P. itamari*, *P. izecksohni*, *P. laticeps*, *P. mantiqueira*, *P. melanopogon*, *P. moheringi*, *P. paviotii*, *P. phyllostomus*, *P. pombali*, *P. renalis*, *P. rondonae*, *P. sanctaritae*, *P. subguttata* and *P. tupinamba* by the absence of a single uni-cuspidate palpebral and rostral appendages (present in those 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 those

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 tubercle in the middle more projected than lateral tubercles in *P. minuta*; small and rounded in *P. redacta*; multiple short and pointed expansions in *P. schirchi*). By having tubercles in the forearm organized in two rows - one row with not fused, enlarged and pointed tubercles, and other with fused and short tubercles – *P. cristiceps* differ from *P. bagnoi*, *P. concavitympanum*, *P. dibernardo* and *P. goyana*, (two rows in *P. bagnoi*, *P. concavitympanum* and *P. dibernardo*, tubercles not organized in rows in *P. goyana*).

Tadpole.—Described by Vieira et al. (2007) based on tadpoles collected at Estação Experimental de São João do Cariri, in the municipality of São João do Cariri (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 in the Coleção Herpetológica do Departamento de Sistemática e Ecologia of the Universidade Federal da Paraíba (UFPB 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 the description of the advertisement call of *P. cristiceps* based on calls from 14 localities (see Table 1 for details).

Variation.—We discuss the color and morphological variation in the present study in the topics “Color and external morphology variation”, “Advertisement call variation”, and in the synonym topics. Descriptive basic statistics of measurement variables from adults is presented in Table 3.

Distribution.—*Proceratophrys cristiceps* occurs in the Caatinga domain and adjacent areas (Cerrado domain in western distribution and Atlantic Forest in the eastern distribution) (Fig. 10).

Proceratophrys cristiceps type locality

Von F. Müller, in 1883, when cataloging the amphibians and reptiles from Naturhistorisches Museum, Basel, Switzerland, described *Ceratophrys cristiceps* (= *Proceratophrys 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 after Nieden (1923) replaced it into the genus *Ceratophrys* and provided some data on morphology and coloration. 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 (U. Wüest, *pers. comm.*). Thus, we are not able to follow the steps of the collector in order to identify where he had collected the individual, because this information does not exist. We tried to associate one population of *Proceratophrys 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, here we define the type locality of *Proceratophrys cristiceps* to the Caatinga biome and adjacent areas, in all its geographic distribution (Article 76, Recommendation 76A.1.4. - ICNZ, 1999) (Fig. 12).

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Appendix I

Specimens examined

Proceratophrys aridus.—BRAZIL: Ceará: Milagres: MNRJ 55349, 55778-822, 75156-68; URCA-H 106, 142-43.

Proceratophrys caramaschii.—BRAZIL: Ceará: Mucuripe: MNRJ 1419-20, 1680, 16470-84, 16487-89, 16591-600.

Proceratophrys concavitympanum.—BRAZIL: Mato Grosso: Alta Floresta: ZUEC 21201.

Aripuanã: MZUFV 9552, 9554-56. Paranaíta: ZUEC 14505-06, 16011-15, 16719, 21201. Pará: Paraopebas: PUC-MG 10561, 11206. Rondônia: Espigão do Oeste: MZUFV 10477.

Proceratophrys cristiceps.—BRAZIL: 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.

Ubajara, Parque Nacional de Ubajara: AAGARDA 10672, 10695, 10698-99, 10703, 10707-09,

10782, 10796, 10907, 10909, 10911-14, 10961, 10974, 10981, 10983. Várzea da Conceição:

UFPB 9661, 9665, 9667. 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 Jundiá: 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 minuta.—BRAZIL: Bahia: Miguel Calmon, Parque Estadual das Sete
Passagens: UFBA 6229-30, 6716-20, 6722, 6725-26.

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.

TABLE 1.—Acoustic parameters of the advertisement call of *Proceratophrys cristiceps*.

Locality/Acoustic parameters	Recording collection label	Duration (s)	Pulse/call	Pulse/sec.	Dominant Frequency (Hz)	Reference
Macaíba, RN (20 calls)	ASUFRN034	0.553 ± 0.06	51 ± 5.47	93.5 ± 1.55	1033.6	Present work (individual 1)
		0.418 – 0.619	40 – 59	90.4 – 95.8		
Macaíba, RN (14 calls)	ASUFRN044	0.592 ± 0.03	55 ± 2.27	92.3 ± 1.40	1033.6	Present work (individual 2)
		0.550 – 0.633	51 – 58	90 – 94.6		
ESEC Seridó, RN (10 calls)	ASUFRN	0.556 ± 0.04	53.5 ± 3.74	96.4 ± 1.81	1125	Present work
		0.506 – 0.633	48 – 60	93.9 – 98.8		
Aiuaba, CE (13 calls)	URCA7396	0.706 ± 0.12	61 ± 9.67	84.4 ± 1.80	1125 ± 82.22	Present work
		0.450 – 0.896	38 – 73	81.9 – 81.5	937.5 – 1125	
Aquiraz, CE (22 calls)		0.492 ± 0.09	42 ± 7.09	87.7 ± 2.30	861.3 ± 79.76	Present work
		0.364 – 0.742	33 – 60	80.7 – 91.9	861.3 – 1033.6	
Barro, CE (27 calls)	URCA7920	0.509 ± 0.08	47 ± 6.96	91.9 ± 1.31	937.5	Present work
		0.354 – 0.638	33 – 57	88.7 – 93.8		
Crato, CE (34 calls)		0.559 ± 0.04	55.5 ± 4.00	99.2 ± 1.00	1205.9 ± 29.53	Present work (individual 1)
		0.511 – 0.648	50 – 65	97.8 – 1027	1205.9 – 1378.1	
Crato, CE (31 calls)		0.526 ± 0.03	52 ± 3.25	99.8 ± 0.87	1205.9	Present work (individual 2)
		0.449 – 0.581	45 – 58	98.5 – 101.5		
Crato, CE		0.460 ± 0.03	45 ± 2.90	98.0 ± 0.83	1033.6 ± 58.20	Present work

(24 calls)		0.430 – 0.533	42 – 53	96.8 – 100.2	1033.6 – 1205.9	(individual 3)
Ipú, CE (12 calls)	URCA9203	0.559 ± 0.04 0.499 – 0.619	50.5 ± 2.84 46 – 55	90.3 ± 1.25 88.5 – 92.2	937.5	Present work
Itapipoca, CE (10 calls)	ASUFRN	0.570 ± 0.08 0.477 – 0.728	54.4 ± 6.81 46 – 68	95.7 ± 1.86 93.4 – 97.8	1125	Present work
Ubajara, CE (25 calls)		0.456 ± 0.03 0.400 – 0.581	35.9 ± 3.08 32 – 46	78.8 ± 0.89 76.0 – 80.6	861.3	Present work (individual 1)
Ubajara, CE (16 calls)		0.674 ± 0.04 0.609 – 0.737	54.2 ± 3.27 49 – 59	80.5 ± 0.83 78.7 – 82.1	861.3	Present work (individual 2)
Planalto do Ibiapaba, Ubajara, CE		0.570 ± 11.0 0.410 – 0.740	45 ± 9.19 33 – 59	80.0 ± 0.86 78.6 – 81.2	860 – 1030	Nunes et al. 2016
Jaguaribe, CE (10 calls)	ASUFRN	0.609 ± 0.08 0.447 – 0.704	59.5 ± 7.57 45 – 68	98.0 ± 1.92 96.6 – 101.9	1125 ± 79.06 937.5 – 1125	Present work (individual 1)
Jaguaribe, CE (3 calls)	ASUFRN	0.650 ± 0.05 0.557 – 0.652	63 ± 5.20 54 – 63	96.9 ± 0.18 96.6 – 96.9	1125	Present work (individual 2)
Jaguaribe, CE (4 calls)	ASUFRN	0.578 ± 0.06 0.531 – 0.645	60.5 ± 4.99 56 – 66	104.3 ± 1.88 102.3 – 106.3	1125	Present work (individual 3)
Quixadá, CE (9 calls)	ASUFRN	0.902 ± 0.14 0.623 – 1.011	77 ± 11.30 55 – 85	86.3 ± 1.61 88.3 – 84.1	861.3	Present work (individual 1)

Floriano, PI (20 calls)	AN0659	0.537 ± 0.05 $0.443 - 0.630$	52.5 ± 4.79 $44 - 62$	98.2 ± 4.12 $81.9 - 101.5$	1033.6	Present work (individual 1)
Floriano, PI (18 calls)	AN0663	0.633 ± 0.04 $0.532 - 0.694$	62 ± 4.23 $52 - 68$	97.7 ± 1.27 $94.9 - 99.1$	1033.6	Present work (individual 2)
Floriano, PI (14 calls)	AN0662	0.892 ± 0.04 $0.814 - 0.955$	90.5 ± 7.92 $82 - 113$	102.7 ± 6.06 $100.1 - 124.0$	1033.6 ± 46.05 $861.3 - 1033.6$	Present work (individual 3)
PARNA Serra das Confusões, PI (9 calls)	ASUFRN	0.740 ± 0.04 $0.653 - 0.782$	53 ± 2.55 $47 - 56$	71.6 ± 0.32 $71.4 - 72.4$	1033.6	Present work (individual 1)
PARNA Serra das Confusões, PI (31 calls)	ASUFRN	0.703 ± 0.05 $0.643 - 0.901$	52 ± 4.01 $48 - 66$	74.3 ± 1.02 $73.5 - 76.9$	1033.6	Present work (individual 2)
PARNA Serra das Confusões, PI (37 calls)	ASUFRN	0.650 ± 0.02 $0.599 - 0.707$	49 ± 2.40 $45 - 58$	74.2 ± 2.63 $73.3 - 89.2$	1033.6	Present work (individual 3)
PARNA Serra das Confusões, PI (24 calls)	ASUFRN	0.680 ± 0.06 $0.591 - 0.781$	52.5 ± 3.92 $47 - 61$	77.9 ± 1.31 $75.9 - 80.5$	1033.6 ± 48.6 $861.3 - 1033.6$	Present work (individual 4)
PARANA Serra das Confusões, PI (40 calls)	ASUFRN	0.686 ± 0.07 $0.570 - 0.811$	51.5 ± 4.23 $44 - 59$	73.5 ± 1.40 $71.6 - 77.2$	1033.6	Present work (individual 5)
Feira de Santana, BA (9 calls)		0.561 ± 0.04 $0.506 - 0.628$	48 ± 4.25 $43 - 55$	84.7 ± 2.63 $83.0 - 92.1$	1033.6 ± 76.0 $861.3 - 1033.6$	Present work (individual 1)
Feira de Santana, BA		0.668 ± 0.09	59 ± 7.28	87.1 ± 0.90	861.3	Present work

(7 calls)	0.520 – 0.800	46 – 69	86.2 – 88.5		(individual 2)
Feira de Santana, BA	0.660 ± 0.05	57.5 ± 6.02	89.5 ± 1.20	940 ± 0.02	Nunes & Juncá, 2006
(29 calls)	0.520 – 0.790	46 – 69	87.4 – 91.5	900 – 990	

TABLE 2.—Uncorrected p -distances of 16S mitochondrial fragment gene for the genus *Proceratophrys*.

	1	2	3	4	5	6	7	8	9	10	11	12
1 <i>Proceratophrys aridus</i> new synonym												
2 <i>Proceratophrys caramaschii</i> new synonym	0.00											
3 <i>Proceratophrys cristiceps</i>	0.00	0.00										
4 <i>Proceratophrys appendiculata</i>	0.06	0.06	0.06									
5 <i>Proceratophrys avelinoi</i>	0.07	0.07	0.07	0.04								
6 <i>Proceratophrys belzebul</i>	0.07	0.07	0.07	0.01	0.05							
7 <i>Proceratophrys bigibbosa</i>	0.07	0.07	0.07	0.03	0.03	0.04						
8 <i>Proceratophrys boiei</i>	0.09	0.09	0.09	0.04	0.06	0.05	0.06					
9 <i>Proceratophrys concavitympanum</i>	0.08	0.08	0.08	0.06	0.06	0.07	0.06	0.06				
10 <i>Proceratophrys cururu</i>	0.06	0.06	0.06	0.03	0.03	0.03	0.03	0.05	0.07			
11 <i>Proceratophrys goyana</i>	0.08	0.08	0.08	0.03	0.04	0.04	0.03	0.04	0.06	0.03		
12 <i>Proceratophrys itamari</i>	0.06	0.06	0.06	0.02	0.03	0.03	0.03	0.04	0.06	0.02	0.04	
13 <i>Proceratophrys izecksohni</i>	0.06	0.06	0.06	0.02	0.03	0.03	0.02	0.05	0.06	0.02	0.03	0.01
14 <i>Proceratophrys laticeps</i>	0.06	0.06	0.06	0.02	0.03	0.03	0.03	0.04	0.06	0.02	0.03	0.02
15 <i>Proceratophrys mantiqueira</i>	0.05	0.05	0.05	0.03	0.03	0.04	0.03	0.05	0.06	0.02	0.04	0.01
16 <i>Proceratophrys melanopogon</i>	0.07	0.07	0.07	0.04	0.04	0.05	0.04	0.06	0.07	0.03	0.04	0.02
17 <i>Proceratophrys minuta</i>	0.05	0.05	0.05	0.05	0.06	0.06	0.05	0.08	0.08	0.07	0.06	0.06
18 <i>Proceratophrys moratoi</i>	0.07	0.07	0.07	0.04	0.06	0.05	0.06	0.06	0.05	0.06	0.06	0.05
19 <i>Proceratophrys pombali</i>	0.07	0.07	0.07	0.03	0.04	0.04	0.04	0.04	0.07	0.03	0.03	0.03
20 <i>Proceratophrys redacta</i>	0.07	0.07	0.07	0.07	0.07	0.08	0.05	0.08	0.08	0.07	0.07	0.06
21 <i>Proceratophrys renalis</i>	0.07	0.07	0.07	0.03	0.04	0.04	0.05	0.05	0.08	0.03	0.04	0.03
22 <i>Proceratophrys schirchi</i>	0.06	0.06	0.06	0.05	0.06	0.06	0.05	0.06	0.08	0.04	0.05	0.04
23 <i>Proceratophrys tupinamba</i>	0.06	0.06	0.06	0.01	0.03	0.02	0.03	0.04	0.05	0.03	0.03	0.02

TABLE 2 (continued).—Uncorrected *p*-distances of 16S mitochondrial fragment gene for the genus *Proceratophrys*.

	13	14	15	16	17	18	19	20	21	22
1 <i>Proceratophrys aridus</i> new synonym										
2 <i>Proceratophrys caramaschii</i> new synonym										
3 <i>Proceratophrys cristiceps</i>										
4 <i>Proceratophrys appendiculata</i>										
5 <i>Proceratophrys avelinoi</i>										
6 <i>Proceratophrys belzebul</i>										
7 <i>Proceratophrys bigibbosa</i>										
8 <i>Proceratophrys boiei</i>										
9 <i>Proceratophrys concavitympanum</i>										
10 <i>Proceratophrys cururu</i>										
11 <i>Proceratophrys goyana</i>										
12 <i>Proceratophrys itamari</i>										
13 <i>Proceratophrys izecksohni</i>										
14 <i>Proceratophrys laticeps</i>	0.02									
15 <i>Proceratophrys mantiqueira</i>	0.01	0.02								
16 <i>Proceratophrys melanopogon</i>	0.02	0.03	0.02							
17 <i>Proceratophrys minuta</i>	0.05	0.06	0.06	0.07						
18 <i>Proceratophrys moratoi</i>	0.06	0.05	0.05	0.07	0.09					
19 <i>Proceratophrys pombali</i>	0.03	0.03	0.03	0.03	0.07	0.06				
20 <i>Proceratophrys redacta</i>	0.06	0.06	0.06	0.07	0.03	0.09	0.08			
21 <i>Proceratophrys renalis</i>	0.03	0.03	0.04	0.05	0.08	0.06	0.04	0.08		
22 <i>Proceratophrys schirchi</i>	0.04	0.04	0.04	0.05	0.06	0.06	0.05	0.07	0.05	
23 <i>Proceratophrys tupinamba</i>	0.02	0.03	0.03	0.04	0.05	0.05	0.03	0.07	0.03	0.05

1 TABLE 3. Measurements of specimens of *Proceratophrys aridus*, *P. caramaschii* and *P.*
 2 *cristiceps*.

	<i>P. cristiceps</i>		<i>P. aridus</i>		<i>P. caramaschii</i>	
	Males (n=160)	Females (n=134)	Males (n=12)	Females (n=21)	Males (n=27)	Females (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 Captions

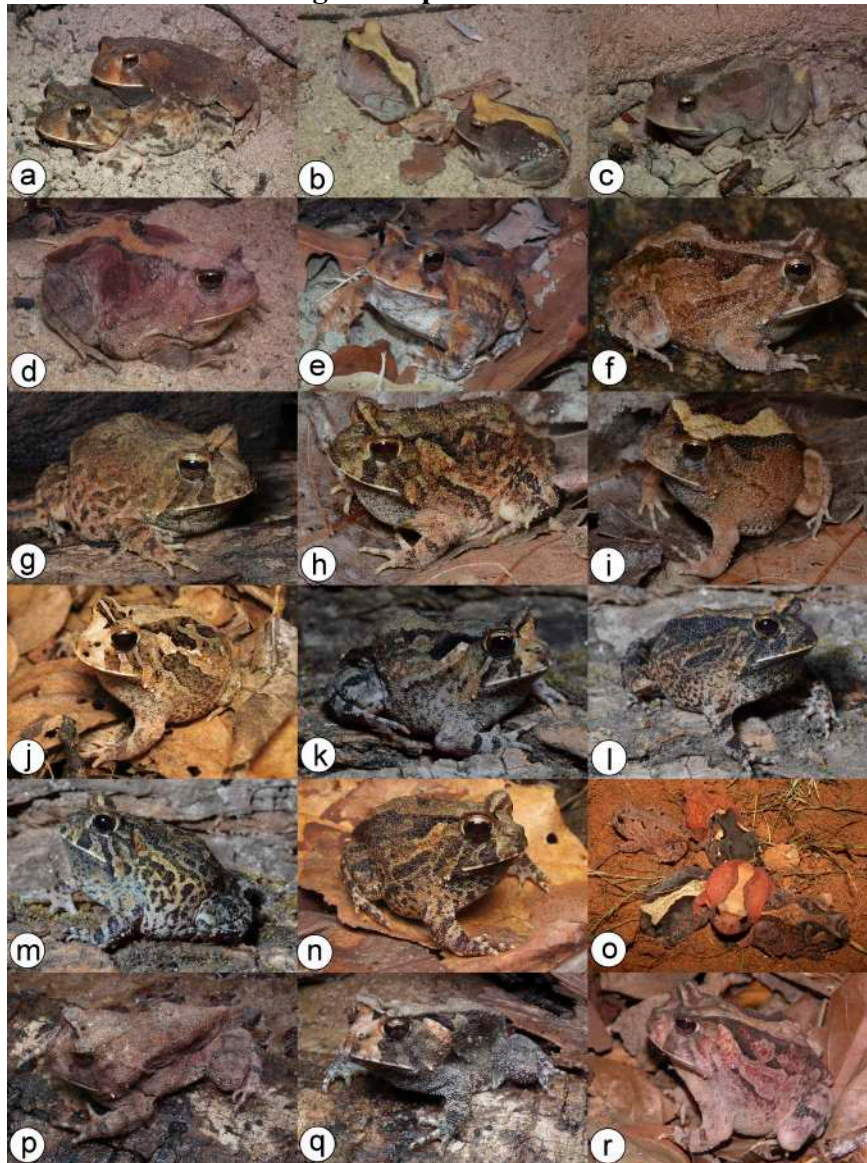


FIG. 1.—Inter and intra-populacional chromatic variation. A–E: individuals from Parque Nacional Serra das Confusões, Piauí State. F–I: individuals from Jaguaribe municipality, Ceará State. J–N: individuals from Macaíba municipality, Rio Grande do Norte State. O: individuals from Milagres municipality, Ceará State. P–Q: individuals from Parque Nacional Serra das Confusões, Piauí State. R: Paraipaba, Ceará State. Photos: D.J. Santana (F–N); I. Joventino (O, R); S. Mângia (A–E, P–Q).

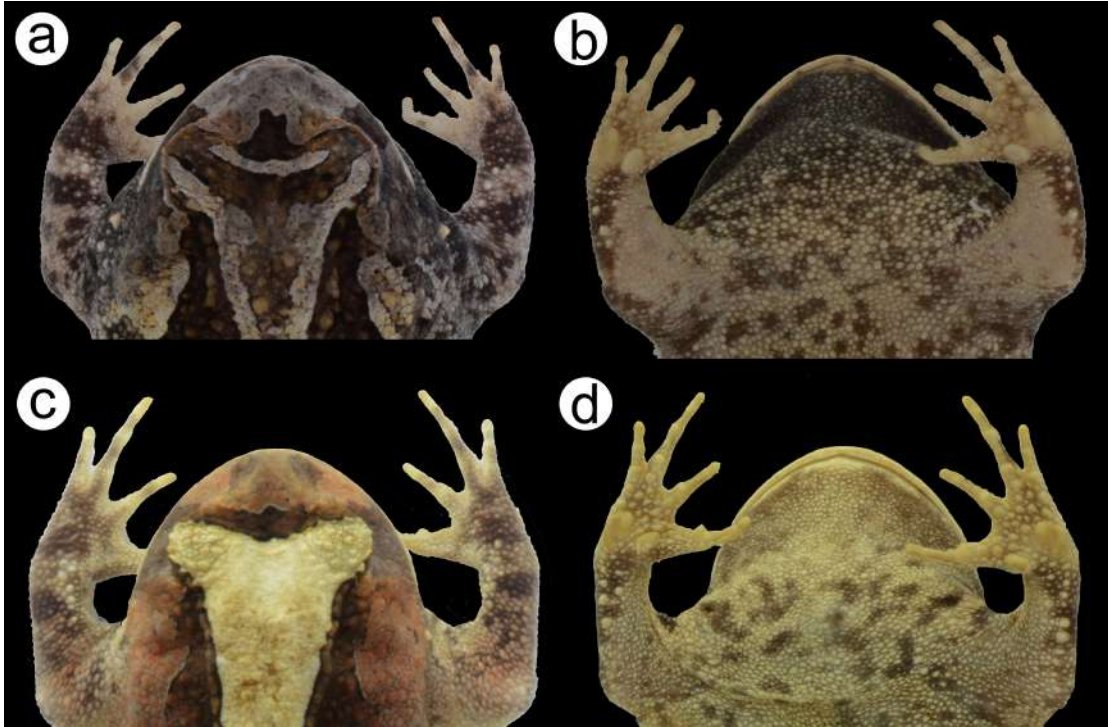


FIG. 2.—Snout shape. A – B: triangular (AAGARDA 10453, Itapipoca, Ceará State). C – D: rounded (AAGARDA 7824, PARNA Catimbau, Pernambuco).

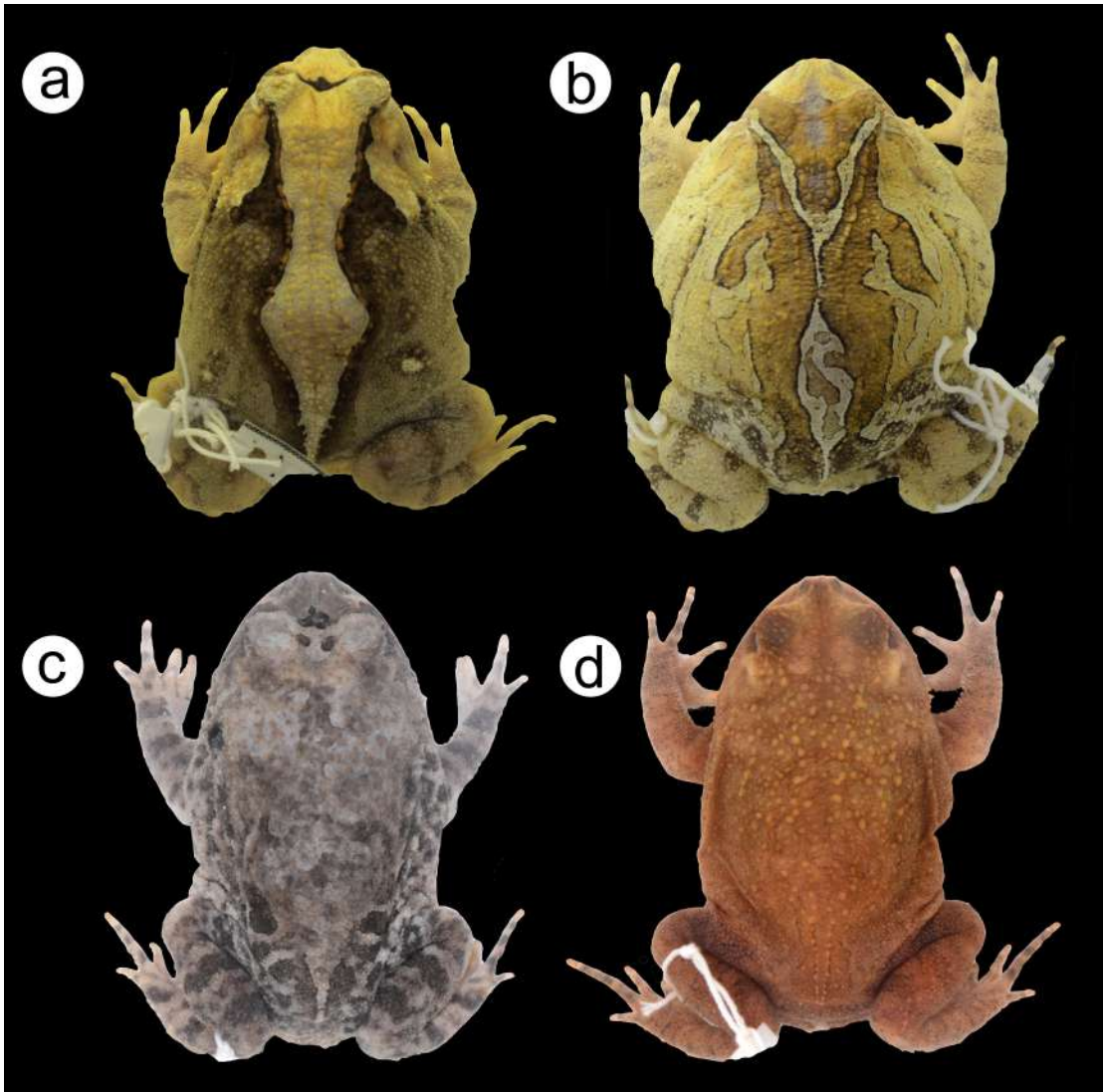


FIG. 3.—Ocular-dorsal ridge of warts. a— continuous extending from the edge of the eyelid to the sacral region (CHUFPB 12126, Poço Redondo, Sergipe State). b— interrupted in the pre-sacral constriction (CHUFPB 10345, Piripiri, Piauí State). c—(AAGARDA 10286, Jaguaribe, Ceará State) and d— absent (AAGARDA 10983, PARNA Ubajara, Ceará State).

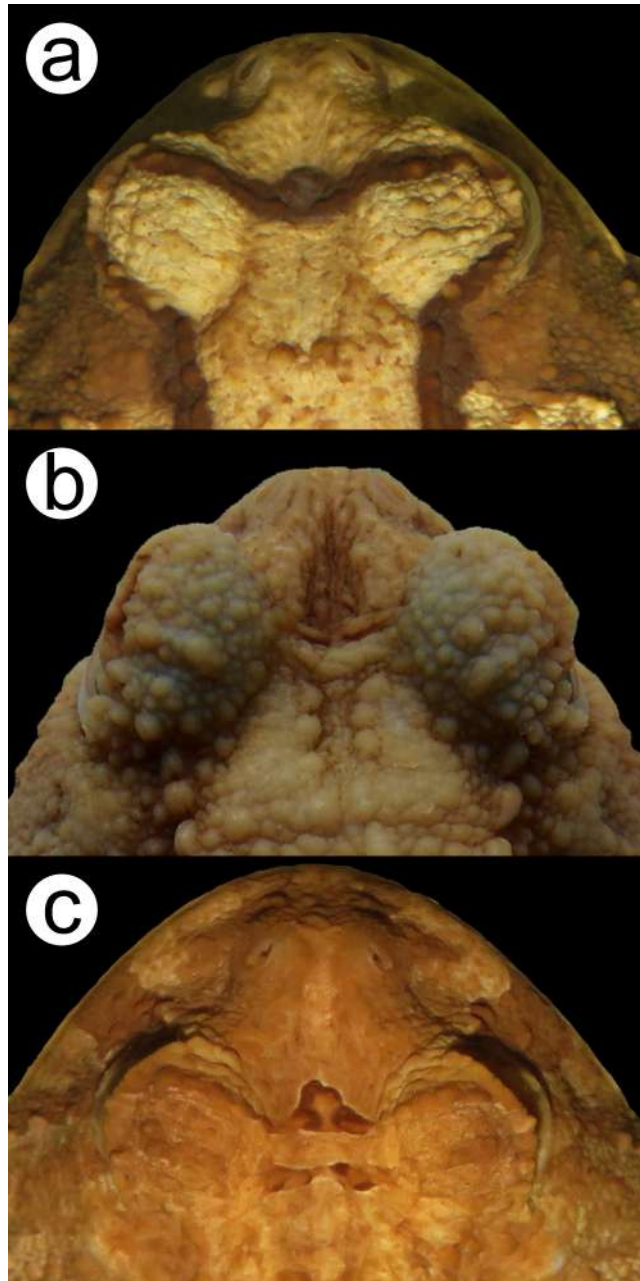


FIG. 4.—Inter-ocular ridge of warts. a— one row (MNRJ 1419, Mucuripe, Ceará State). b— (NHMB 1503, *P. cristiceps* holotype). c— two rows (MNRJ 16487, Mucuripe, Ceará State).

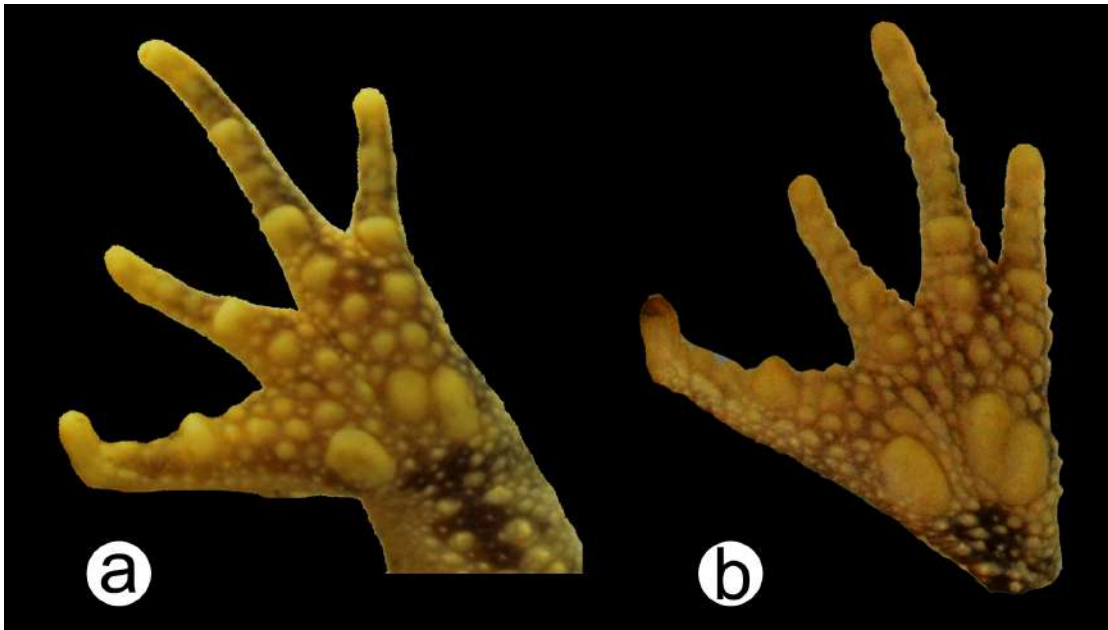


FIG. 5.—Inner part of metacarpal tubercle. A – smaller than the outer (CHUFPB 10344, Piripiri, Piauí, State). B – similar size of the outer (CHUFPB 10146, Piripiri, Piauí, State).

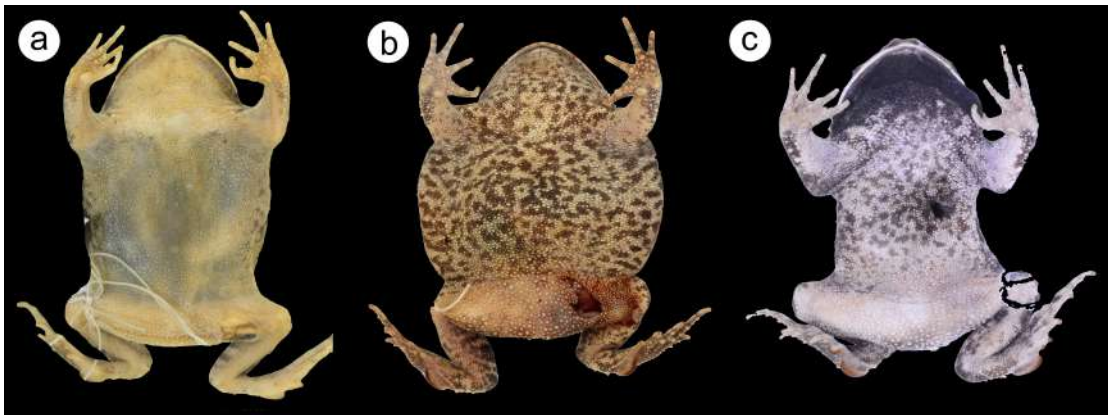


FIG. 6.—Ventral region coloration. A – cream without blotches (URCA 5773, Paracuru, Ceará State). B – 2 cream with brown spots or blotches (AAGARDA 10796, Ubajara, Ceará State). C – males present darkish gular region (AAGARDA AAGARDA 9809, João Câmara, Rio Grande do Norte State).

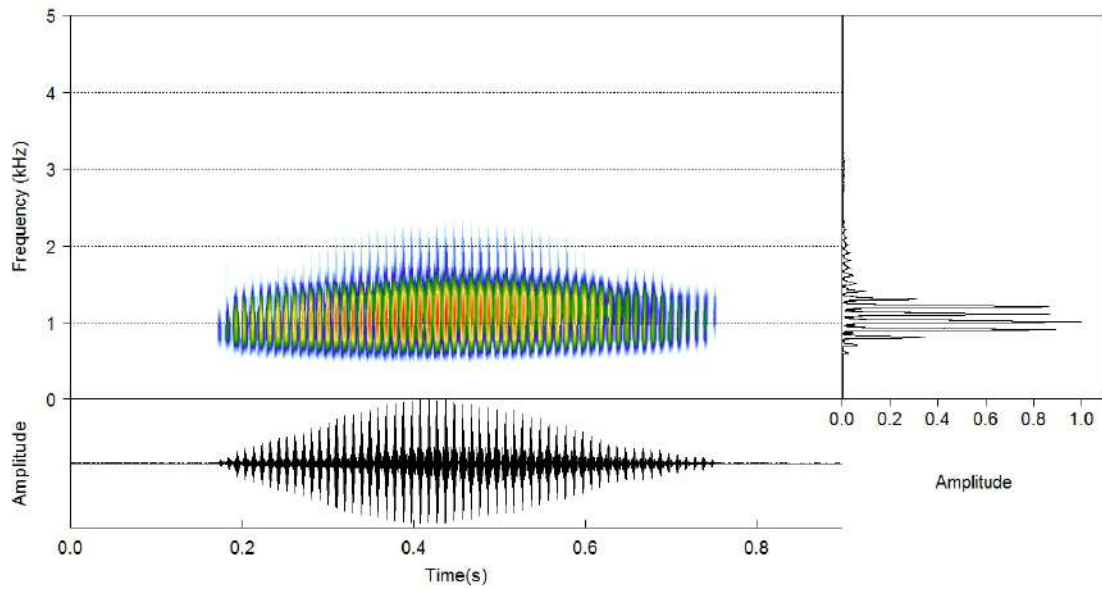


FIG. 7.—Advertisement call of *Proceratophrys cristiceps* (AAGARDA 10176, Jaguaribe, Ceará State).

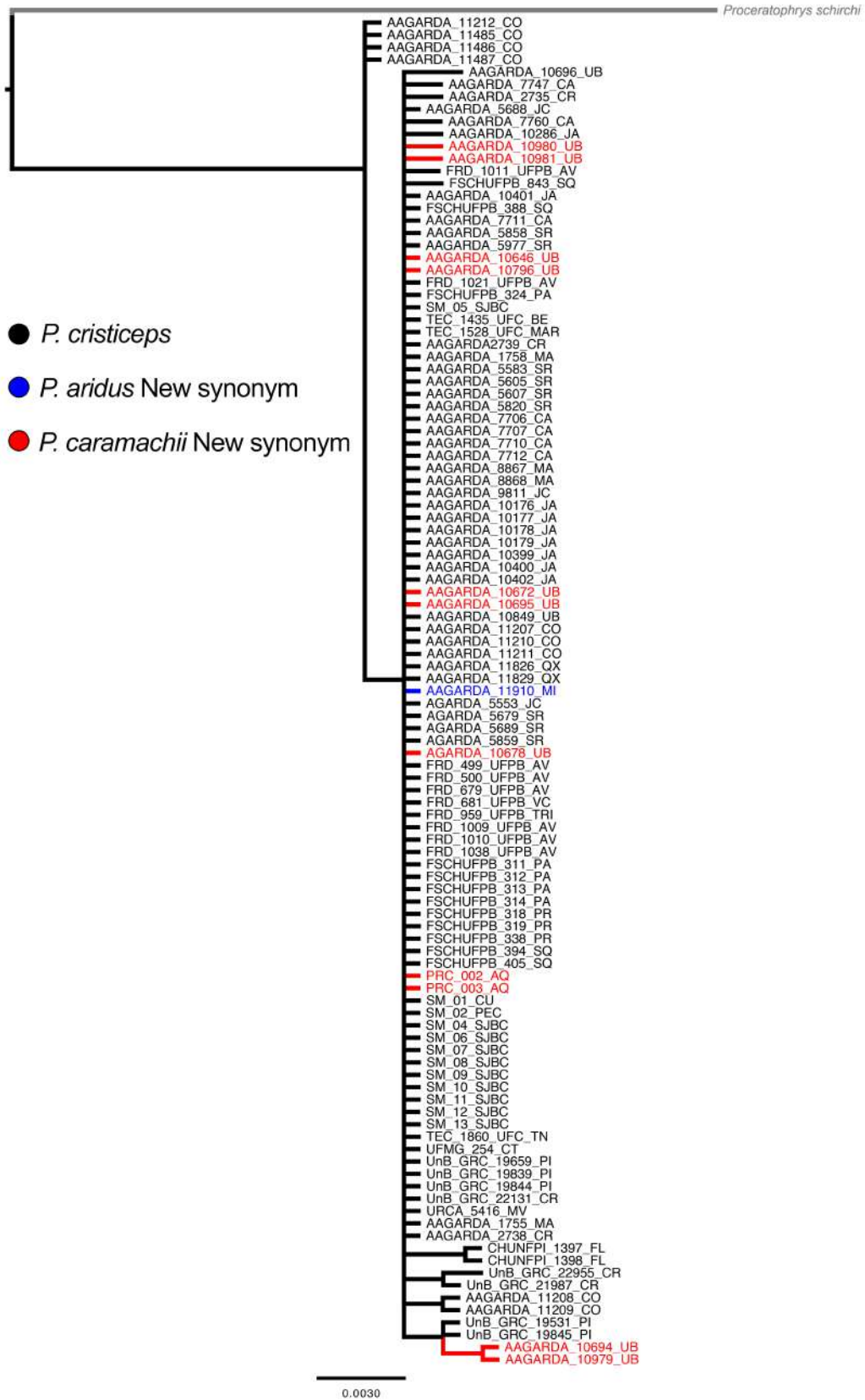


FIG. 8.— Bayesian tree topology obtained from the 16S mtDNA.



FIG. 9.—Type locality of *Proceratophrys caramaschii* new synonym. Mucuripe municipality, Ceará State, Brazil.

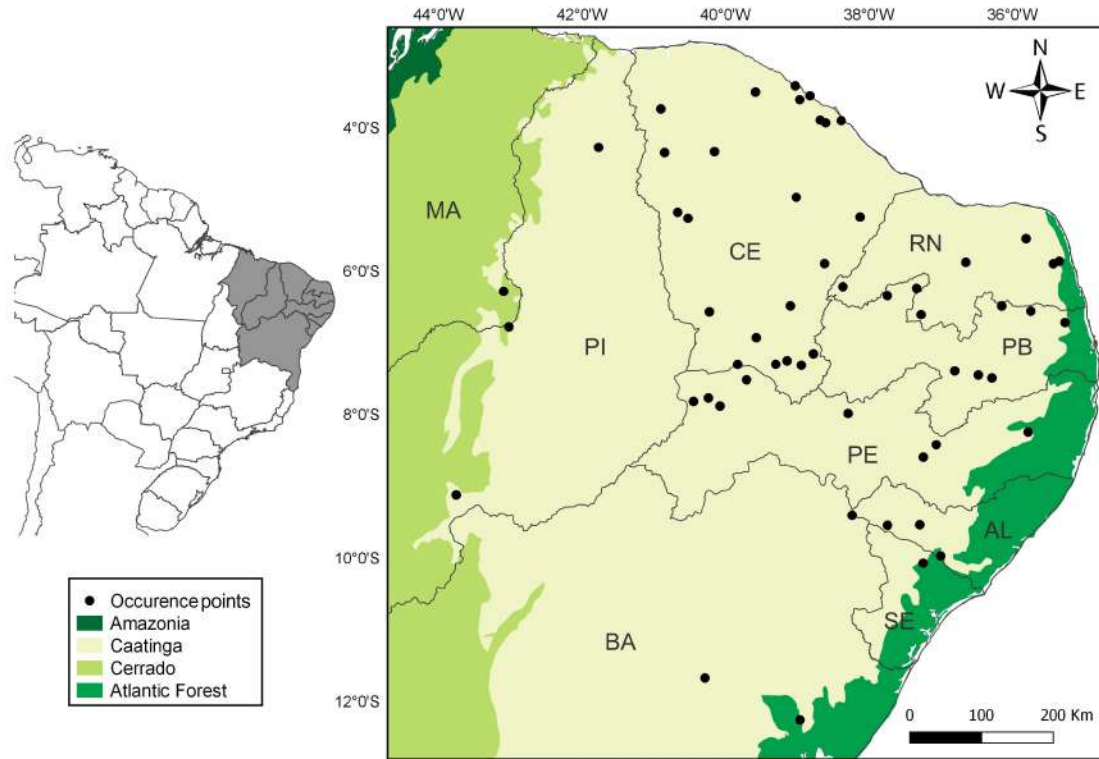


FIG. 10.—Geographic distribution of *Proceratophrys cristiceps* in Northeastern Brazil.

Artigo II. Mângia, S., Koroiva, R., Nunes, P.M. S., Roberto, I.J., Santa'Anna, A.C., Santana, D.J. and Garda, A.A. A new and endangered species of *Proceratophrys* (Amphibia: Anura: Odontophrynidae) from Araripe Plateau, Ceará State, Northeastern Brazil.
(Submetido na revista Herpetologica)

1 **A new and endangered species of *Proceratophrys* (Amphibia: Anura: Odontophrynidae)**
2 **from the Araripe Plateau, Ceará State, Northeastern Brazil**

3
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20

21 RRH: MÂNGIA ET AL.—A NEW SPECIES OF *PROCERATOPHRY*S

22 ABSTRACT: Natural Rainforest enclaves in Northeastern Brazil (called *Brejos de Altitude*)
23 are present in isolated nuclear areas surrounded by the semi-arid Caatinga vegetation. Among
24 these high altitude areas, the Araripe Plateau in Ceará State harbors physiognomies from
25 Caatinga, Cerrado, and Atlantic Forest remnants and is considered a priority area for biodiversity
26 conservation within the Caatinga domain. Herein, we described a new species of *Proceratophrys*
27 endemic to the Araripe Plateau. We show, based on nuclear and mitochondrial markers, that this
28 new species is phylogenetically related to the Amazonian species *P. concavitympanum*. The new
29 species occurs in a small area (ca. 3.100 km²), severely impacted by deforestation and
30 canalization of local streams for agriculture and recreational use. This same region harbors other
31 endemic vertebrate species, several of which, for the same reasons (human impact and restricted
32 distribution), are listed in local and IUCN red lists. Therefore, we recommend this species be
33 consider Endangered based on IUCN criteria.

34 **Key words:** Molecular; Morphology; Advertisement call; Phylogeny; Caatinga domain.

35
36 THE CAATINGA domain is a semi-arid region in Brazil characterized by high solar
37 radiation, low nebulosity and relative humidity, and especially by low and unpredictable rainfall
38 patterns (Prado 2003). Several species well adapted to arid conditions are found in the Caatinga
39 (Brazão et al. 1992), which covers most of the Brazilian northeastern region. During Pleistocene
40 climatic cycles, the Amazon and Atlantic Forests expanded and contracted repeatedly,
41 establishing connections across the diagonal of open formations that cuts South America from
42 southwest to northeast (Por 1992). Such intermittent connections enabled faunal exchanges
43 between these biomes, as supported by phylogenetic relationships in different vertebrate groups.
44 During glacial periods, forested environments are believed to have retracted while the dry areas
45 expanded (Costa 2003; Werneck et al. 2011; Batalha-Filho et al. 2014). Nowadays, rainforest

46 natural enclaves (called *Brejos de Altitude*) are present in isolated nuclear areas surrounded by
47 the Caatinga semi-arid vegetation (Ab'Sáber 1977). Such remnants persist at the slopes of higher
48 altitude areas such as Baturité, Borborema, Ibiapaba, and Araripe plateau in northeastern Brazil,
49 where lower temperatures and orographic rains allow the maintenance of mesic habitats within
50 the semi-arid Caatinga (Andrade-Lima 1982).

51 Because such rainforest enclaves were isolated during the Pleistocene, vicariance events
52 likely promoted high diversification rates within these areas (Werneck et al. 2011). Indeed,
53 several amphibian (e.g. *Rhinella casconi*, *Adelophryne baturitensis*, *A. maranguapensis*) and
54 squamate (e.g. *Atractus ronnie*, *Leposoma baturitensis*, *Placosoma limaverdorum*) species are
55 endemic to these high altitude regions (Hoogmoed et al. 1994; Rodrigues and Borges 1997;
56 Loebmann et al. 2009; Roberto et al. 2014; Borges-Nojosa et al. 2016; Roberto and Loebmann
57 2016). Among these high altitude areas, the Araripe Plateau harbors physiognomies from
58 Caatinga, Cerrado, and Atlantic Forest (MMA 2000) and is considered a priority area for
59 biodiversity conservation within the Caatinga domain (MMA 2007; Camardelli and Napoli
60 2012).

61 Currently, the genus *Proceratophrys* harbors 40 recognized species that are organized into
62 four morphological groups (*Proceratophrys bigibbosa* and *P. cristiceps* groups, and *P. boiei*, and
63 *P. appendiculata* complexes, Giaretta et al. 2000; Kwet and Faivovich 2001; Prado and Pombal
64 2008). During different field expeditions to the Araripe Plateau, the authors collected several
65 specimens belonging to the genus *Proceratophrys*. Herein, we described an endemic population
66 from the Araripe Plateau as a new species related to the Amazonian species *P. concavitympanum*
67 and belonging to the *P. cristiceps* species group.

68

69

MATERIAL AND METHODS

Araripe Plateau

The humid forests along the slopes of the Araripe Plateau are about 200 km long and mostly composed of 8 – 15 m high trees (Girão and Souto 2005). The region has two marked seasons: the dry season from May to November with precipitation not exceeding 60 mm, and a rainy season from December to April, with a mean rainfall of 1.033 mm. The mean annual temperature is 25.7°C (Girão and Souto 2005; Linhares et al. 2010). The area is inserted in the sustainable Environment Protected Area of Chapada do Araripe (a sustainable use protected area).

Morphological and morphometric assessments

Specimens used in the description and examined for comparisons are housed at the following collections: UFRN (Coleção Herpetológica da Universidade Federal do Rio Grande do Norte), CHUFPE (Coleção Herpetológica da Universidade Federal de Pernambuco), MZUFBA (Museu de Zoologia da Universidade Federal da Bahia), MZFS (Museu de Zoologia da Universidade Estadual de Feira de Santana), CHUFMG (Coleção Herpetológica da Universidade Federal de Minas Gerais), MCNAM (Museu de Ciências Naturais, Pontifícia Universidade Católica de Minas Gerais), MNRJ (Museu Nacional do Rio de Janeiro, Universidade Federal do Rio de Janeiro), and URCA-H (Coleção de Herpetologia da Universidade Regional do Cariri) (Appendix).

We follow the terminology for morphological diagnosis of Cruz and Napoli (2010), Teixeira et al. (2012), Brandão et al. (2013) and Mângia et al. (2014). Measurements of 11 adult specimens (eight males, three females) follow Prado and Pombal-Jr. (2008) and are presented in millimeters throughout the paper: SVL (snout-vent length), HL (head length), HW (head width), DICS (distance from the interocular crest to the tip of snout), IND (internarial distance), END (eye-nostril distance), ED (eye diameter), UEW (upper eyelid width), IOD (interorbital distance),

94 THL (thigh length), TL (tibia length), FL (foot + tarsus length), and FHL (forearm and hand
95 length).

96 Vocalizations

97 We recorded the advertisement calls of three males (total of 89 calls) at Sítio Caianas
98 (7°16'36''S, 39°26'42''W), Crato Municipality, Ceará State. Calls were recorded with a Sony
99 TCM 5.000 EV tape recorder coupled to a Sennheiser ME 66 directional microphone, totaling 89
100 calls. We analyzed calls in Raven Pro 1.5 for Mac (Bioacoustics Research Program 2012) and
101 constructed audio spectrograms with the following parameters: FFT window width = 256, Frame
102 = 100, Overlap = 75, and flat top filter. We analyzed acoustic parameters typically evaluated in
103 anuran taxonomy studies (e.g. Brasileiro et al. 2008; Mângia et al. 2014): call duration, pulse
104 number per call, pulse number per second, and dominant frequency. Terminology on call
105 descriptions follows Duellman and Trueb (1994). Comparative data for other species were
106 obtained from the available literature (see Santana et al. 2010; Nunes et al. 2016) (Table 1).
107 Voucher specimens were not collected, but were photographed and the species identity was
108 confirmed based on morphological characters (see below).

109 Molecular data

110 We sequenced fragments of 16S ribosomal RNA mitochondrial gene (14 individuals) and
111 of the nuclear gene rhodopsin (seven individuals) (Table 2). We extracted genomic DNA from
112 muscle and/or liver samples using the phenol-chloroform protocol (Sam Brooks et al., 1989). We
113 used the 16Sa/16Sb primer of Palumbi (2002), following PCR conditions described by Costa et
114 al. (2016). For the nuclear gene rhodopsin, we used primers Rhod1A and Rhod1C of Bossuyt and
115 Milinkovitch (2000). PCR conditions for amplification consisted of 1× buffer, dNTP at 0.2 mM,
116 each primer at 0.2 µM, MgCl₂ at 2mM, 1U Taq polymerase and 2 µl of template DNA, in a total
117 reaction volume of 25 µl. We used the following PCR cycling program: 94°C for 2 min, followed

118 by 35 cycles of 94°C for 30 s, 59°C for 1 min, and 72°C for 1 min, and concluding with a 5 min
119 extension at 72°C. We purified PCR products with Ethanol/Sodium Acetate and sequenced at
120 ABI 3730 XL DNA Analyzer (Applied Biosystems, Foster City, California (CA)). Resulting
121 sequences were edited by aligning forward and reverse reads using Geneious 9.1.2 with
122 MUSCLE algorithm using default parameters (Edgar 2004). The final dataset was 453 bp for 16S
123 and 350 bp for rhodopsin. We deposited final sequences in GenBank (Table 2).

124 For phylogenetic analyses, we included 16S and rhodopsin sequences from 21
125 *Proceratophrys* species available in GenBank along with *Macrogenioglottus alipioi*,
126 *Odontophrynus americanus* and *Cycloramphus acangatan* as outgroups (Table 2). To determine
127 the most probable pair of alleles for rhodopsin, we used the PHASE algorithm (Stephens et al.
128 2001) implemented in the DNASP 5.10 software (Librado & Rozas 2009) using default options.
129 We also determined the model of nucleotide substitution for each gene with jModelTest (Darriba
130 et al. 2012) using the Akaike Information Criterion (AIC). The best-fit models were GTR+I+G
131 for 16S and K80+I+G for rhodopsin.

132 First, we performed a Bayesian phylogenetic analysis for 16S using BEAST v.1.8
133 software (Drummond et al. 2012). We performed a run with 30 million generations, sampling
134 every 1,000 steps using a Yule Process prior tree. We checked for stationarity by visually
135 inspecting trace plots and ensuring that all values for effective sample size were above 200 in
136 Tracer v1.5 (Rambaut and Drummond 2007). To infer the timing of speciation of the new
137 sequenced species, we used *Proceratophrys* species and outgroups as terminal taxa for a species
138 tree in *BEAST, as implemented in BEAST v1.8 (Drummond 2012). Due to the lack of fossil
139 calibrations for this group, we used the mtDNA 16S mutation rate of 0.0028 per lineage per
140 million years (Lemmon et al. 2007; Lymberakis et al. 2007). We ran 250 million generations,
141 sampling at every 10,000 generations. Stationarity was determined by visually inspecting trace

142 plots and ensuring that all ESS values were above 200 in TRACER v1.5. The first 10% sampled
143 genealogies were discarded as burn-in, and the most credible clade was inferred with
144 TreeAnnotator v1.8 (Drummond 2012). We also calculated sequence divergences (uncorrected p-
145 values) using Mega v 6.06 (Tamura et al. 2013).

146

147

SPECIES DESCRIPTION

148

Proceratophrys ararype sp. nov.

149

Proceratophrys cristiceps Ribeiro et al. 2012

150

Figs. (1 – 3)

151

152

Holotype.—CHUFPE 156, adult female, collected at Mata do Clube Recreativo

153

Grangeiro, Crato Municipality, Ceará State (7°16'49"S, 39°26'22" W; 720m a.s.l.), on December

154

14, 2014 by P.M.S. Nunes; M.P.L. Castro; K.M. Kameoka; M.C. Guarnieri.

155

Paratopotypes.—CHUFPE 160, CHUFPE 161 (adult males), CHUFPE 152 (juvenile)

156

collected with the holotype, CHUFPE 226 and CHUFPE 227 (juvenile) collected on May 2015

157

by P.M.S. Nunes, A.V.A. Mello, M P.L. Castro, A.N. Cabral; A.R.M. Gonzaga; M.C. Guarnieri.

158

Paratypes.—AAGARDA 2736, AAGARDA 2741 (adult males) collected at Clube

159

Serrano Atlético Cratense, Crato Municipality, Ceará State (7°16'10"S, 39°27'35"W; 740m

160

a.s.l.), on May 2011 by D.J. Santana and A.A. Garda. URCA-H 114 (adult female) collected at

161

Nascente, Chapada do Araripe, Crato Municipality, Ceará State, on May 1st 2008 by S.C.

162

Ribeiro; URCA-H 4028 – 29 (adult males), collected at Nascente, Chapada do Araripe, Crato

163

Municipality, Ceará State, on January 1st 2009; URCA-H 1030 (adult female) URCA-H 1031 –

164

34 (adult males) collected at Mata do Clube Recreativo Grangeiro, Crato Municipality, Ceará

165

State, on December 13, 2011 by S.C. Ribeiro.

166 **Diagnosis.**—The new species is characterized by (1) medium size (38.4–42.2 mm in
167 males, 44.3 mm female); (2) snout rounded in dorsal and ventral views, obtuse and slightly
168 vertical in profile; (3) presence of short, fused but not pointed warts on upper eyelid border (L 1,
169 2/5, 3; R 1/2/5, 2); (4) one row of small and pointed tubercles on the forearm; (5) dorsal region
170 with pointed tubercles on the head, arms, legs, and pre-sacral region; the flanks, sacral-urostyle
171 region and coccyx end with shorter and fewer tubercles than the rest of the dorsal region; (6)
172 ventral region cream with dark-brown vermiculations on the gular region, chest and belly; (7)
173 advertisement call consisting of a multipulsed note with 0.526 ± 0.05 seconds of duration (0.430–
174 0.649), 52 ± 5.3 pulses per note (42–65), 99.2 ± 0.9 pulses per second (96.8–102.7), and
175 dominant frequency of 1205.9 ± 99.5 Hz (1033.6–1205.9).

176 **Comparison with other species.**—*Proceratophrys ararype* differs from *P.*
177 *appendiculata*, *P. belzebul*, *P. boiei*, *P. gladius*, *P. itamari*, *P. izecksohni*, *P. laticeps*, *P.*
178 *mantiqueira*, *P. melanopogon*, *P. moheringi*, *P. paviotii*, *P. phyllostomus*, *P. pombali*, *P. renalis*,
179 *P. rondonae*, *P. sanctaritae*, *P. subguttata* and *P. tupinamba* by the absence of single uni-
180 cuspidate palpebral and rostral appendages (present in those species; single, short and multi-
181 cuspidate in *P. rondonae*). Additionally, *P. ararype* can also be distinguished from *P.*
182 *appendiculata*, *P. gladius*, *P. itamari*, *P. izecksohni*, *P. laticeps*, *P. mantiqueira*, *P. melanopogon*,
183 *P. moheringi*, *P. phyllostomus*, *P. pombali*, *P. sanctaritae*, *P. subguttata* and *P. tupinamba* by
184 lacking a rostral appendage (present in those species).

185 From *P. avelinoi*, *P. bigibbosa*, *P. brauni* and *P. palustris*, *P. ararype* differs by lacking
186 postocular swellings (present in these species). *Proceratophrys ararype* presents short, fused and
187 not pointed warts on upper eyelid border (fused with small points in *P. goyana*, *P. strussmannae*,
188 *P. carranca*, *P. branti* and *P. concavitympanum*; small, rounded and not fused in *P. cururu* and
189 *P. rotundipalpebra*; slightly fused without appendage in *P. huntingtoni*, *P. vielliardi* and *P.*

190 *moratoi*; conical and pointed in *P. bagnoi*; enlarged, pointed and with the largest tubercle in the
191 middle more projected than lateral tubercles in *P. minuta*; small and rounded in *P. redacta*;
192 multiple short and pointed expansions in *P. schirchi*). From *P. bagnoi*, *P. concavitympanum*, *P.*
193 *dibernardo* and *P. goyana*, *P. ararype* differs by the presence a single row of tubercles on the
194 forearm (two rows in *P. bagnoi*, *P. concavitympanum* and *P. dibernardo*, tubercles not organized
195 in rows in *P. goyana*). From *P. concavitympanum*, *P. ararype* differs by the number, size, and
196 disposition of tubercles on the dorsal region: tubercles more concentrated on the head, arms, legs
197 and pre-sacral constriction; less concentrated and smaller tubercles on the flanks, sacral-urostyle,
198 and coccyx regions (tubercles uniformly distributed on the dorsal region with no variation in size
199 in *P. concavitympanum*).

200 A cream ventral region with dark-brown vermiculations on the gular region, chest and
201 belly, distinguishes *P. ararype* from *P. branti*, *P. carranca*, *P. cristiceps*, *P. redacta* and *P.*
202 *strussmannae* (dark spots in *P. branti* and *P. carranca*, light-brown dots on the gular region and
203 chest, or ventral surface cream in *P. cristiceps*, gular region blackish, scattered small, dark-brown
204 dots on the chest in *P. redacta*, and on the chest and belly in *P. strussmannae*).

205 *Proceratophrys ararype* can be distinguished from the sympatric species *P. cristiceps* by
206 the advertisement call with higher number of pulses/second (96.8–102.7; 87.4–91.9 pulses/s in *P.*
207 *cristiceps*) and higher dominant frequency (1033.6 – 1205.9 Hz; 900 – 990 Hz in *P. cristiceps*).
208 The advertisement call also distinguishes *P. ararype* from *P. huntingtoni* and *P. moratoi* by the
209 longer duration (0.430 – 0.649 s; 0.200 – 0.320 s in *P. huntingtoni*, 0.179 – 0.335 s in *P.*
210 *moratoi*), the higher number of pulses/call (96.8 – 102.7; 19 – 25 in *P. huntingtoni*, 12 – 26
211 pulses/call in *P. moratoi*) and higher number of pulses/second (96.8 – 102.7; 78 – 95 in *P.*
212 *huntingtoni*, 69 – 86 pulses/second in *P. moratoi*).

213 **Description of the holotype.**—Head wider than long, head length 46% of SVL, snout
214 rounded in dorsal and ventral views, obtuse and vertical in profile; nares elliptical and prominent,
215 canthal crests marked, prominent and covered by small tubercles; no preocular crests; eyes
216 directed anterolaterally, eye diameter 22% of head length and 92% of the palpebral appendage;
217 eyelid with short and fused warts, five warts on the left eyelid and four on the right (L 1, 2/5, 3; R
218 1/2/5, 2), with one larger and more prominent; presence of one row of tubercles on the eyelid;
219 indistinct tympanum; vomerine teeth in two short rows between the choanae; frontoparietal
220 crests well developed; region between frontoparietal crests shallow; interocular ridge of warts
221 organized in a row, markedly curved; ocular-dorsal ridge of warts complete from the eyes to the
222 coccyx region; two parallel rows of warts on the supra-scapulae region. Dorsal surface, including
223 flanks, arms and legs, with various warts of different sizes and shapes, one row of close and
224 pointed forearm tubercles, reaching the hands; ventral surfaces, except hands and feet, covered by
225 numerous small, rounded, uniform warts. Finger lengths I > II < III > IV (Fig. 1); interdigital
226 webbing absent; inner metacarpal tubercle large and elliptical; outer metacarpal divided in two
227 parts, the internal oval and the external elliptical; scarce small rounded supernumerary tubercles;
228 subarticular tubercles large, rounded, but grooved anteriorly and posteriorly. Thigh length longer
229 than tibia length, the sum of thigh and tibia lengths 79% of snout-vent length; toe lengths I < II <
230 III < IV > V; inner metatarsal tubercle large, elliptical, spatulated; outer metatarsal tubercle
231 small, rounded; scarce small rounded supernumerary tubercles; subarticular tubercles large,
232 nearly rounded, grooved anteriorly and posteriorly.

233 **Measurements of the holotype (mm).**—SVL 44.3; HL 20.6; HW 13.0; DICS 10.4; IND
234 2.6; END 4.2; ED 4.5; UEW 4.9; IOD 2.9; THL 18.8; TL 16.2; FL 24.8; FHL 23.2.

235 **Color in life of the holotype.**—Based on photographs of the holotype (Fig. 3A). Dorsal
236 background color grey, maculated with variegate brown and dark-brown pattern, resembling dead

237 leaves. Area delimited by the symmetrical dorsal crest light brown, bordered along the external
238 sides by a dark-brown band with four wave-like blotches on each side. Two brown bands from
239 the eye to the upper lip. From two to three transverse dark-grey bars on the fingers and toes.

240 **Color in preservative of the holotype.**—Dorsal background color dark-grey. Area
241 delimited by the symmetrical dorsal crest light grey, bordered along external sides by a black
242 band with four wave-like blotches on each side. Two dark-grey bands from the eye to the upper
243 lip. From two to three transverse dark-grey bars on the fingers and toes. Ventral surface
244 background color cream with vermiculations dark-brown in the gular region, chest and belly (Fig.
245 1B).

246 **Variation.**—Some individuals do not present a complete ocular-dorsal ridge of warts
247 (CHUFPE 161, adult male; CHUFPE 227, juvenile). One individual (CHUFPE 227, juvenile)
248 presents a cream dorsal background color. The measurements of the type series are provided in
249 Table 3.

250 **Advertisement call.**—The advertisement call of *Proceratophrys ararype* consists of a
251 pulsed note (Fig. 4) with a duration of 0.526 ± 0.05 seconds ($0.430 - 0.649$), emitted sporadically
252 with 52 ± 5.3 pulses/note ($42 - 65$), 99.2 ± 0.9 pulses/s ($96.8 - 102.7$), and a dominant frequency
253 of $1.205.9 \pm 99.5$ Hz ($1033.6 - 205.9$).

254 **Geographic distribution.**—*Proceratophrys ararype* **sp. nov.** is known from the type
255 locality at Mata do Clube Recreativo Grangeiro, from Clube Serrano Atlético Cratense, and from
256 Sítio Caianas, Crato Municipality, Ceará State, Brazil (Fig. 5). A phylogeographic project being
257 conducted by SM (in preparation) has analyzed *Proceratophrys* populations throughout
258 northeastern Brazil, comparing specimens morphologically and molecularly. With 51 localities
259 analyzed, including several other “Brejos de Altitude”, *P. ararype* only occurs at Chapada do
260 Araripe.

261 **Etymology.**—The Araripe Plateau limits the Ceará, Pernambuco and Piauí States.
262 "Araripe", from the ancient tupi, *ararype*, means "on the araras river" (*arara*, arara + 'y, river +
263 *pe*, on the. Arara means Macaw in tupi and in Portuguese). Because the new species is known
264 only from the Araripe region, we name it after its distribution area. The specific epithet *ararype*
265 is treated as a noun in apposition.

266 **Environment and habitat.**—The holotype and paratopotypes were collected in a forest
267 remnant at the slopes of Araripe Plateau, within the limits of a recreational club (Clube
268 Grangeiro). The area has suffered significant anthropogenic modification, including the
269 plantation of exotic trees (such as coconut, *Cocus nocifera*) and the use of trails for hiking. Palm
270 trees (Arecaceae) cover most of the area and the local drainage is composed of several permanent
271 streams that emerge from springs on the slopes of Chapada do Araripe. Many of these streams
272 and rivulets are partially or fully channeled for local supply, presenting a problem for *P. ararype*,
273 which uses lotic environments for reproduction. Some specimens (CHUFPE 152, 156, 160 and
274 161) were collected on December 2014, around 20:00h to 22:00h. Males were calling along a
275 permanent stream with males of other species (*Physalaemus cuvieri*, *Adenomera* sp. and
276 *Dendropsophus minutus*). The specimens registered on May 2015 (CHUFPE 226, 227) were
277 collected in pitfall traps. We also recorded the species in Sítio Caianas, located at the slopes of
278 Chapada do Araripe. There, the main habitat is humid forest alongside the streams, where males
279 of *Proceratophrys ararype* were found calling in the leaf litter near to the streams (10 - 50cm).
280 Other species found in calling activity were *Physalaemus cuvieri*, *Adenomera* sp. and *Scinax* gr.
281 *ruber*.

282 **Molecular phylogeny and genetic divergence of the new species.**—Our 16S mtDNA
283 (Fig. 6) confirmed that *P. cristiceps* populations occur at the Araripe plateau region, showing that
284 the *P. ararype* sp. nov. occurs sympatrically with *P. cristiceps*. However, both species belong to

285 two distant lineages within *Proceratophrys*. Sequence divergences between *P. ararype* and *P.*
286 *cristiceps* for 16S mtDNA is about 8% with uncorrected p-distance; and 4% between *P. ararype*
287 and *P. concavitympanum* from Aripuanã Municipality, Mato Grosso State (Table 4).

288 The topology of the species tree (Fig. 7) recovered *Proceratophrys ararype* as sister taxa
289 of *P. concavitympanum* from Palmas Municipality, Tocantins State, Brazil. *Proceratophrys*
290 *concavitympanum* from Aripuanã Municipality, Mato Grosso State, Brazil, forms a well-
291 supported clade with the previous species (1.00 pp). The molecular dating analyses indicated that
292 the divergence between *P. ararype* and *P. concavitympanum* from Palmas took place
293 approximately 1.4 Mya during Pleistocene. The clade formed by these two species diverge from
294 *P. concavitympanum* from Aripuanã approximately 5.2 Mya, during the end of Miocene and
295 beginning of Pliocene (Fig. 7).

296

297 DISCUSSION

298 Some species occurring in natural rainforest enclaves in Northeastern Brazil (*Brejos de*
299 *altitude*) support preterit connections between these remnants and the Amazon and Atlantic
300 Forests. For example, phylogenetic and comparative studies recovered *Adelophryne baturitensis*,
301 *A. maranguapensis*, and *Rhinella casconi* more closely related to forest congeners (Fouquet et al.
302 2012; Roberto and Loebmann 2016). Likewise, some snake species reinforce preterit connections
303 of Chapada do Araripe with the Amazon, including *Anylius scitale*, *Bothrops* aff. *atrox*, and
304 *Drymoluber dichrous* (Ribeiro et al. 2012; Roberto and Loebmann 2016). The same pattern was
305 recovered for *Proceratophrys ararype*, a sister to the Amazonian *P. concavitympanum*,
306 supporting that Araripe forests were once previously connected with the Amazon forest.

307 *Proceratophrys ararype* diverged from an ancestor shared with *P. concavitympanum*
308 during the Pleistocene, probably as a result of forest expansions and contractions resulting from

309 climatic cycles (Por 1992). High altitude areas in Ceará State are frequently inhabited by moist
310 forest species, which likely originated from ancestors from the Amazon and/or Atlantic forests
311 (Roberto and Loebmann, 2016). Therefore, *P. concavitympanum* was likely more widespread and
312 one population became isolated in forest remnants around the Araripe Plateau as forests retreated
313 in response to the overall cooling of the planet during the Pleistocene. This population remained
314 surrounded by the Caatinga semi-arid vegetation leading to its divergence in isolation from *P.*
315 *concavitympanum*.

316 *Proceratophrys cristiceps* occurs sympatrically with *P. ararype* in the Araripe Plateau
317 region. Previous works considered *P. cristiceps* to occur in the Caatinga and in the humid forest
318 slopes of the Araripe Plateau (Ribeiro et al., 2016). However, *P. cristiceps* seems to be restricted
319 to the Cerradão phytophysionomy (drymorphic subdeciduous foliage tropical forest – Figueiredo
320 1990; Bezerra et al. 2003) at the top of the Araripe Plateau and to the Caatinga at lower elevation
321 areas, whereas *P. ararype* is endemic to the humid forests.

322 The phylogenetic diversity within *P. concavitympanum* is likely underestimated. The first
323 phylogenetic tree reconstructed for the genus *Proceratophrys* used a sample from Palmas
324 Municipality (Tocantins State, Brazil) representing *P. concavitympanum* (Amaro et al. 2009). In
325 our study, we use samples from Aripuanã Municipality, Mato Grosso State (see Fig. 6), which is
326 much closer (200km versus 1400km in straight line) to the type locality of *P. concavitympanum*
327 (Espigão d'Oeste Municipality, Rondônia State, Brazil). Based on morphology, genetic and
328 geographic distances, and previous morphologic and acoustic analyses (Santana et al. 2010) we
329 consider the population of Aripuanã as *P. concavitympanum*. Hence, our results indicate that the
330 population of *Proceratophrys* from Palmas is yet another undescribed species. Such
331 underestimation may have important consequences for phylogenetic reconstructions, ecology,
332 and conservation.

333 Indeed, the new species described herein occurs in the same habitat as the critically
334 endangered Araripe manakin (*Antilophia bokermanni*), the endangered freshwater crab *Kingsleya*
335 *attenboroughi*, and the endangered snake *Atractus ronnie*, the humid forests of Chapada do
336 Araripe (BirdLife International 2015; Pinheiro and Santana 2016; Roberto and Loebmann 2016).
337 Although the area is inserted in the sustainable use Environment Protected Area of Araripe
338 Plateau, this protection category is the least restrictive of the Brazilian legislation. Furthermore,
339 this region is highly deforested and streams have been canalized for agriculture and recreational
340 uses. These are the main threats for the humid forest in the slopes of Araripe Plateau, and
341 consequently for the maintenance of the water aquifers in the region (Linhares et al. 2010) where
342 *P. ararype* occurs and reproduces. The conservation of these humid forests is therefore critical
343 for the species long-term survival. Furthermore, *P. ararype* known range is less than 5.000 km²
344 (3.100 km²). Thus, based on the criteria and categories of the IUCN Red List B1 (Version 3.1,
345 2012), we consider *P. ararype* endangered (EN).

346
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APPENDIX

Specimens examined

Proceratophrys aridus.—BRAZIL: Ceará: Milagres: MNRJ 55349, 55778-822, 75156-68; URCA-H 106, 142-43.

Proceratophrys caramaschii.—BRAZIL: Ceará: Mucuripe: MNRJ 1419-20, 1680, 16470-84, 16487-89, 16591-600.

Proceratophrys concavitympanum.—BRAZIL: Mato Grosso: Alta Floresta: ZUEC 21201. Aripuanã: MZUFV 9552, 9554-56. Paranaíta: ZUEC 14505-06, 16011-15, 16719, 21201. Pará: Paraopebas: PUC-MG 10561, 11206. Rondônia: Espigão do Oeste: MZUFV 10477.

Proceratophrys cristiceps.—BRAZIL: 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. Ubajara, Parque Nacional de Ubajara: AAGARDA 10672, 10695, 10698-99, 10703, 10707-09, 10782, 10796, 10907, 10909, 10911-14, 10961, 10974, 10981, 10983. Várzea da Conceição: UFPB 9661, 9665, 9667. 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,

549 9701. Betânia: UFC 3331. Bezerros: UFPB 7098. Exu: URCA 1462-63; UFPB 7214-17.
550 Nascente: UFPB 9670. Ouricuri: URCA 2988-89. Buíque, Parque Nacional do Catimbau:
551 AAGARDA 7706-12, 7747, 7760-61, 7765, 7799, 7802, 7804-05, 7824, 7886, 7975, 8056, 8362,
552 8417, 8435, 8437-40, 8450, 8463. Serra Talhada: UFPB 9656, 9659, 9660. Trindade: UFPB 974,
553 9673-77. Piauí: Floriano: UFPI 214-16, 222, 236. Piripiri: UFPB 10340, 10342-46. Rio Grande
554 do Norte: Serra Negra do Norte, Estação Ecológica do Seridó: AAGARDA 5447, 5528, 5583,
555 5689, 6061, 6790. João Câmara: AAGARDA 8913-15, 9806-11; URCA 422, 427, 483-85, 487-
556 88, 493, 498, 501. Macaíba, Escola Agrícola de Jundiá: AAGARDA 1013-14, 1019-20, 1753-
557 71, 1773, 1776, 1778, 1786-91, 1935, 2495-96, 2583, 3757, 5447, 5528, 5554, 5583, 5689, 6061,
558 6790, 8866-71, 8913-15, 9806-11. Sergipe: Poço Redondo: UFPB 12120-21, 12125-27.
559 *Proceratophrys minuta*.—BRAZIL: Bahia: Miguel Calmon, Parque Estadual das Sete
560 Passagens: UFBA 6229-30, 6716-20, 6722, 6725-26.
561 *Proceratophrys redacta*.—BRAZIL: Bahia: Morro do Chapéu: PUC-MG 7910-11, 7913;
562 UFMG 6049-57.
563 *Proceratophrys schirchi*.—BRAZIL: Minas Gerais: Santa Maria do Salto: PUC-MG
564 4021.

TABLE 1.—Acoustic parameter of the advertisement calls of species from the *Proceratophrys cristiceps* group.

Species	Duration (s)	Notes/call	Pulses/note	Pulses/s	Dominant frequency (Hz)	Location	Source
<i>P. ararype</i> sp. nov.	0.526 ± 0.05 (0.430 – 0.648)	1	52 ± 5.3 (42 – 65)	99.2 ± 0.9 (96.8 – 102.7)	1205.9 ± 99.5 (1033.6 – 1205.9)	Crato, CE	Present work
<i>P. caramaschii</i>	0.570 ± 0.01 (0.410 – 0.740)	1	45 ± 9.2 (33 – 59)	80.0 ± 0.9 (78.6 – 81.8)	860 or 1030	Planalto do Ibiapaba, CE	Nunes et al. (2016)
<i>P. carranca</i>	0.107 ± 0.04 (0.045 – 0.191)	1 – 10	12.2 ± 3.9 (5 – 21)	111.1 – 109.9	1178.0 ± 65.5 (1033.6 – 1378.1)	Buritizeiro, MG	Godinho et al. (2013)
<i>P. concavitympanum</i>	0.367 ± 0.06 (0.230 – 0.500)	1	38.7 ± 7.4 (23 – 51)	106.3 ± 3.1 (100 – 112.3)	948.2 ± 66.7 (851.0 – 1116.4)	Aripuanã, MT	Santana et al. (2010)
<i>P. concavitympanum</i>	0.278 ± 0.04 (0.178 – 0.326)	1	30.9 ± 4.8 (19 – 37)	110.9 ± 5.2 (100.7 – 119.3)	819.2 ± 62.2 (754.3 – 874.5)	Espigão do Oeste, RO	Santana et al. (2010)
<i>P. cristiceps</i>	0.660 ± 0.05 (0.520 – 0.790)	1	57.5 ± 6.0 (46 – 69)	89.5 ± 1.2 (87.4 – 91.9)	940 ± 20 (900 – 990)	Feira de Santana, BA	Nunes & Jucá (2006)
<i>P. cururu</i>	0.600 – 1.000	1	40	45	900 (600 – 1000)	Serra do Cipó, MG	Eterovick & Sazima (1998)
<i>P. goyana</i>	0.104 ± 0.15 (0.071 – 0.195)	1 – 34	99.3 ± 4.4 (83.3 – 120.5)	10.2 ± 1.4 (7 – 18)	1005.9 ± 28.4 (937.3 – 1125.0)	Chapada dos Veadeiros, GO	Martins & Giaretta (2013)
<i>P. huntingtoni</i>	0.270 ± 0.01 (0.200 – 0.320)	1	21.3 ± 1.2 (19.0 – 25.0)	78 – 95	1250.2 ± 49.9 (1095.0 – 1344.5)	Chapada dos Guimarães, MT	Ávila et al. (2012)
<i>P. moratoi</i>	0.245 ± 0.03 (0.185 – 0.307)	1	20.5 ± 2.5 (15 – 26)	81 – 85	1343.0 ± 73.7 (1174 – 1444)	Itirapina, SP	Brasileiro et al. (2008)
<i>P. moratoi</i>	0.207 ± 0.02 (0.146 – 0.238)	1	17.5 ± 1.5 (12 – 20)	82 – 84	1348.7 – 86.6 (1153 – 1420)	Botucatu, SP	Brasileiro et al. (2008)
<i>P. moratoi</i>	0.232 ± 0.02 (0.181 – 0.268)	1	19 ± 3.0 (14 – 23)	77 – 86	1440 ± 50 (1406 – 1594)	Ituiutaba, MG	Martins e Giaretta (2012)
<i>P. moratoi</i>	0.253 ± 0.04 (0.179 – 0.335)	1	19 ± 2.0 (14 – 23)	69 – 78	1327 ± 108 (1219 – 1464)	Uberlândia, MG	Martins e Giaretta (2012)
<i>P. rotundipalpebra</i>	0.093 ± 0.04	1 – 24	9.3 ± 1.7	101.0 ± 8.9	1287 ± 54.4	Chapada dos	Martins & Giaretta

	(0.050 – 0.200)		(5 – 19)	(78.1 – 130.4)	(1125.0 – 1453.1)	Veadeiros, GO	(2013)
<i>P. vielliardi</i>	0.059 ± 0.08	3 – 20	6.4 ± 0.9	107.7 ± 6.2	1133.8 ± 93.3	Caldas Novas, GO	Martins & Giaretta (2011)

TABLE 2.—Information of all specimens examined in the molecular section of this study (16s rRNA and Rhodopsin).

Species	Municipality, State	Voucher	Genbank		Reference
			Accession number		
			Rhodopsin	16S	
<i>Proceratophrys ararype</i> sp. nov.	Crato, Ceará	CHUFPE156	XXX	KX858852	This study
<i>Proceratophrys ararype</i> sp. nov.	Crato, Ceará	CHUFPE152	XXX	KX858854	This study
<i>Proceratophrys ararype</i> sp. nov.	Crato, Ceará	CHUFPE160	XXX	KX858853	This study
<i>Proceratophrys ararype</i> sp. nov.	Crato, Ceará	AAGARDA2736	XXX	KX855986	This study
<i>Proceratophrys ararype</i> sp. nov.	Crato, Ceará	AAGARDA2741	XXX	KX855987	This study
<i>Proceratophrys concavitympanum</i>	Aripuanã, Mato Grosso	FMT-A 11698	XXX	KX858855	This study
<i>Proceratophrys concavitympanum</i>	Palmas, Tocantins	AF1094	KF214207	FJ685694	Amaro et al. 2009
<i>Proceratophrys moratoi</i>	Itirapina, São Paulo	CFBH6515	KF214213	FJ685689	Amaro et al. 2009
<i>Proceratophrys tupinamba</i>	Ilha Grande, Rio de Janeiro	MNRJ54541	KF214236	KF214158	Dias et al. 2013
<i>Proceratophrys goyana</i>	Petrolina de Goiás, Goiás	AF1188	KF214210	FJ685697	Amaro et al. 2009
<i>Proceratophrys mantiqueira</i>	Araponga, Minas Gerais	MZUFV10139	KF214222	KF214143	Dias et al. 2013
<i>Proceratophrys avelinoi</i>	Misiones, Argentina	DB1246	KF214204	FJ685691	Amaro et al. 2009
<i>Proceratophrys laticeps</i>	Linhares, Espírito Santo	MTR12156	KF214211	FJ685698	Amaro et al. 2009
<i>Proceratophrys boiei</i>	São Paulo, São Paulo	AF1587	KF214206	FJ685693	Amaro et al. 2009
<i>Proceratophrys itamari</i>	Campos do Jordão, São Paulo	MZUSP135186	KF214226	KF214147	Dias et al. 2013
<i>Proceratophrys izecksohni</i>	Paraty, Rio de Janeiro	MNRJ64584	KF214235	KF214157	Dias et al. 2013
<i>Proceratophrys belzebul</i>	São Sebastião, São Paulo	MTR9456	KF214233	KF214233	Dias et al. 2013
<i>Proceratophrys melanopogon</i>	São José do Barreiro, São Paulo	TG3295	KF214228	KF214149	Dias et al. 2013
<i>Proceratophrys pombali</i>	Bertioga, São Paulo	AF1988	KF214223	KF214148	Dias et al. 2013
<i>Proceratophrys cururu</i>	Cardeal Mota, Minas Gerais	FSFL580	KF214209	FJ685696	Amaro et al. 2009
<i>Proceratophrys appendiculata</i>	São Sebastião, São Paulo	MNRJ53936	KF214231	FJ685690	Amaro et al. 2009
<i>Proceratophrys bigibbosa</i>	Misiones, Argentina	DB2313	KF214205	FJ685692	Amaro et al. 2009
<i>Proceratophrys renalis</i>	Brejo da Madre de Deus, Pernambuco	ZUFRJ8682	KF214213	FJ685700	Amaro et al. 2009
<i>Proceratophrys schirchi</i>	Santa Tereza, Espírito Santo	371	KF214214	FJ685701	Amaro et al. 2009
<i>Proceratophrys cristiceps</i>	Macaíba, Rio Grande do Norte	AAGARDA1754	-	KX855989	This study

<i>Proceratophrys cristiceps</i>	Crato, Ceará	AAGARDA2739	XXX	KX855993	This study
<i>Proceratophrys cristiceps</i>	Crato, Ceará	AAGARDA2740	XXX	KX855992	This study
<i>Proceratophrys cristiceps</i>	Crato, Ceará	AAGARDA2735	XXX	KX855990	This study
<i>Proceratophrys cristiceps</i>	Crato, Ceará	AAGARDA2736	-	KX855986	This study
<i>Proceratophrys cristiceps</i>	Crato, Ceará	AAGARDA2738	-	XXX	This study
<i>Proceratophrys cristiceps</i>	Crato, Ceará	AAGARDA2741	-	KX855987	This study
<i>Proceratophrys cristiceps</i>	Crato, Ceará	UnB_GRC_21987	-	XXX	This study
<i>Proceratophrys cristiceps</i>	Crato, Ceará	UnB_GRC_22131	-	XXX	This study
<i>Proceratophrys cristiceps</i>	Crato, Ceará	UnB_GRC_22955	-	XXX	This study
<i>Proceratophrys minuta</i>	Miguel Calmon, Bahia	MZUSP146499	KF214215	JX982965	Teixeira-Jr et al. 2012
<i>Proceratophrys redacta</i>	Morro do Chapéu, Bahia	MZUSP150266	KF214216	JX982967	Teixeira-Jr et al. 2012
<i>Odontophrynus americanus</i>	Poços de Caldas, Minas Gerais	AF665	KF214201	FJ685686	Amaro et al. 2009
<i>Macrogenioglottus alipioi</i>	Jussari, Bahia	AF919	KF214199	FJ685684	Amaro et al. 2009
<i>Cycloramphus acangatan</i>	Cotia, São paulo	AF1605	KF214198	FJ685683	Amaro et al. 2009

TABLE 3.—Measurements (mm) of specimens of *Proceratophrys ararype*.

	<i>P. ararype</i> sp. nov.	
	Males (n=8)	Females (n=3)
SLV	38.7 ± 2.6 35.6 – 42.2	54.1 ± 4.1 51.3 – 57.0
HW	16.6 ± 1.4 15.1 – 19.0	24.3 ± 1.9 23.0 – 25.6
HL	11.7 ± 1.3 10.5 – 13.9	16.7 ± 0.9 16.1 – 17.3
DICS	8.1 ± 1.1 7.9 – 10.3	11.8 ± 0.5 11.5 – 12.2
IND	2.6 ± 0.6 2.1 – 3.7	3.6 ± 0.8 3.0 – 4.1
END	3.6 ± 0.2 3.3 – 3.9	4.4 ± 0.1 4.4 – 4.5
ED	5.4 ± 0.5 4.7 – 6.0	5.6 ± 0.8 5.0 – 6.2
UEW	4.9 ± 0.4 4.2 – 5.2	6.6 ± 1.1 5.8 – 7.4
IOD	3.0 ± 0.2 2.8 – 3.3	4.0 ± 0.1 3.9 – 4.0
THL	14.7 ± 1.4 13.1 – 16.9	20.7 ± 2.1 19.2 – 22.2
TL	14.5 ± 1.1 12.8 – 15.4	20.3 ± 2.2 18.7 – 21.8
FL	22.4 ± 1.3 21.0 – 24.0	30.7 ± 2.9 28.7 – 32.8
FHL	19.4 ± 1.2 18.1 – 21.3	25.0 ± 0.5 24.6 – 25.4

TABLE 4.—Uncorrected p-distances of 16S mitochondrial fragment gene of the new species described *Proceratophrys ararype* and 28 other related specimens taken from GenBank.

		1	2	3	4	5	6	7	8	9	10	11	12
1	KX855986 <i>P. ararype</i> sp. nov.												
2	KX855987 <i>P. ararype</i> sp. nov.	0.000											
3	KX858854 <i>P. ararype</i> sp. nov.	0.002	0.002										
4	KX858852 <i>P. ararype</i> sp. nov.	0.002	0.002	0.000									
5	KX858853 <i>P. ararype</i> sp. nov.	0.002	0.002	0.000	0.000								
6	FJ685683 <i>C. acangatan</i>	0.122	0.122	0.124	0.124	0.124							
7	FJ685684 <i>M. alipioi</i>	0.105	0.105	0.107	0.107	0.107	0.127						
8	FJ685686 <i>O. americanus</i>	0.088	0.088	0.090	0.090	0.090	0.112	0.063					
9	FJ685689 <i>P. moratoi</i>	0.046	0.046	0.049	0.049	0.049	0.134	0.112	0.095				
10	FJ685691 <i>P. avelinoi</i>	0.071	0.071	0.073	0.073	0.073	0.134	0.095	0.085	0.071			
11	FJ685692 <i>P. bigibbosa</i>	0.076	0.076	0.078	0.078	0.078	0.134	0.098	0.083	0.076	0.041		
12	FJ685693 <i>P. boiei</i>	0.076	0.076	0.078	0.078	0.078	0.139	0.088	0.090	0.071	0.068	0.068	
13	FJ685694 <i>P. concavitypanu</i> TO	0.012	0.012	0.015	0.015	0.015	0.124	0.105	0.085	0.054	0.071	0.076	0.076
14	FJ685696 <i>P. cururu</i>	0.080	0.080	0.083	0.083	0.083	0.132	0.098	0.078	0.071	0.046	0.039	0.066
15	FJ685697 <i>P. goyana</i>	0.066	0.066	0.068	0.068	0.068	0.127	0.088	0.078	0.066	0.054	0.039	0.049
16	FJ685698 <i>P. laticeps</i>	0.071	0.071	0.073	0.073	0.073	0.120	0.080	0.071	0.059	0.044	0.039	0.051
17	FJ685700 <i>P. renalis</i>	0.090	0.090	0.093	0.093	0.093	0.144	0.100	0.085	0.076	0.059	0.056	0.063
18	FJ685701 <i>P. schirchi</i>	0.083	0.083	0.085	0.085	0.085	0.132	0.078	0.076	0.066	0.063	0.061	0.076
19	JX982965 <i>P. minuta</i>	0.085	0.085	0.088	0.088	0.088	0.124	0.110	0.090	0.095	0.078	0.056	0.093
20	JX982967 <i>P. redacta</i>	0.090	0.090	0.093	0.093	0.093	0.124	0.110	0.090	0.100	0.076	0.054	0.093
21	KF214143 <i>P. mantiqueira</i>	0.068	0.068	0.071	0.071	0.071	0.127	0.100	0.073	0.063	0.039	0.037	0.066
22	KF214144 <i>P. pombali</i>	0.080	0.080	0.083	0.083	0.083	0.129	0.085	0.085	0.068	0.054	0.049	0.054

23	KF214147 <i>P. itamari</i>	0.071	0.071	0.073	0.073	0.073	0.127	0.090	0.073	0.063	0.039	0.034	0.056
24	KF214149 <i>P. melanopogon</i>	0.076	0.076	0.078	0.078	0.078	0.137	0.100	0.080	0.076	0.046	0.046	0.068
25	KF214151 <i>P. appendiculata</i>	0.063	0.063	0.066	0.066	0.066	0.127	0.088	0.073	0.051	0.044	0.039	0.049
26	KF214154 <i>P. belzebul</i>	0.073	0.073	0.076	0.076	0.076	0.129	0.100	0.083	0.061	0.051	0.051	0.063
27	KF214157 <i>P. izecksohni</i>	0.073	0.073	0.076	0.076	0.076	0.127	0.095	0.073	0.068	0.034	0.029	0.061
28	KF214158 <i>P. tupinamba</i>	0.061	0.061	0.063	0.063	0.063	0.124	0.090	0.071	0.056	0.041	0.037	0.051
29	KX858855 <i>P. concavitympanum</i> MT	0.041	0.041	0.044	0.044	0.044	0.132	0.105	0.095	0.068	0.071	0.073	0.085
30	KX855989 <i>P. cristiceps</i> RN	0.085	0.085	0.088	0.088	0.088	0.127	0.093	0.088	0.083	0.078	0.078	0.098
31	KX855993 <i>P. cristiceps</i> CE	0.085	0.085	0.088	0.088	0.088	0.127	0.093	0.088	0.083	0.078	0.078	0.098
32	KX855990 <i>P. cristiceps</i> CE	0.088	0.088	0.090	0.090	0.090	0.129	0.095	0.090	0.085	0.080	0.080	0.100
33	KX855992 <i>P. cristiceps</i> CE	0.085	0.085	0.088	0.088	0.088	0.127	0.093	0.088	0.083	0.078	0.078	0.098
34	XXXXXX <i>P. cristiceps</i> CE_21987	0.085	0.085	0.088	0.088	0.088	0.127	0.093	0.088	0.083	0.078	0.078	0.098
35	XXXXXX <i>P. cristiceps</i> CE_22131	0.085	0.085	0.088	0.088	0.088	0.127	0.093	0.088	0.083	0.078	0.078	0.098
36	XXXXXX <i>P. cristiceps</i> CE_22955	0.088	0.088	0.090	0.090	0.090	0.129	0.090	0.090	0.085	0.080	0.080	0.095

TABLE 4.—(continued) Uncorrected p-distances of 16S mitochondrial fragment gene of the new species described

Proceratophrys ararype and 28 other related specimens taken from GenBank.

	12	13	14	15	16	17	18	19	20	21	22	23
1 KX855986 <i>P. ararype</i> sp. nov.												
2 KX855987 <i>P. ararype</i> sp. nov.												
3 KX858854 <i>P. ararype</i> sp. nov.												
4 KX858852 <i>P. ararype</i> sp. nov.												
5 KX858853 <i>P. ararype</i> sp. nov.												
6 FJ685683 <i>C. acangatan</i>												
7 FJ685684 <i>M. alipioi</i>												
8 FJ685686 <i>O. americanus</i>												
9 FJ685689 <i>P. moratoi</i>												
10 FJ685691 <i>P. avelinoi</i>												
11 FJ685692 <i>P. bigibbosa</i>												
12 FJ685693 <i>P. boiei</i>												
13 FJ685694 <i>P. concavitypanu</i> TO	0.076											
14 FJ685696 <i>P. cururu</i>	0.066	0.078										
15 FJ685697 <i>P. goyana</i>	0.049	0.063	0.037									
16 FJ685698 <i>P. laticeps</i>	0.051	0.068	0.024	0.032								
17 FJ685700 <i>P. renalis</i>	0.063	0.088	0.032	0.044	0.037							
18 FJ685701 <i>P. schirchi</i>	0.076	0.083	0.056	0.063	0.051	0.066						
19 JX982965 <i>P. minuta</i>	0.093	0.083	0.073	0.068	0.068	0.090	0.071					
20 JX982967 <i>P. redacta</i>	0.093	0.088	0.078	0.076	0.071	0.093	0.076	0.027				
21 KF214143 <i>P. mantiqueira</i>	0.066	0.073	0.027	0.046	0.034	0.049	0.054	0.073	0.071			
22 KF214144 <i>P. pombali</i>	0.054	0.080	0.039	0.037	0.039	0.056	0.061	0.078	0.083	0.037		

23	KF214147	<i>P. itamari</i>	0.056	0.071	0.024	0.041	0.024	0.046	0.049	0.068	0.066	0.010	0.032	
24	KF214149	<i>P. melanopogon</i>	0.068	0.080	0.034	0.049	0.037	0.059	0.059	0.080	0.078	0.022	0.034	0.017
25	KF214151	<i>P. appendiculata</i>	0.049	0.063	0.039	0.041	0.029	0.044	0.049	0.063	0.071	0.039	0.044	0.029
26	KF214154	<i>P. belzebul</i>	0.063	0.073	0.041	0.049	0.037	0.051	0.061	0.073	0.080	0.041	0.046	0.032
27	KF214157	<i>P. izecksohni</i>	0.061	0.073	0.024	0.037	0.029	0.046	0.049	0.063	0.061	0.010	0.032	0.005
28	KF214158	<i>P. tupinamba</i>	0.051	0.061	0.037	0.039	0.032	0.041	0.049	0.061	0.068	0.037	0.041	0.032
29	KX858855	<i>P. concavitympanum</i> MT	0.085	0.046	0.080	0.071	0.076	0.095	0.088	0.080	0.088	0.073	0.076	0.073
30	KX855989	<i>P. cristiceps</i> RN	0.098	0.088	0.076	0.088	0.068	0.078	0.071	0.059	0.073	0.071	0.080	0.076
31	KX855993	<i>P. cristiceps</i> CE	0.098	0.088	0.076	0.088	0.068	0.078	0.071	0.059	0.073	0.071	0.080	0.076
32	KX855990	<i>P. cristiceps</i> CE	0.100	0.090	0.078	0.090	0.071	0.080	0.073	0.061	0.076	0.073	0.083	0.078
33	KX855992	<i>P. cristiceps</i> CE	0.098	0.088	0.076	0.088	0.068	0.078	0.071	0.059	0.073	0.071	0.080	0.076
34	XXXXXX	<i>P. cristiceps</i> CE_21987	0.098	0.088	0.076	0.088	0.068	0.078	0.071	0.059	0.073	0.071	0.080	0.076
35	XXXXXX	<i>P. cristiceps</i> CE_22131	0.098	0.088	0.076	0.088	0.068	0.078	0.071	0.059	0.073	0.071	0.080	0.076
36	XXXXXX	<i>P. cristiceps</i> CE_22955	0.095	0.090	0.078	0.085	0.071	0.080	0.073	0.056	0.071	0.073	0.078	0.078

TABLE 4.—(continued) Uncorrected p-distances of 16S mitochondrial fragment gene of the new species described

Proceratophrys ararype and 28 other related specimens taken from GenBank.

	24	25	26	27	28	29	30	31	32	33	34	35
1 KX855986 <i>P. ararype</i> sp. nov.												
2 KX855987 <i>P. ararype</i> sp. nov.												
3 KX858854 <i>P. ararype</i> sp. nov.												
4 KX858852 <i>P. ararype</i> sp. nov.												
5 KX858853 <i>P. ararype</i> sp. nov.												
6 FJ685683 <i>C. acangatan</i>												
7 FJ685684 <i>M. alipioi</i>												
8 FJ685686 <i>O. americanus</i>												
9 FJ685689 <i>P. moratoi</i>												
10 FJ685691 <i>P. avelinoi</i>												
11 FJ685692 <i>P. bigibbosa</i>												
12 FJ685693 <i>P. boiei</i>												
13 FJ685694 <i>P. concavitypanu</i> TO												
14 FJ685696 <i>P. cururu</i>												
15 FJ685697 <i>P. goyana</i>												
16 FJ685698 <i>P. laticeps</i>												
17 FJ685700 <i>P. renalis</i>												
18 FJ685701 <i>P. schirchi</i>												
19 JX982965 <i>P. minuta</i>												
20 JX982967 <i>P. redacta</i>												
21 KF214143 <i>P. mantiqueira</i>												
22 KF214144 <i>P. pombali</i>												

23	KF214147	<i>P. itamari</i>												
24	KF214149	<i>P. melanopogon</i>												
25	KF214151	<i>P. appendiculata</i>	0.046											
26	KF214154	<i>P. belzebul</i>	0.049	0.017										
27	KF214157	<i>P. izecksohni</i>	0.017	0.034	0.037									
28	KF214158	<i>P. tupinamba</i>	0.044	0.007	0.020	0.032								
29	KX858855	<i>P. concavitympanum</i> MT	0.083	0.063	0.073	0.076	0.061							
30	KX855989	<i>P. cristiceps</i> RN	0.083	0.063	0.076	0.076	0.066	0.093						
31	KX855993	<i>P. cristiceps</i> CE	0.083	0.063	0.076	0.076	0.066	0.093	0.000					
32	KX855990	<i>P. cristiceps</i> CE	0.085	0.066	0.078	0.078	0.068	0.095	0.002	0.002				
33	KX855992	<i>P. cristiceps</i> CE	0.083	0.063	0.076	0.076	0.066	0.093	0.000	0.000	0.002			
34	XXXXXX	<i>P. cristiceps</i> CE_21987	0.083	0.063	0.076	0.076	0.066	0.093	0.000	0.000	0.002	0.000		
35	XXXXXX	<i>P. cristiceps</i> CE_22131	0.083	0.063	0.076	0.076	0.066	0.093	0.000	0.000	0.002	0.000	0.000	
36	XXXXXX	<i>P. cristiceps</i> CE_22955	0.085	0.066	0.078	0.078	0.068	0.090	0.002	0.002	0.005	0.002	0.002	0.002

FIGURES

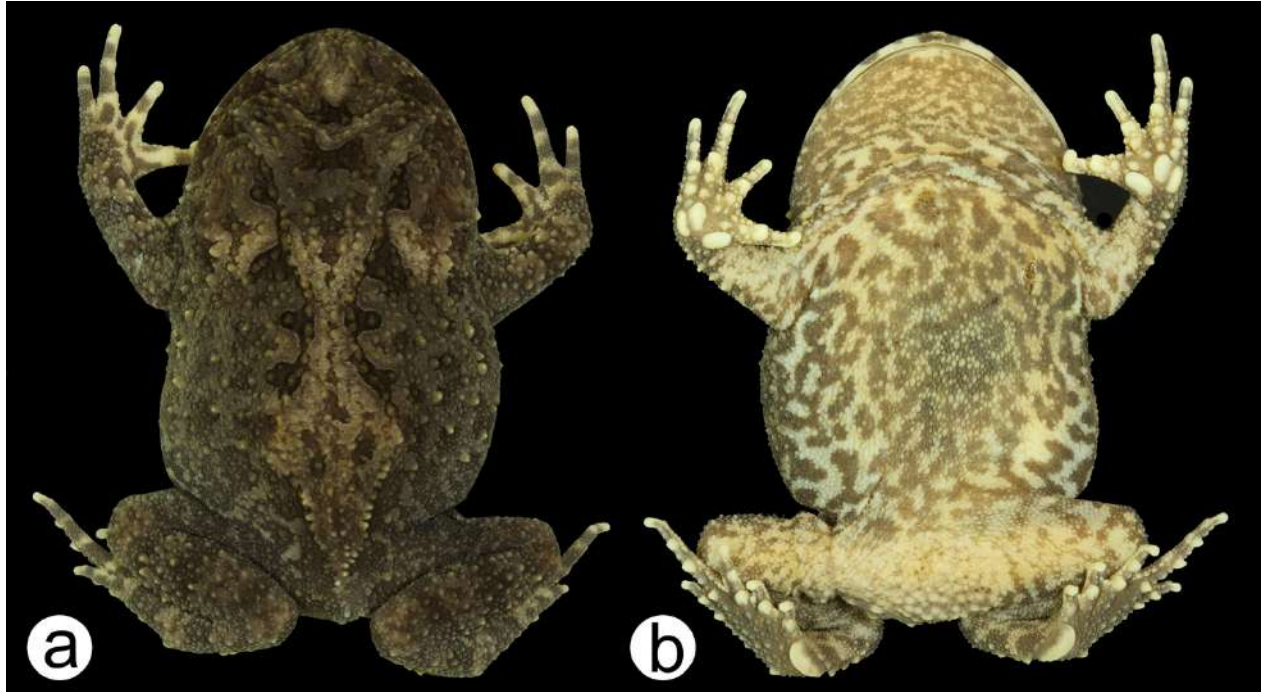


FIG. 1.— *Proceratophrys ararype* (holotype, adult female, CHUFPE 156, SLV 44.3 mm): (A) dorsal and (B) ventral views. Photos: B.F. Santos.

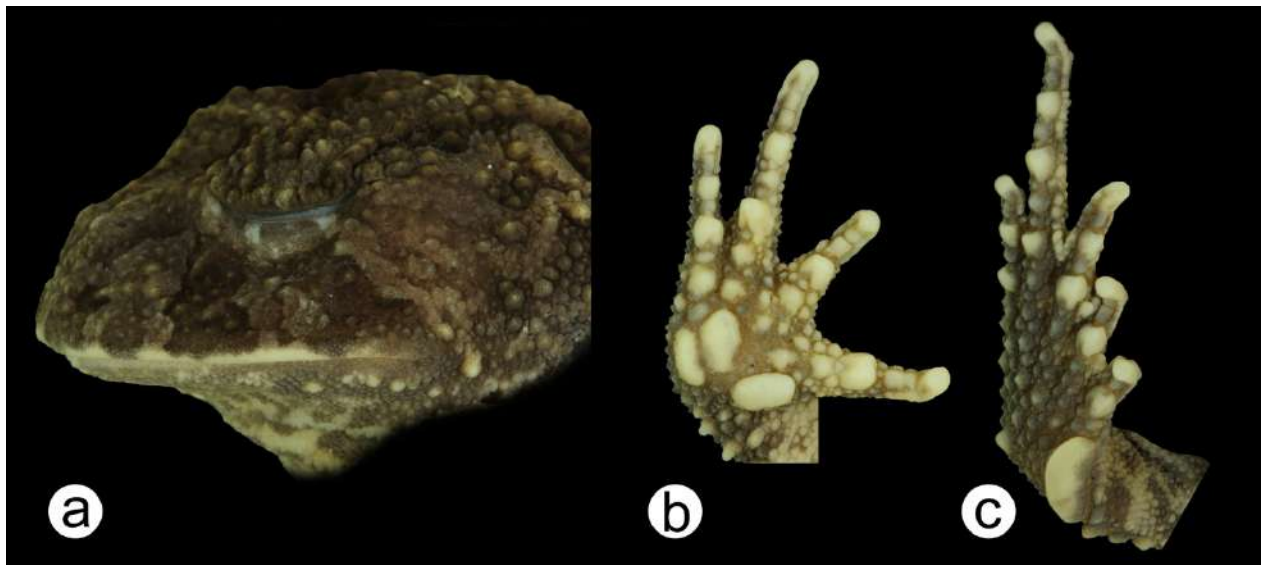


FIG. 2.— *Proceratophrys ararype* (holotype, adult female, CHUFPE 156, SLV 44.3 mm): (A) lateral view of the head; ventral views of (B) hand and (C) foot. Photos: B.F. Santos.



FIG. 3.— Live specimens of *Proceratophrys ararype* from Crato Municipality, Ceará State, Brazil: (A) adult female (holotype, CHUFPE 156) from Mata do Clube Recreativo Grangeiro, and (B) male calling at Sítio Caianas. Photos: P.M.S. Nunes (A); I.J. Roberto (B).

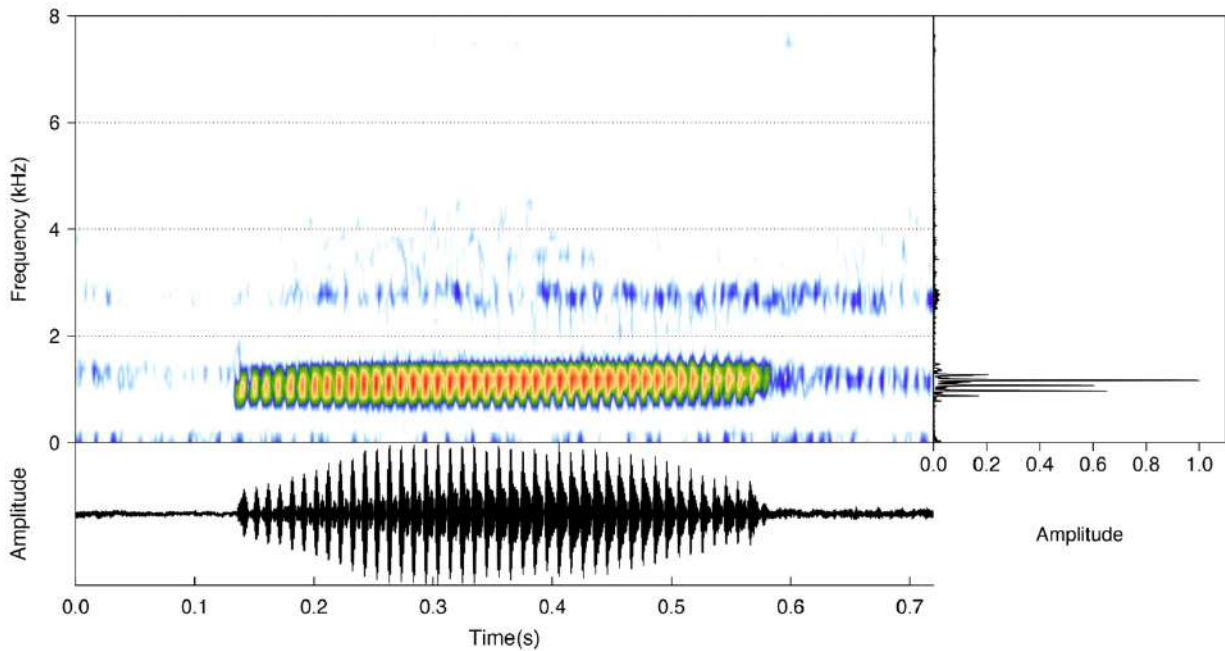


FIG. 4.— Advertisement call of *Proceratophrys ararype*: (A) oscillogram and (B) audiospectrogram of a single call.

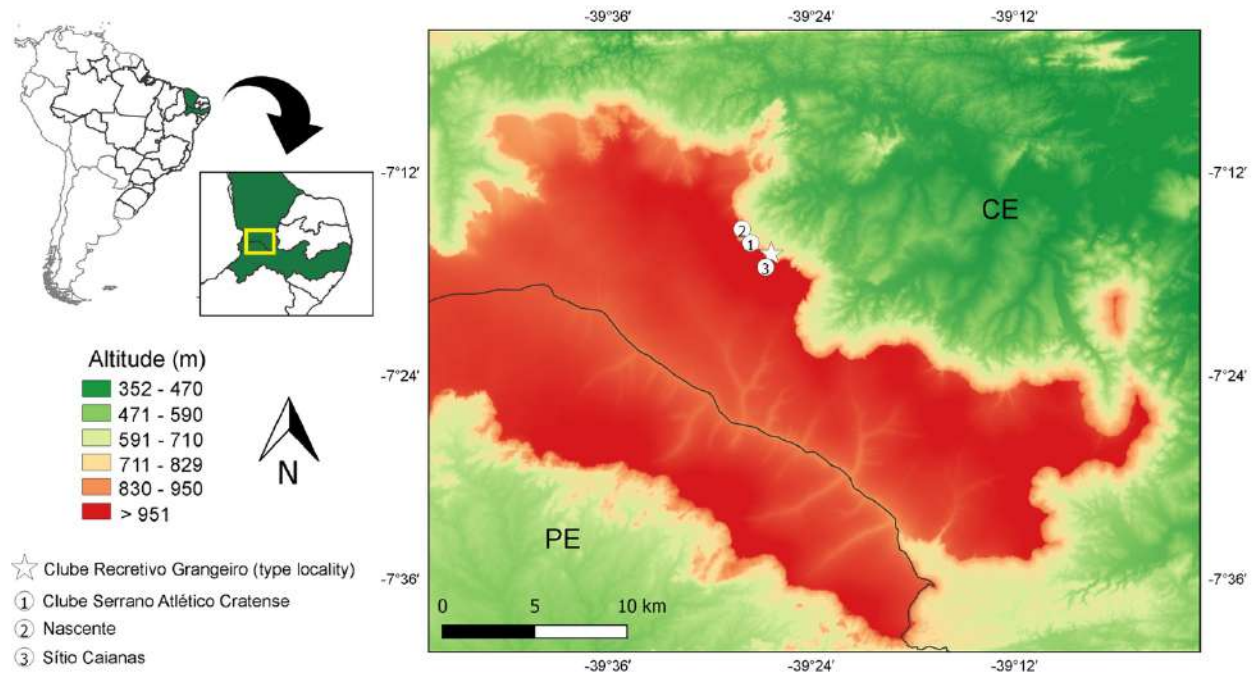


FIG. 5.— Geographic distribution of *Proceratophrys ararype*.

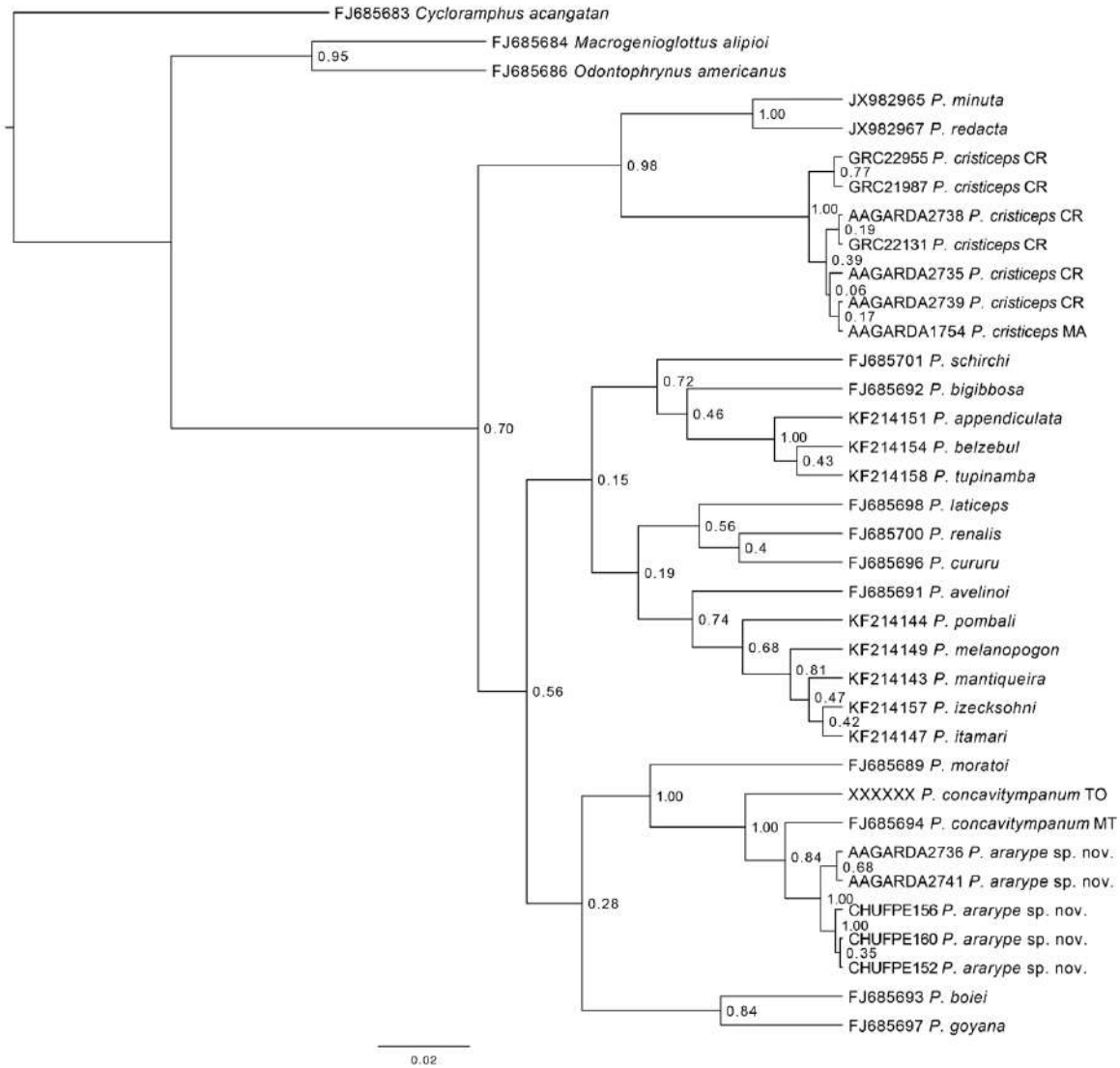


FIG. 6.— Phylogenetic relationship among *Proceratophrys*, inferred through a Bayesian analysis based on the 16S mitochondrial gene. Values above branches indicate posterior probabilities. Scale bar represents number of substitutions per site.

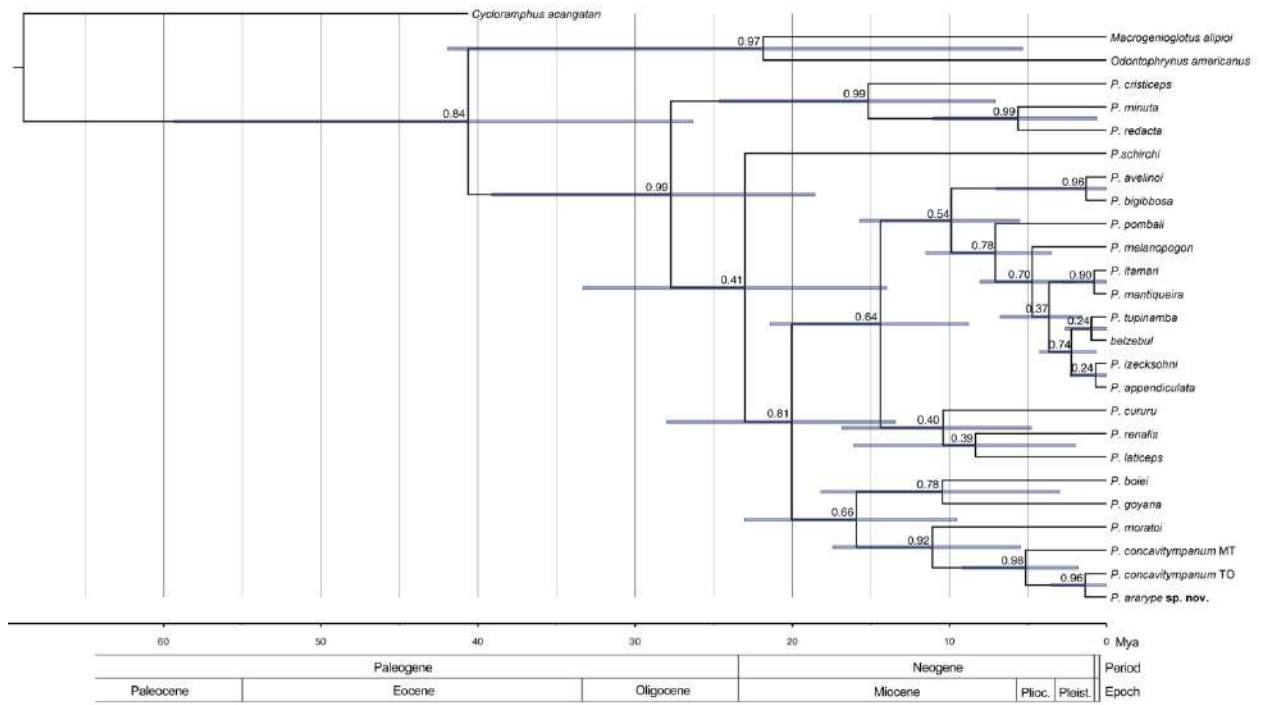


FIG. 7.— Bayesian species tree topology (based on the 16S and rhodopsin genes). Values above branches indicate posterior probabilities. Scale bar represents number of substitutions per site.

Artigo III. Mângia, S., Koroiva, R., Oliveira, E.F., Leite, F.S.F., Del-Grande, M.L., Napoli, M.F., Juncá, F.A., Santana, D.J. and Garda, A.A. Speciation in dwarf *Proceratophrys* (Miranda-Ribeiro, 1920) from the sky islands of Chapada Diamantina, Bahia, Brazil.

Speciation in dwarf *Proceratophrys* (Miranda-Ribeiro, 1920) from the sky islands of Chapada
Diamantina, Bahia, Brazil

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Abstract. The genus *Proceratophrys* is currently composed by 38 species. Only two of them, *P. minuta* and *P. redacta*, occur in the highland areas at the northern portion of the Espinhaço Mountain Range (EMR), known as Chapada Diamantina. The Chapada Diamantina represents a set of disjoint mountains, with isolated peaks at higher elevations. We found three new populations that fit criteria to be considered candidate species from Campo Formoso, Pindobaçu, and Palmeiras Municipalities, aside from those recently described for Chapada Diamantina. Based on molecular, morphometric, and morphological data, we revised the taxonomy of highland populations of *Proceratophrys* in the region. Our results show that each mountaintop studied to herein in the Chapada Diamantina contain restricted lineages, separated by warmer and more xeric valleys, which may act as gene flow barriers. We consider each one as distinct species, except the population from Pindobaçu Municipality, which must be studied further to confirm its possible species status.

Key-words. Espinhaço, Taxonomy, Mountain Range, Anura, Phylogeny.

Introduction

Molecular data has help uncover morphologically cryptic species in many species groups (Prado et al., 2012; Werneck et al., 2012; Costa et al., 2012; Giugliano et al., 2013; Machado et al., 2014). In amphibians, which have highly conserved morphologies, molecular data has assisted in the recognition of new and cryptic species as well (e.g. Garda et al., 2010; Vences et al., 2012; Dias et al., 2013; de Sá et al., 2015; Santana et al., 2015; Fouquet et al., 2016). Currently, the integration of more than one database (such as DNA sequences, morphology, acoustic, behavior, etc.) has proved to be an efficient way to discover and validate new species (e.g. Guicking et al., 2009; Vasconcelos et al., 2012; Gehara et al., 2013).

In the genus *Proceratophrys* Miranda-Ribeiro, 1920, currently with 38 species (Mângia et al., manuscript 1), three species were recently described using molecular data combined with morphological, morphometric, and osteological characteristics (Teixeira et al., 2012; Dias et al., 2013). These descriptions demonstrate that the genus *Proceratophrys* possesses a rich cryptic diversity still uncovered, highlighting the need for taxonomic review (Dias et al., 2013). The 38 species of *Proceratophrys* are usually grouped in three species complex based only on external morphological similarities (Giaretta et al., 2000; Prado and Pombal, 2008). However, gene trees for the genus present two main clades (Teixeira et al., 2012; Dias et al., 2013). The first is composed of four species: *P. cristiceps* (Müller, 1884 “1883”), *P. schirchi* (Miranda-Ribeiro, 1937), *P. minuta* Napoli, Cruz, Abreu & Del Grande, 2011, and *P. redacta* Teixeira, Amaro, Recoder, Vechio & Rodrigues, 2012. The other clade encompasses the remaining species of *Proceratophrys*. Thus, the monophyly of the morphological groups is not supported by molecular data.

Proceratophrys minuta and *P. redacta* occur in few highland areas at the northern Espinhaço Mountain Range (EMR). *Proceratophrys minuta* is known from two localities, Miguel

Calmon and Palmeiras Municipalities (Napoli et al., 2011), both in Bahia State and 160 km apart. *Proceratophrys redacta* is known only from Morro do Chapéu, Bahia State (Teixeira et al., 2012). The EMR extends from the southern Minas Gerais until central region of Bahia State. It is formed by inselbergs, which can reach about 1,000m asl (Derby, 1906). Characterized by a great environmental heterogeneity, the EMR presents several phytophysionomies such as rock outcrops, gallery forests, cloudy forests, savannas, and floodplain grasslands (Kamino et al., 2008). The northern portion of the EMR, popularly known as Chapada Diamantina, represents a set of disjoint mountains, with isolated peaks at higher elevations (Rocha et al., 2005). These clusters of mountains in which environmental conditions at the mountain tops differ markedly from those in the intervening valleys are termed sky islands (Shepard and Burbrink, 2008). When *P. redacta* was described, Teixeira et al. (2012) suggested that the disjoint distribution of *P. minuta* at Miguel Calmon and Palmeiras Municipalities, with *P. redacta* in the middle, deserves further investigation in order to verify their specific identity, because lowlands covered with Caatinga vegetation separate these areas.

Recently, we found three new populations in Campo Formoso, Pindobaçu, and Palmeiras Municipalities. Herein, based on molecular, morphometric and morphological data, we revise the taxonomy of highland populations of *Proceratophrys* and describe two new species.

Material and Methods

Morphological and morphometric assessment. Specimens used in the description and examined for comparisons are housed at the following collections: Coleção Herpetológica da Universidade Federal do Rio Grande do Norte (UFRN), Coleção Herpetológica da Universidade Federal de Pernambuco (CHUFPE), Museu de Zoologia da Universidade Federal da Bahia (MZUFBA), Museu de Zoologia da Universidade Estadual de Feira de Santana (MZFS), Coleção

de Anfíbios, Universidade Estadual do Sudoeste da Bahia (UESB), Coleção Herpetológica da Universidade Federal de Minas Gerais (CHUFMG), Museu de Ciências Naturais, Pontifícia Universidade Católica de Minas Gerais (MCNAM), Museu Nacional do Rio de Janeiro, Universidade Federal do Rio de Janeiro (MNRJ), and Coleção de Herpetologia da Universidade Regional do Cariri (URCA-H).

We analyzed morphological and morphometric data of preserved specimens of *Proceratophrys* (Appendix). We follow the terminology for diagnostic features and morphological structures of Cruz and Napoli (2010), Teixeira et al. (2012), Brandão et al. (2013), and Mângia et al. (2014). Measurements of adult specimens follows Prado and Pombal-Jr. (2008) and are in millimeters: snout-vent length (SVL), head length (HL), head width (HW), distance from the interocular crest to the tip of snout (DICS), internarial distance (IND), eye-nostril distance (END), eye diameter (ED), upper eyelid width (UEW), interorbital distance (IOD), thigh length (THL), tibia length (TL), foot + tarsus length (FL), and forearm and hand length (FHL).

Molecular data. We sequenced a fragment of 16S ribosomal RNA of mitochondrial DNA (mtDNA) and the nuclear gene (nuDNA) rhodopsin from three individuals (Table 1). We extracted genomic DNA from muscle and/or liver samples using the phenol-chloroform protocol (Sam Brooks et al., 1989). We used the 16Sa/16Sb primer of Palumbi (1996), following PCR conditions described by Costa et al. (2016). For the nuclear gene rhodopsin, we used the primers Rhod1A and Rhod1C (Bossuyt and Milinkovitch 2000). PCR conditions for amplification consisted of 1× buffer, dNTP at 0.2 mM, each primer at 0.2 μM, MgCl₂ at 2mM, 1U Taq polymerase, and 2 μl of template DNA, in a total reaction volume of 25 μl. We used the following PCR cycling program: 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 59°C for 1 min, and 72°C for 1 min, and concluding with a 5 min extension at 72°C. We purified PCR products with Ethanol/Sodium Acetate and sequenced at ABI 3730 XL DNA Analyzer (Applied

Biosystems, Foster City, California (CA)). Resulting sequences were edited by aligning forward and reverse reads in the Geneious Pro v9.1.2 program using the MUSCLE algorithm with default parameters (Edgar, 2004). The final dataset was 590 bp for 16S and 350 bp for rhodopsin, which are deposited in GenBank (Table 1).

For Bayesian phylogenetic analysis, we included 16S and rhodopsin sequences from other 21 *Proceratophrys* species available on GenBank along with *Macrogenioglottus alipioi*, *Odontophrynus americanus*, and *Cycloramphus acangatan* as outgroups (Table 1). We determined the model of nucleotide substitution for each gene with jModelTest (Darriba et al. 2012) using the Bayesian Information Criterion (BIC). The best-fit models were HKY + I for 16S and K80 + I for rhodopsin. We performed a Bayesian phylogenetic analysis for 16S and for rhodopsin using BEAST v1.8 software (Drummond et al. 2012). We ran 30 million generations, sampling every 1,000 steps using a Yule Process prior tree. We checked for stationarity by visually inspecting trace plots and ensuring that all values for effective sample size (ESS) were above 200 in Tracer v1.5 (Rambaut and Drummond 2007). We calculated genetic distances (uncorrected p-values) for both genes using Mega v 6.06 (Tamura et al. 2013).

Results

Both topologies generated from mitochondrial and nuclear markers (16S and rhodopsin) recovered a monophyletic group formed by *Proceratophrys cristiceps*, *P. minuta*, *P. redacta*, and the populations from Campo Formoso, Pindobaçu, and Palmeiras Municipalities (Figs. 1 and 2). Each population from Chapada Diamantina was recovered as a distinct lineage corresponding to a specific mountain top. However, the relationship among these lineages is different for each marker. The topology of the mtDNA 16S tree (Fig. 1) recovered the lineage from Palmeiras as sister of *P. redacta*, and the lineage from Pindobaçu as sister of *P. minuta*. *Proceratophrys*

cristiceps was recovered as the outgroup to these four lineages, while the remaining lineage from Campo Formoso is sister to the clade formed by the previous species. The nuclear marker rhodopsin recovered two well-defined clades among these lineages. The first is formed by *P. cristiceps* and Campo Formoso lineages as sister of Pindobaçu lineage, and the second by *P. minuta* and *P. redacta* as sister of Palmeiras lineage.

Genetic distance among lineages from Chapada Diamantina for 16S mtDNA ranges from 2 to 8% uncorrected *p*-distance, except between the lineages of Pindobaçu and *P. minuta* (Table 2). However, the genetic distance between the population of Pindobaçu and *P. minuta* for rhodopsin is 3%.

As each lineage corresponds to specific mountain blocks that are separated by warmer and more xeric valleys, which may act as gene flow barriers, we considered each one as distinct species (see below comments about Pindobaçu population). Furthermore, we also observed morphological differences among these lineages that produced diagnostic characters (see descriptions below) (Fig. 3).

Taxonomic accounts

Proceratophrys minuta Napoli, Cruz, Abreu and Del Grande, 2011

Holotype - UFBA 6721, adult male, collected at the Riacho do Dandá (11°26'S, 40°33'W, ca. 800 m above sea level), Parque Estadual das Sete Passagens (an environmental State protected area), Miguel Calmon Municipality, Bahia State, Brazil, on 22 November 2006, by Rafael Oliveira de Abreu and Heverton Cardona.

Paratypes - MNRJ 75410 (ex-UFBA 6229, adult female), 75411 (ex-UFBA 6230, adult male), collected at Parque Estadual das Sete Passagens, Miguel Calmon Municipality, Bahia State, Brazil, on 22–25 July 2006, by A. Xavier, M.B. Santos, and R. Burger; UFBA 6287, adult male,

collected on 12–16 October 2006, by A.L. Xavier, D. Cruz, M. Camardelli, P.M. Fonseca, and R.O. Abreu; UFBA 6289 (adult male), 6290 (adult female), collected on 12–16 October 2006, by A.L. Xavier, D. Cruz, M. Camardelli, P.M. Fonseca, and R.O. Abreu; UFBA 6356–6358 (adult males), 6353–6355 (adult females), MNRJ 75417 (ex-UFBA 6351), UFBA 6349, 6350, 6352 (juveniles), collected on 23 July 2006, by A.L. Xavier, D. Cruz, M. Camardelli, P.M. Fonseca, and R.O. Abreu; MNRJ 75412–75416 (adult males, ex-UFBA 6718, 6722, 6723, 6726, 6728 [stained and cleared], respectively), UFBA 6716, 6720, 6725, 6727 (adult males), 6724 (adult female), 6717, 6719 (juveniles), collected on 18–23 November 2006, by A.L. Xavier, D. Cruz, M. Camardelli, P.M. Fonseca, R.O. Abreu, and W. Fahning; UFBA 7156, 7158 (adult males), 7155, 7157 (juveniles), collected on 20 January 2007, by A. Xavier, D. Cruz, R.A. Abreu, N. Menezes, and T. Jordão.

Diagnosis (modified from Napoli et al. 2011) - Diagnosed by the following combination of characters: (1) small size (adult males SVL 20.4–25.2 mm, adult females 28.3–31.9 mm); (3) presence of not fused and pointed warts on upper eyelid border, the largest tubercle in the middle more projected than lateral tubercles; (4) snout rounded from above, vertical or oblique in profile; (5) a complete sagittal ridge of warts extending from eyelid to sacral region; (6) presence of a inter-ocular ridge of warts complete or not organized in a row, extending to the border of each eyelid; (7) eyelid and ocular-dorsal ridge contact point at the posterior third of the eyelid and (8) outer metacarpal tubercle single or partially grooved; (9) ventral region cream without spots.

Geographic distribution - *Proceratophrys minuta* was known from Miguel Calmon and Palmeiras Municipalities, Bahia State. Here, we restrict the occurrence of the species only to the type locality (Miguel Calmon) (Fig. 3). The population from Palmeiras corresponds to a new species (described below).

Proceratophrys redacta Teixeira, Amaro, Recoder, Vechio and Rodrigues, 2012

Holotype - MZUSP 150266, an adult male, field number MTR 22579, collected at Morro do Chapéu Municipality, Bahia State, Brazil (11°35'26.02"S, 41°12'32.87"W, 1254 m a.s.l., datum WGS-84), by M. Teixeira Jr, F. Dal Vechio, I. Prates, R.P. Damasceno and M.T. Rodrigues, on 30 December 2011.

Paratopotypes - MZUSP 150263, 150264, 150265, 150268, 150269, adult males, field numbers MTR 22474, 22554, 22564, 22582, 22583; MZUSP 150267, adult female, field number MTR 22580. Collected between 27 and 30 December 2011.

Paratypes - MZUSP 150272, an adult male, field number MTR 22628; MZUSP 150270, 150271, 150273 adult females, field numbers MTR 22623, MTR 22626, MTR 22629. All from an old road (11°33'58.3"S, 41°07'32.3"W, 929 m a.s.l., datum SAD69).

Diagnosis - (*modified from Teixeira-Jr. et al. 2012*) - Diagnosed by the following combination of characters: (1) small size (26.9–29.7 mm of SVL in males; 33.6–34.9 mm of SVL in females); (2) snout rounded from above and vertical in profile; (3) presence of not fused and rounded warts on upper eyelid border, (4) an incomplete sagittal ridge of warts extending from eyelid to sacral region, interrupted at pre-sacral region; (5) eyelid and ocular-dorsal ridge contact point at the posterior third of the eyelid; (6) one row of small, pointed, and not fused tubercles in the forearm.

Geographical distribution - *Proceratophrys redacta* is known only from the type locality (Morro do Chapéu; Fig. 3).

***Proceratophrys* “Palmeiras” sp. nov.**

Proceratophrys minuta Napoli, Cruz, Abreu and Del Grande, 2011 (part).

Figures 4–6

Holotype - AAGARDA 6915, adult female, collected at Trilha das Águas Claras, Morrão, Parque Nacional da Chapada Diamantina, Palmeiras Municipality, Bahia State, Brazil (12°32'27"S; 41°29'32"W; ca. 950 m a.s.l.), on January 2013 by F.M. Magalhães, F.A. Juncá, and A.A. Garda.

Paratopotypes - AAGARDA 6812, AAGARDA 6913, AAGARDA 6896 (adult males), AAGARDA 6705, AAGARDA 6741, AAGARDA 6814, AAGARDA 6815, AAGARDA 6944, AAGARDA 6971 (adult females), and AAGARDA 6813 (juvenile) collected with the holotype.

Paratypes – Morro do Pai Inácio, Palmeiras Municipality, Bahia State (12°27'45"S; 41°28'30"W, ca. 840 m a.s.l.), UFBA 10755 collected on 28 September 2006, and UFBA 10751-10754 collected on 01 May 2006 by M.L. Del-Grande.

Diagnosis - Diagnosed by the following combination of characters: (1) small size (19.3 – 24.5 mm in males, 26.8 – 32.6 mm in females); (2) snout rounded in dorsal and ventral views, obtuse and vertical in profile; (3) presence of not fused and pointed warts on upper eyelid border (L 3,1/5,4; R 3,1/5,4); (4) an incomplete sagittal ridge of warts extending from eyelid to sacral region, interrupted at pre-sacral region; (5) eyelid and ocular-dorsal ridge contact point at the posterior third of the eyelid; (6) one row of small and pointed tubercles in the forearm; (7) ventral region cream without spots.

Comparisons with other species - *Proceratophrys* “Palmeiras” **sp. nov.** is promptly distinguished from *P. appendiculata*, *P. belzebul*, *P. boiei*, *P. gladius*, *P. itamari*, *P. izecksohni*, *P. laticeps*, *P. mantiqueira*, *P. melanopogon*, *P. moheringi*, *P. paviotii*, *P. phyllostomus*, *P. pombali*, *P. renalis*, *P. rondonae*, *P. sanctaritae*, *P. subguttata* and *P. tupinamba* by the absence of a single unicuspidate palpebral and rostral appendages (present in those species, single, short and multi- cuspidate in *P. rondonae*). Additionally, *P.* “Palmeiras” **sp. nov.** can 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 those species).

From *P. avelinoi*, *P. bigibbosa*, *P. brauni*, and *P. palustris*, *P. "Palmeiras" sp. nov.* differs by lacking postocular swellings (present). *Proceratophrys "Palmeiras" sp. nov.* presents not fused and pointed warts on upper eyelid border (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 tubercle in the middle more projected than lateral tubercles in *P. minuta*; small and rounded in *P. redacta*; multiple short and pointed expansions in *P. schirchi*). By having an incomplete sagittal ridge of warts extending from eyelid to sacral region, *P. "Palmeiras" sp. nov.* can be distinguished from *P. bagnoi*, *P. goyana*, *P. minuta*, *P. rotundipalpebra*, *P. strussmannae* and *P. vielliardi* (complete in *P. bagnoi*, *P. goyana*, *P. minuta*, *P. rotundipalpebra* and *P. strussmannae*, absent in *P. vielliardi*). From *P. bagnoi*, *P. dibernardoi* and *P. goyana*, *P. "Palmeiras" sp. nov.* differs by the presence of only one row of tubercles in the forearm (two rows in *P. bagnoi* and *P. dibernardoi*, tubercles not organized in rows in *P. goyana*). *Proceratophrys "Palmeiras" sp. nov.* also differs from *P. bagnoi* by the eyelid and ocular-dorsal ridge contact point at the posterior third of the eyelid (medial in *P. bagnoi*). From *P. bagnoi*, *P. branti*, *P. carranca*, *P. redacta*, and *P. strussmannae*, *P. "Palmeiras" sp. nov.* differs by the presence of ventral region cream without spots (dark spots in *P. branti* and *P. carranca*, vermiculations in *P. bagnoi*, gular region blackish, scattered small, dark-brown dots in the chest in *P. redacta*, and in the chest and belly in *P. strussmannae*).

Description of holotype - Head wider than long, head length 30% of SVL, snout rounded in dorsal and ventral views (Fig. 4), obtuse and slightly vertical in profile (Fig. 5A); nares elliptical and prominent, canthal crests marked, prominent and covered by small tubercles, no preocular crests; eye directed anterolaterally, eye diameter 38% of head length; palpebral appendage short, 28% of the head length, eyelid with pointed, elongated and not fused warts, nine warts in the left eyelid and ten in the right; indistinct tympanum; vomerine teeth in two short rows between the choanae; frontoparietal crest poorly developed, region between the frontoparietal crest shallow; ocular-dorsal ridge of warts interrupted at pre-sacral constriction region, and the coccyx end region with tubercles; two parallel rows of warts in the supra-scapulae region; interocular ridge of warts present but not organized in a row; the ocular-dorsal ridge and eyelid contact is in the posterior third region. Dorsal surface, including flanks, arms and legs, with various warts of different sizes and shapes, one row of very close and pointed forearm tubercles; ventral surfaces, except hands and feet, covered by numerous small, rounded, uniform warts. Finger lengths I > II < III > IV (Fig. 5B); interdigital webbing absent; inner metacarpal tubercle large and elliptical; outer metacarpal tubercle large, single, in a shape of a upside down heart (partially grooved); scarce small rounded supernumerary tubercles; subarticular tubercles large, nearly rounded, grooved anteriorly and posteriorly. Thigh length longer than tibia length, the sum of thigh and tibia lengths 13% of snout-vent length; toe lengths I < II < III < IV > V; inner metatarsal tubercle large, elliptical, spatulated; outer metatarsal tubercle small, rounded; scarce small rounded supernumerary tubercles; subarticular tubercles large, nearly rounded, grooved anteriorly and posteriorly (Fig. 5C).

Measurements of holotype (mm) - SVL 28.8; HL 12.3; HW 8.5; DICS 6.4; IND 1.3; END 2.6; ED 2.9; UEW 3.5; IOD 2.5; THL 12.7; TL 10.6; FL 17.0; FHL 15.6

Color in life - Based on color photographs of the holotype and a paratype (Fig. 6). *Proceratophrys* “Palmeiras” **sp. nov.** presents a variation of the dorsal coloration pattern. The dorsal color pattern is marbled with several shades of light brown and cream on a dark brown and/or reddish hue background.

Color in preservative - Based on the type series. The dorsal surface is dark brown. Two individuals (AAGARDA 6705, adult female; AAGARDA 6812, adult male) present light brown spots in the arms and dorsal region. Ventral surface background color cream.

Variation - The number of tubercles on the upper eyelid vary from seven to ten among the individuals of the type series, and between the eyelids in the same individual. All individuals do not present a complete ocular-dorsal ridge of warts. However, in some individuals (AAGARDA 6705, AAGARDA 6814, AAGARDA 6971, adult females and AAGARDA 6912, adult male) is possible to observe this adornment in the dorsal region, even with an interruption of the tubercles at pre-sacral region. The outer metacarpal tubercle can be completely divided in two (AAGARDA 6705, AAGARDA 6741, AAGARDA 6791, adult females, AAGARDA 6913, adult male, AAGARDA 6813, juvenile). Variation in the dorsal and ventral background colors were described above (color in life and in preservative items). Descriptive statistics of measurement variables from adult males and females are in Table 3.

Natural History - The holotype and type series from Palmeiras Municipality were collected using pitfall traps in a gallery forest at rock fields, with a stream nearby. The series from Morro do Pai Inácio were collected in a gallery forest on 01 May 2006 and from September to October of 2006. The individuals were emitting advertisement calls positioned on the leaf litter and always near (1-5 m distance) of shallow waters.

Geographic distribution - *Proceratophrys* “Palmeiras” **sp. nov.** is known only from the type locality at Chapada Diamantina, Palmeiras Municipality, Bahia State, Brazil (Fig. 3).

***Proceratophrys* “Campo Formoso” sp. nov.**

Figures 7–9

Holotype - UFMG 6225 (adult female), Campo Formoso Municipality, Bahia State (10°19'2.27"S, 41°9'11.62"W), collected on 16 December 2010 by F. Leite, M. Lindermann and C. Rievers.

Paratopotypes – UFMG 6224 (adult female), UFMG 6220 (juvenile) collected with the holotype.

Diagnosis – Diagnosed by the following combination of characters: (1) small to medium size (37.7 – 40.4mm in females); (2) snout rounded in dorsal and ventral views, obtuse and vertical in profile; (3) absence of prominent warts on upper eyelid border; (4) absence of inter-ocular and ocular-dorsal ridge of warts (5) dorsal skin smooth, with short and few warts concentrated in the inter-ocular region and in the upper eyelid; (6) canthal crest poorly developed; (7) one row of small, rounded and not fused tubercles in the forearm; (8) presence of a prominent rounded tubercle on the tarsus; (9) ventral region cream without spots.

Comparisons with other species – *Proceratophrys* “Campo Formoso” **sp. nov.** is promptly diagnosed from all species of *Proceratophrys* by the presence of a smooth dorsal skin (dorsum scattered with several small pointed and/or rounded granules in other *Proceratophrys*), by the presence of a small and rounded tubercle in the buccal commissure (longer, prominent and generally triangular), and by the absence of palpebral ornamentation (presence of single unicuspidate palpebral appendage, postocular swellings or a large marginal row of tubercles in the eyelid).

Proceratophrys “Campo Formoso” **sp. nov.** 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 those species). The absence of a complete ocular-dorsal ridge of warts differs *Proceratophrys* “Campo Formoso” **sp. nov.** from *P. bagnoi*, *P. goyana* and *P. redacta* (complete ocular-dorsal ridge of warts). From *P. bagnoi*, *P. concavitympanum*, *P. dibernardo* and *P. goyana*, *P.* “Campo Formoso” **sp. nov.** differs by the presence of only one row of tubercles in the forearm (two rows in *P. bagnoi*, *P. concavitympanum*, *P. cristiceps* and *P. dibernardo*, tubercles not organized in rows in *P. goyana*). The presence of ventral region cream, without spots or vermiculations, differs *P.* “Campo Formoso” **sp. nov.** from *P. branti*, *P. carranca*, *P. redacta* and *P. strussmannae* (dark spots in *P. branti* and *P. carranca*, gular region blackish, scattered small, dark-brown dots in the chest in *P. redacta*, and in the chest and belly in *P. strussmannae*).

Description of holotype - Head wider than long, head length 28% of SVL, snout rounded in dorsal and ventral views (Fig. 7), obtuse and vertical in profile (Fig. 8A); nares elliptical and prominent, canthal crests poorly marked and smooth, no preocular crests; eye directed anterolaterally, eye diameter 19% of head length and 51% of the palpebral appendage; eyelid without prominent warts; indistinct tympanum; vomerine teeth in two short rows between the choanae; frontoparietal crests poorly developed; region between frontoparietal crests shallow; absence of inter-ocular and ocular-dorsal ridge of warts. Dorsal surface smooth, without prominent warts, one row of rounded and not fused forearm tubercles, reaching the hands; ventral surfaces, except hands and feet, covered by numerous small, rounded, uniform warts. Finger lengths I > II < III > IV (Fig. 8B); interdigital webbing absent; inner metacarpal tubercle large and elliptical; outer metacarpal divided in two oval parts; scarce small rounded supernumerary tubercles; subarticular tubercles large, nearly rounded, grooved anteriorly and posteriorly. Thigh length longer than tibia length, the sum of thigh and tibia lengths 14.7% of snout-vent length; toe lengths I < II < III < IV > V; inner metatarsal tubercle large, elliptical, espatulated; outer

metatarsal tubercle small, rounded, presence of a prominent rounded tubercle on the tarsus; scarce small rounded supernumerary tubercles; subarticular tubercles large, nearly rounded, grooved anteriorly and posteriorly (Fig. 8C).

Color in life of the holotype - Based on photographs of the holotype (Fig. 9). Dorsal background color grey, maculated with irregular stains dark-brown and cream looking like dead leaves. Three cream bands from eye to upper lip. One transverse cream band in the inter-ocular region. Two transverse dark-brown bars on the tibia. Fingers, toes and ventral surface are cream.

Color in preservative of the holotype – Dorsal background color light-grey, maculated with irregular stains dark-grey. One transverse light-grey band in the inter-ocular region. Three light-grey bands from eye to upper lip. Two transverse grey bars on the tibia. Fingers, toes and ventral surface are cream.

Measurements of holotype (mm) - SVL 37.7; HL 10.7; HW 15.3; DICS 7.0; IND 1.8; END 3.4; ED 4.4; UEW 4.1; IOD 2.1; THL 14.4; TL 12.5; FL 19.6; FHL 15.9.

Variation – Specimens are congruent with respect to morphological characters. The dorsal background color varies in adult specimens from grey to cream. The irregular stains in the dorsal surface varies from dark-brown, cream and reddish.

Geographic distribution - *Proceratophrys* “Campo Formoso” **sp. nov.** is known only from the type locality at Chapada Diamantina, Campo Formoso Municipality, Bahia State, Brazil (Fig. 3).

***Proceratophrys* sp.**

Figures 10–11

Material examined. Brazil. BAHIA. Pindobaçu Municipality (10°41'26.81"S, 40°22'51.42"W), collected on 11 December 2010 by F. Leite, M. Lindermann and C. Rievers.

Diagnosis - Diagnosed by the following combination of characters: (1) small size (23.6 – 26.0 mm in males, 30.3 – 33.9 mm in females); (2) snout rounded in dorsal and ventral views, obtuse and vertical in profile; (3) presents enlarged, pointed, not fused, and with the largest tubercle in the middle more projected than lateral tubercles on the upper eyelid border (L 4,1/5,3; R 4,1/5,3); (4) presence of a prominent row of tubercles from the middle of the eye to the arm insertion; (5) a complete sagittal ridge of warts extending from eyelid to sacral region, connected at pre-sacral region; (6) eyelid and ocular-dorsal ridge contact point at the posterior third of the eyelid; (7) one row of small and pointed tubercles in the forearm (or not organized in row); (8) ventral region cream without spots (males present gular and chest region scattered with brown spots).

Comparisons with other species - *Proceratophrys* sp. is promptly distinguished from *P. appendiculata*, *P. belzebul*, *P. boiei*, *P. gladius*, *P. itamari*, *P. izecksohni*, *P. laticeps*, *P. mantiqueira*, *P. melanopogon*, *P. moheringi*, *P. paviotii*, *P. phyllostomus*, *P. pombali*, *P. renalis*, *P. rondonae*, *P. sanctaritae*, *P. subguttata* and *P. tupinamba* by the absence of a single unicuspidate palpebral and rostral appendages (present in those species, single, short and multi- cuspidate in *P. rondonae*). Additionally, *Proceratophrys* sp. can 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 those species).

From *P. avelinoi*, *P. bigibbosa*, *P. brauni*, and *P. palustris*, *Proceratophrys* sp. differs by lacking postocular swellings (present). *Proceratophrys* sp. presents enlarged, pointed, not fused, and with the largest tubercle in the middle more projected than lateral tubercles (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*; small and rounded

in *P. redacta*; multiple short and pointed expansions in *P. schirchi*). By having a complete sagittal ridge of warts extending from eyelid to sacral region, *Proceratophrys* sp. can be distinguished from *P. “Palmeiras”*, *P. redacta* and *P. vielliardi* (incomplete in *P. “Palmeiras”*, *P. redacta*, and absent in *P. vielliardi*). From *P. bagnoi* and *P. dibernardoi*, *Proceratophrys* sp. differs by the presence of only one row of tubercles in the forearm or not organized in row (two rows in *P. bagnoi* and *P. dibernardoi*). *Proceratophrys* sp. also differs from *P. bagnoi* by the eyelid and ocular-dorsal ridge contact point at the posterior third of the eyelid (medial in *P. bagnoi*). From *P. bagnoi*, *P. branti*, *P. carranca*, *P. redacta*, and *P. strussmannae*, *Proceratophrys* sp. differs by the presence of ventral region cream without spots, males with brown spots in the gular and chest region (dark spots in *P. branti* and *P. carranca*, vermiculations in *P. bagnoi*, gular region blackish, scattered small, dark-brown dots in the chest in *P. redacta*, and in the chest and belly in *P. strussmannae*).

Proceratophrys sp. can also be distinguished from *P. “Palmeiras”* by the presence of a prominent row of tubercles from the middle of the eye to the arm insertion (absent or tubercles with the same size of the other from the body not organized in row in *P. “Palmeiras”*). From *P. redacta*, *Proceratophrys* sp. can also be distinguished by its smaller size in adult males (SVL ranging from 23.6 – 26.0 mm; 26.9 – 29.7 mm in *P. redacta*).

Comments. Although the uncorrected *p*-distance for rhodopsin between *Proceratophrys* sp. and *P. minuta* is 3%, the uncorrected *p*-distance for 16S is 0% and so far we have not found morphologic characteristics that distinguish them. Because we do not have enough material to access diagnostic characters to distinguish these two populations, we choose to keep the population from Pindobaçu unnamed.

Discussion

Much of the species diversity of *Proceratophrys* from Chapada Diamantina is unknown, what may have important implications for conservation because many genetically distinct taxa have small ranges and usually are restricted to single mountaintops (Salerno et al. 2012; Firkowski et al. 2016). Proper identification and recognition of independent evolutionary lineages is imperative to conserve biodiversity (Shepard et al., 2011). Despite its importance for conservation, northern Espinhaço in the Bahia State (Chapada Diamantina) remains virtually unexplored for amphibians (Leite et al. 2008).

Environmental conditions at high and low elevations in Chapada Diamantina are discrepant, especially related to the temperature and humidity. This disparity promotes a sky island situation (He and Jiang, 2014), and may create barriers to gene flow. Some amphibians occurring in mountain ranges present such pattern of distribution, such as the *Plethodon* salamanders in the Ouachita Mountains, United States (Shepard and Burbrink, 2008), treefrogs of the genus *Tepuhyla* in the Tepuis, northern South America (Salerno et al., 2012), and species of the genus *Brachycephalus* and *Melanophryniscus* from Serra da Mar, in the southern Brazilian Atlantic Forest (Firkowski et al., 2016). Our study is the first to indicate a similar pattern for Chapada Diamantina. Although many species are recognized for this area, it is unknown if these mountains act as sky islands for other anuran species in this region. Some groups deserve special attention such as the recently described *Bokermannohyla* species (Lugli and Haddad, 2006a, 2006b, Napoli and Juncá, 2006, Leite et al., 2012), which present similar distribution patterns. This type of information is essential for efficient conservation initiatives because many species at the Chapada Diamantina may be actually complexes of morphologically cryptic species.

Correspondence between genetic and morphological data can easily support the recognition of species or lineages as distinct taxa, and provide insights into patterns of morphological diversification during the evolutionary history of this clade. However, in some

cases, speciation is not accompanied by morphological changes, because the intraspecific recognition is not always visual. This might be the case of *Proceratophrys* sp. “Pindobaçu”, which we have not observed morphological differences with respect to *P. minuta* (see comments above). Besides that, the mechanisms that generate cryptic speciation are still unknown (Bickford *et al.* 2007). Thus, integrative taxonomy approaches are required and recommended once the use of various sources of characters may provide a better resolution of the taxonomic and evolutionary status of organisms.

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Appendix

Specimens examined

Proceratophrys aridus: **BRAZIL. CEARÁ.** Milagres: MNRJ 55349, 55778-822, 75156-68.

URCA-H 106, 142-43.

Proceratophrys caramaschii: **BRAZIL. CEARÁ.** Mucuripe: MNRJ 1419-20, 1680, 16470-84, 16487-89, 16591-600.

Proceratophrys concavitympanum: **BRAZIL. MATO GROSSO.** Alta Floresta: ZUEC 21201; Aripuanã: MZUFV 9552, 9554-56; Paranaíta: ZUEC 14505-06, 16011-15, 16719, 21201. **PARÁ.** Paraopebas: PUC-MG 10561, 11206. **RONDÔNIA.** Espigão do Oeste: MZUFV 10477.

Proceratophrys cristiceps: **BRAZIL. 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 7366, 7385, 7393, 7396, 7408, 7416, 7418. Barbalha:

URCA 4293, 4571. Baturité: UFC3722. Crateús: URCA 4744. General Sampaio: UFC 5351.

Itapipoca: AAGARDA 9817, 10453-55. Ipu: UFPB 6117-19, 6121, 6123, 6125. Jaguaribe:

AAGARDA 10176-79, 10286, 10398-402. Milagres: URCA 106, 142-43. Pacajus: UFC 4562.

Paracuru: URCA 5773-74. Pentecoste: UFC 5001, 5018-19, 5193. São Gonçalo do Amarante:

URCA 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. Ubajara, Parque

Nacional de Ubajara: AAGARDA 10672, 10695, 10698-99, 10703, 10707-09, 10782, 10796,

10907, 10909, 10911-14, 10961, 10974, 10981, 10983. Várzea da Conceição: UFPB 9661, 9665,

9667. **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.** Arco Verde: UFPB 9678-82, 9684, 9686-88, 9692, 9701;

Betânea: UFC 3331; Bezerras: UFPB 7098; Exú: URCA 1462-63, UFPB 7214-17; Nascente: UFPB 9670; Ouricuri: URCA 2988-89; 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. **PIAUI**. Floriano: UFPI 214-16, 222, 236; Piripiri: UFPB 10340, 10342-46; Ribeiro Gonçalves: URCA 2358. **RIO GRANDE DO NORTE**. ESEC 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 Jundiá: 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 minuta: BRAZIL. **BAHIA**. Miguel Calmon, Parque Estadual das Sete Passagens: UFBA 6229-30, 6716-20, 6722, 6725-26.

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 4021.

Figures

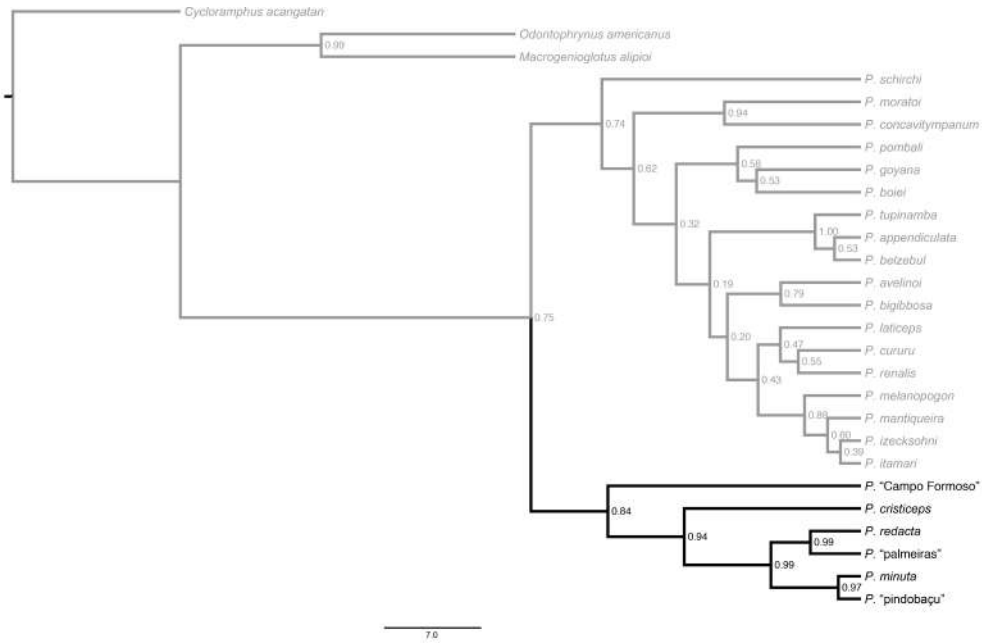


Figure 1. Bayesian tree topology obtained from mitochondrial 16S gene. Posterior probability values are shown above the branches.

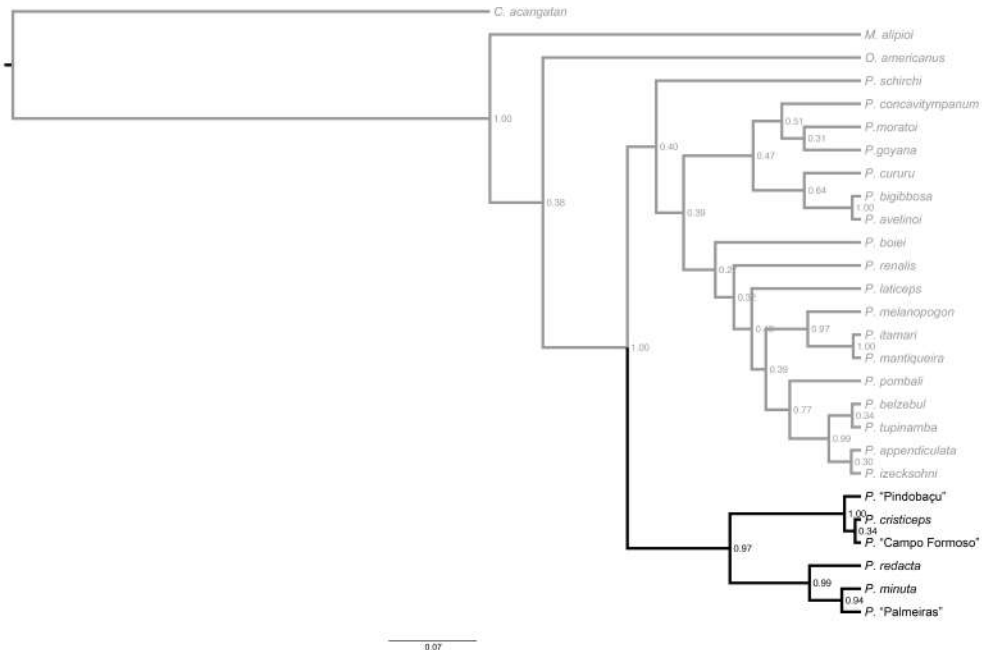


Figure 2. Bayesian tree topology obtained from nuclear (rhodopsin) gene. Posterior probability values are shown above the branches.

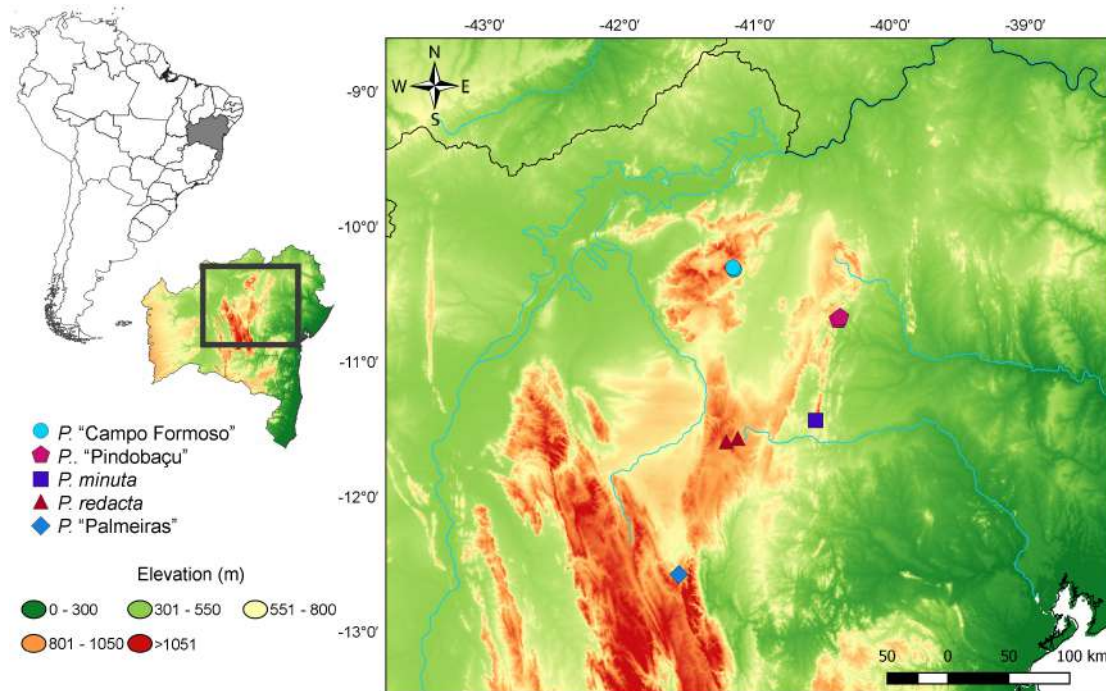


Figure 3. Geographic distribution of *Proceratophrys* from Chapada Diamantina, Bahia State, Brazil.

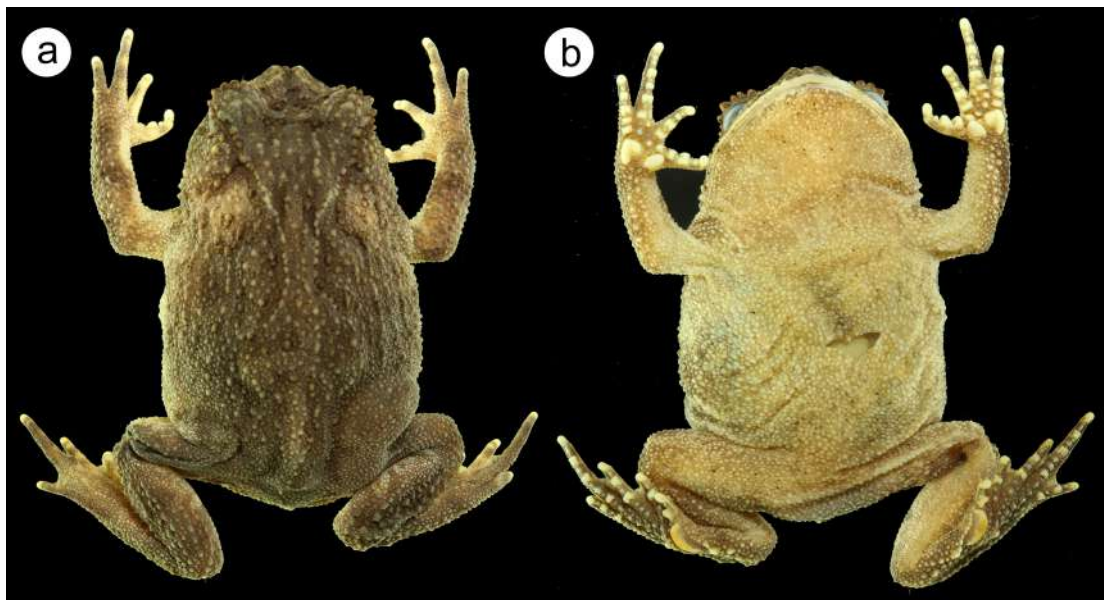


Figure 4. *Proceratophrys "Palmeiras" sp. nov.* (holotype, adult female, AAGARDA 6915, SLV 28.8 mm): (A) dorsal and (B) ventral views. Photos: B.F. Santos.

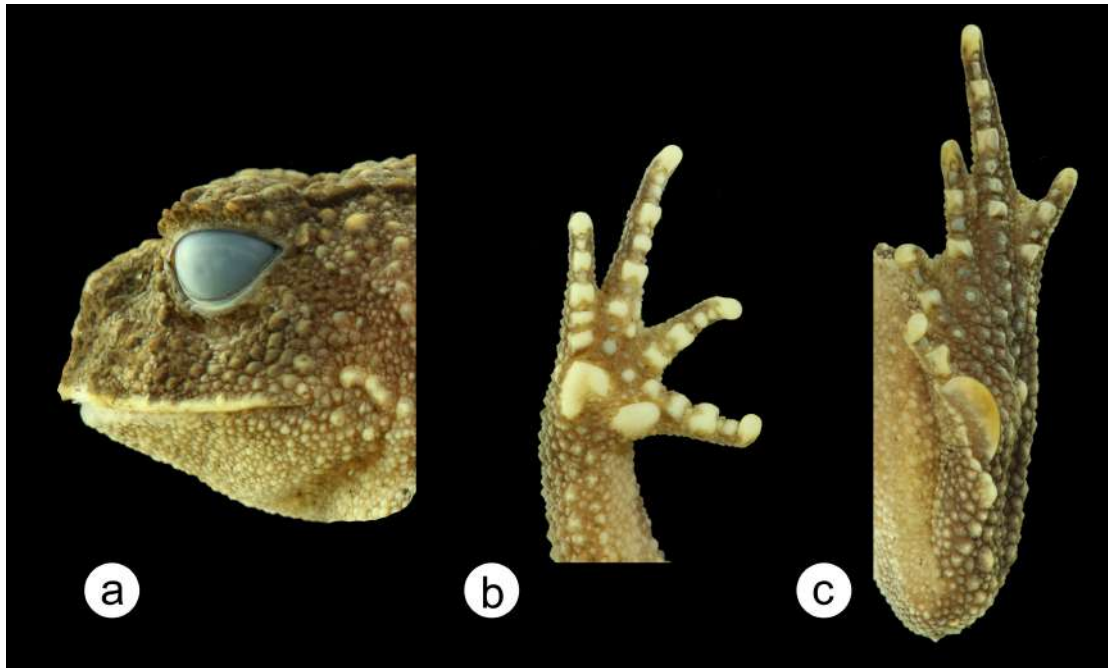


Figure 5. *Proceratophrys* “*Palmeiras*” **sp. nov.** (holotype, adult female, AAGARDA 6915, SLV 28.8 mm): (A) lateral view of the head; ventral views of (B) hand and (C) foot. Photos: B.F. Santos.



Figure 6. Live specimens of *Proceratophrys* “*Palmeiras*” **sp. nov.**: (A) adult female (holotype, AAGARDA 6915), and (B) adult individual from the type series, from Palmeiras Municipality, Bahia State, Brazil. Photos: A.A. Garda.

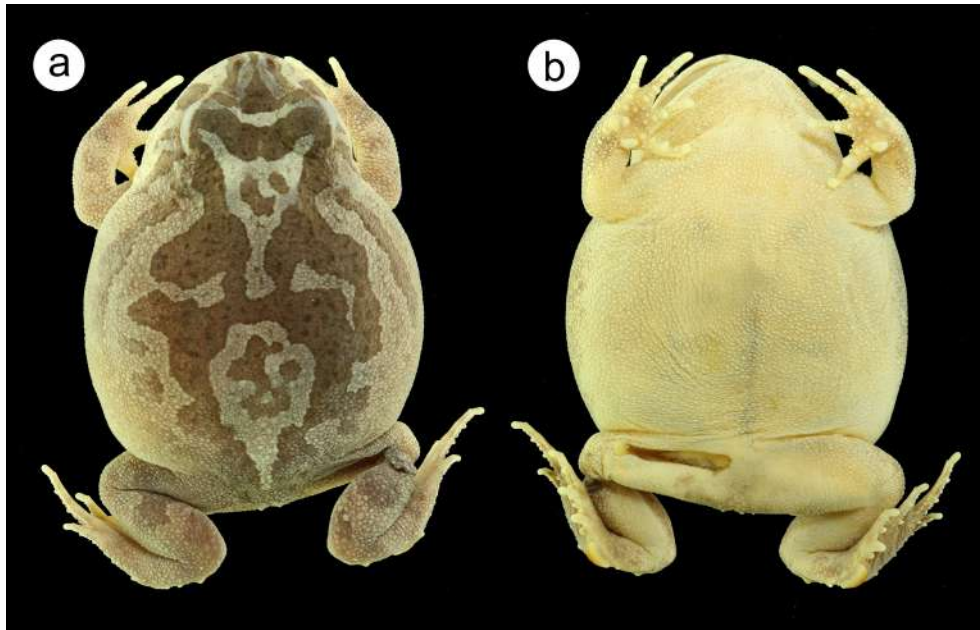


Figure 7. *Proceratophrys* “Formoso” **sp. nov.** (holotype, adult female, UFMG 6225, SLV 37.7 mm): (A) dorsal and (B) ventral views. Photos: B.F. Santos.

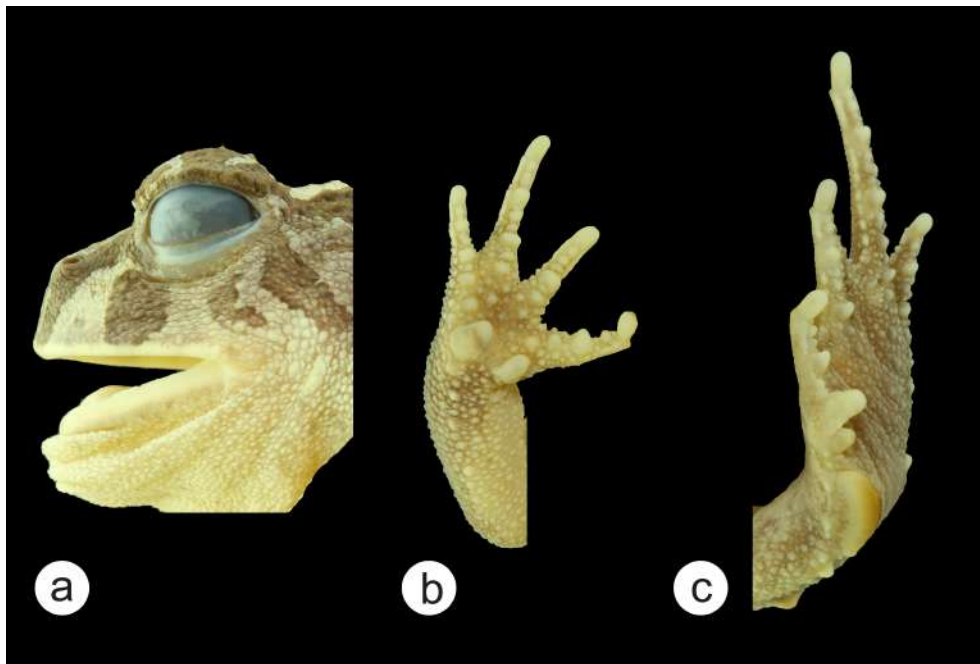


Figure 8. *Proceratophrys* “Formoso” **sp. nov.** (holotype, adult female, UFMG 6225, SLV 37.7 mm): (A) lateral view of the head; ventral views of (B) hand and (C) foot. Photos: B.F. Santos.



Figure 9. Live specimens of *Proceratophrys* “Formoso” **sp. nov.**: (A) adult female (holotype, UFMG 6225), and (B) adult female (UFMG 6224) from Campo Formoso Municipality, Bahia State, Brazil. Photos: F.S.F. Leite.

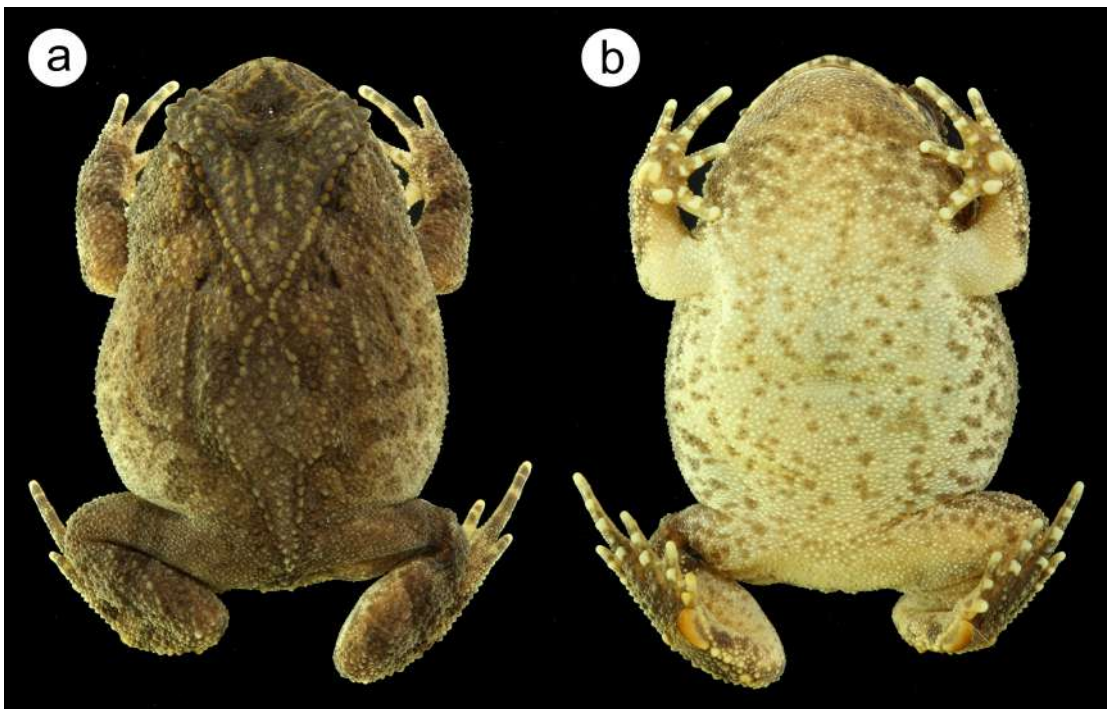


Figure 10. *Proceratophrys* sp. (adult male, UFMG 6055, SLV 26.0 mm): (A) dorsal and (B) ventral views. Photos: B.F. Santos.



Figure 11. Live specimens of *Proceratophrys* sp.: adult females from Pindobaçu Municipality, Bahia State, Brazil. Photos: F.S.F. Leite.

Table 1. Information of all specimens examined in the molecular section of this study (16s rRNA and Rhodopsin).

Species	Municipality, State	Voucher	Genbank accession number		Reference
			Rhodopsin	16S	
<i>Proceratophrys</i> “Palmeiras” sp. nov.	Palmeiras, Bahia	AAGARDA6705	*	*	This study
<i>Proceratophrys</i> “Pindobaçu” sp. nov.	Pindobaçu, Bahia	FSFL4802	*	*	This study
<i>Proceratophrys</i> “ Campo Formoso” sp. nov.	Campo Formoso, Bahia	FSFL4837	*	*	This study
<i>Proceratophrys concavitympanum</i>	Aripuanã, Mato Grosso	*	*	KX858855	This study
<i>Proceratophrys concavitympanum</i>	Palmas, Tocantins	AF1094	KF214207	FJ685694	Amaro et al. 2009
<i>Proceratophrys moratoi</i>	Itirapina, São Paulo	CFBH6515	KF214212	FJ685689	Amaro et al. 2009
<i>Proceratophrys tupinamba</i>	Ilha Grande, Rio de Janeiro	MNRJ54541	KF214236	KF214158	Dias et al. 2013
<i>Proceratophrys goyana</i>	Petrolina de Goiás, Goiás	AF1188	KF214210	FJ685697	Amaro et al. 2009
<i>Proceratophrys mantiqueira</i>	Araponga, Minas Gerais	MZUFV10139	KF214222	KF214143	Dias et al. 2013
<i>Proceratophrys avelinoi</i>	Misiones, Argentina	DB1246	KF214204	FJ685691	Amaro et al. 2009
<i>Proceratophrys laticeps</i>	Linhares, Espírito Santo	MTR12156	KF214211	FJ685698	Amaro et al. 2009
<i>Proceratophrys boiei</i>	São Paulo, São Paulo	AF1587	KF214206	FJ685693	Amaro et al. 2009
<i>Proceratophrys itamari</i>	Campos do Jordão, São Paulo	MZUSP135186	KF214226	KF214147	Dias et al. 2013
<i>Proceratophrys izecksohni</i>	Paraty, Rio de Janeiro	MNRJ64584	KF214235	KF214157	Dias et al. 2013
<i>Proceratophrys belzebul</i>	São Sebastião, São Paulo	MTR9456	KF214233	KF214233	Dias et al. 2013
<i>Proceratophrys melanopogon</i>	São José do Barreiro, São Paulo	TG3295	KF214228	KF214149	Dias et al. 2013
<i>Proceratophrys pombali</i>	Bertioga, São Paulo	AF1988	KF214223	KF214148	Dias et al. 2013
<i>Proceratophrys cururu</i>	Cardeal Mota, Minas Gerais	FSFL580	KF214209	FJ685696	Amaro et al. 2009
<i>Proceratophrys appendiculata</i>	São Sebastião, São Paulo	MNRJ53936	KF214231	FJ685690	Amaro et al. 2009
<i>Proceratophrys bigibbosa</i>	Misiones, Argentina	BD2313	KF214215	FJ685692	Amaro et al. 2009
<i>Proceratophrys renalis</i>	Brejo Madre de Deus, Pernambuco	ZUFRJ8682	KF214213	FJ685700	Amaro et al. 2009
<i>Proceratophrys schirchi</i>	Santa Tereza, Espírito Santo	371	KF214214	FJ685701	Amaro et al. 2009
<i>Proceratophrys cristiceps</i>	Macaíba, Rio Grande do Norte	AAGARDA1754		KX855989	This study
<i>Proceratophrys minuta</i>	Miguel Calmon, Bahia	MZUSP146499	KF214215	JX982965	Teixeira-Jr et al. 2012
<i>Proceratophrys redacta</i>	Morro do Chapéu, Bahia	MZUSP150266	KF214216	JX982967	Teixeira-Jr et al. 2012

<i>Odontophrynus americanus</i>	Poços de Caldas, Minas Gerais	AF665	KF214201	FJ685686	Amaro et al. 2009
<i>Macrogenioglottus alipioi</i>	Jussari, Bahia	AF919	KF214199	FJ685684	Amaro et al. 2009
<i>Cycloramphus acangatan</i>	Cotia, São Paulo	AF1605	KF214198	FJ685683	Amaro et al. 2009

* Submitted in Genbank (numbers not available yet)

Table 2. Uncorrected *p*-distances of mtDNA 16S (inferior diagonal) and nuDNA rhodopsin (superior diagonal) among *Proceratophrys cristiceps*, *P. minuta*, *P. redacta*, *Proceratophrys* sp. “Pindobaçu”, *P.* “Campo Formoso” sp. nov., and *P.* “Palmeiras” sp. nov.

	1	2	3	4	5	6	7
1) <i>P. cristiceps</i>	-	0.03	0.02	0.03	0.00	0.00	0.03
2) <i>P. minuta</i>	0.06	-	0.00	0.02	0.03	0.03	0.00
3) <i>P. redacta</i>	0.07	0.03	-	0.02	0.02	0.02	0.00
4) <i>P. schirchi</i>	0.07	0.07	0.08	-	0.03	0.03	0.02
5) <i>P.</i> sp. “Pindobaçu”	0.06	0.00	0.03	0.07	-	0.00	0.03
6) <i>P.</i> “Campo Formoso” sp. nov.	0.08	0.07	0.07	0.06	0.07	-	0.03
7) <i>P.</i> “Palmeiras” sp. nov.	0.07	0.03	0.03	0.07	0.04	0.07	-

Table 3. Measurements (mm) of specimens of *Proceratophrys minuta*, *P. redacta*, *P. “Palmeiras”* sp. nov., *P. sp. “Pindobaçu”*, and *P. “Campo Formoso”* sp. nov.

	<i>P. minuta</i>		<i>P. redacta</i>		<i>P. “Palmeiras”</i> sp. nov.		<i>Proceratophrys</i> sp.		<i>P. “Formoso”</i> sp. nov.
	Males (n=18)	Females (n=6)	Males (n=6)	Males (n=4)	Males (n=3)	Females (n=4)	Males (n=4)	Females (n=3)	Females (n=2)
SLV	22.7 ± 1.2	30.3 ± 1.5	28.4	24.3 ± 1.2	31.8 ± 1.9	34.4	23.8 ± 1.2	31.2 ± 1.9	24.3 ± 1.2
	20.4 – 25.2	28.3 – 31.9	26.9 – 29.7	23.6 – 26.0	30.3 – 34.0	33.5 – 34.9	23.6 – 26.0	30.3 – 34.0	23.6 – 26.0
HW	11.1 ± 0.6	14.5 ± 1.1	13.0	11.1 ± 0.5	14.1 ± 0.8	15.7	11.0 ± 0.5	14.5 ± 0.8	11.1 ± 0.5
	10.4 – 12.9	13.5 – 16.1	12.2 – 13.8	10.7 – 11.8	13.2 – 14.6	15.2 – 16.2	10.7 – 11.8	13.2 – 14.6	10.7 – 11.8
HL	9.6 ± 0.4	12.1 ± 0.6	11.6	8.9 ± 1.7	9.7 ± 0.7	13.3	8.4 ± 1.7	9.9 ± 0.7	8.9 ± 1.7
	9.0 – 10.4	11.5 – 13.0	10.8 – 12.4	11.4 – 7.4	8.9 – 10.2	2.7 – 14.1	7.4 – 11.4	8.9 – 10.2	11.4 – 7.4
DICS	*	*	*	5.5 ± 0.2	7.0 ± 0.1	*	5.5 ± 0.2	7.0 ± 0.1	5.5 ± 0.2
				5.1 – 5.7	6.9 – 7.1		5.1 – 5.7	6.9 – 7.1	5.1 – 5.7
IND	1.7 ± 0.3	2.3 ± 0.2	1.9	1.6 ± 0.2	1.6 ± 0.2	2.1	1.6 ± 0.2	1.6 ± 0.2	1.6 ± 0.2
	1.4 – 2.5	2.0 – 2.5	1.7 – 2.2	1.4 – 1.8	1.4 – 1.8	2.0 – 2.5	1.4 – 1.8	1.4 – 1.8	1.4 – 1.8
END	2.1 ± 0.2	2.7 ± 0.3	2.5	2.3 ± 0.2	2.8 ± 0.5	2.9	2.3 ± 0.2	2.9 ± 0.5	2.3 ± 0.2
	1.5 – 2.5	2.5 – 3.1	2.4 – 2.6	2.2 – 2.6	2.3 – 3.3	2.7 – 3.1	2.2 – 2.6	2.3 – 3.3	2.2 – 2.6
ED	2.9 ± 0.2	3.7 ± 0.1	4.1	2.9 ± 0.3	3.3 ± 0.1	4.6	2.9 ± 0.3	3.9 ± 0.1	2.9 ± 0.3
	2.7 – 3.2	3.5 – 3.8	3.7 – 4.4	2.6 – 3.3	3.1 – 3.4	4.5 – 4.7	2.6 – 3.3	3.1 – 3.4	2.6 – 3.3
UEW	3.4 ± 0.3	4.3 ± 0.3	3.4	3.7 ± 0.4	4.6 ± 0.4	4	3.8 ± 0.3	4.9 ± 0.4	3.7 ± 0.4
	3.0 – 3.8	3.9 – 4.7	3.3 – 3.7	3.2 – 4.0	4.2 – 4.9	3.8 – 4.2	3.2 – 4.0	4.2 – 4.9	3.2 – 4.0
IOD	2.7 ± 0.2	3.3 ± 0.3	3	2.1 ± 0.3	2.5 ± 0.1	3.4	2.1 ± 0.3	2.5 ± 0.1	2.1 ± 0.3
	2.3 – 3.0	3.0 – 3.7	2.4 – 3.4	1.8 – 2.3	2.4 – 2.5	3.1 – 3.8	1.8 – 2.3	2.4 – 2.5	1.8 – 2.3
THL	9.9 ± 0.5	12.9 ± 0.7	12.1	10.3 ± 0.5	12.4 ± 0.5	13.9	10.2 ± 0.5	12.1 ± 0.5	10.3 ± 0.5
	8.8 – 10.5	12.0 – 13.7	11.3 – 12.8	9.9 – 11.1	12.0 – 12.9	13.1 – 14.5	9.9 – 11.1	12.0 – 12.9	9.9 – 11.1
TL	8.8 ± 0.3	11.5 ± 0.7	10.2	9.0 ± 0.5	11.4 ± 0.4	12	9.2 – 0.5	11.1 ± 0.4	9.0 ± 0.5
	8.3 – 9.5	10.5 – 12.3	9.8 – 10.5	8.4 – 9.4	11.1 – 11.8	11.9 – 12.2	8.3 – 9.4	10.1 – 11.8	8.4 – 9.4
FL	14.0 ± 0.4	18.1 ± 0.9	*	14.4 ± 0.4	18.5 ± 0.5	*	14.3 ± 0.4	18.4 ± 0.5	14.4 ± 0.4
	13.2 – 15.0	17.0 – 19.2		14.0 – 15.0	18.1 – 19.0		14.0 – 15.0	18.1 – 19.0	14.0 – 15.0
FHL	*	*	*	12.4 ± 0.6	15.6 ± 0.4	*	12.3 ± 0.7	15.5 ± 0.4	12.4 ± 0.6
				11.7 – 13.1	16.1 – 15.3		11.7 – 13.1	15.3 – 16.1	11.7 – 13.1

Artigo IV. Mângia, S., Oliveira, E.F., Santana, D.J., Koroiva, R. and Garda, A.A. Population genetics and historical demography of the Caatinga frog *Proceratophrys cristiceps* (Müller, 1883) (Amphibia: Anura: Odontophryidae).

**Population genetics and historical demography of the Caatinga frog *Proceratophrys
cristiceps* (Müller, 1883) (Amphibia: Anura: Odontophryidae)**

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Abstract: The Caatinga is characterized by deciduous xerophytic and thorny vegetation, and by a severe water deficit caused by intense and unpredictable dry seasons. Around 210,000 years ago, pulses of moister climate allowed the replacement of part of the semi-arid vegetation of the Caatinga by forests. These climatic fluctuations during the Pleistocene may have affected the geographic genetic structure and demographic history on endemic Caatinga taxa. Herein, we evaluate geographic structure, population genetics, and demographic history of the widespread Caatinga frog *Proceratophrys cristiceps*. We recovered *P. cristiceps* as a single population widely distributed in the Caatinga, with low levels of genetic diversity for mitochondrial and nuclear genes. Our results indicate that *P. cristiceps* experienced significant variation on its effective population size, with a rapid and recent expansion throughout the upper Pleistocene. Our data support the hypothesis that the fauna from the Caatinga may have experienced and been affected by intense climatic fluctuations during Pleistocene.

Key-words: Caatinga, biogeography, Pleistocene, climatic fluctuations.

Introduction

The Caatinga biome was first considered a homogeneous landscape poor in biodiversity (Vanzolini, 1963). This biased view likely contributed to the fact that most studies on Neotropical biodiversity have focused on Rainforests (Furley and Metcalfe, 2007). The Caatinga is characterized by deciduous xerophytic and thorny vegetation such as cactus, shrubs, and small trees, and by a severe water deficit caused by intense and unpredictable dry seasons (Cole, 1960; Ab'Saber, 1998). These current environmental conditions were not permanent in the past, once the Caatinga passed through wetter climates associated with humid vegetation during the Pleistocene (Auler et al., 2004). Around 210,000 years ago, pulses of moister climate allowed the replacement of part of the semi-arid vegetation of the Caatinga by forests. Meanwhile,

expansions of the Caatinga occurred during colder and drier climates (Wang et al., 2004). The occurrence of wetter periods and the existence of rainforest migration routes in this xeric domain are widely recognized based on a variety of evidences (e.g. pollen records – De Oliveira et al., 1999; dated fossil bats bones – Czaplewski and Cartelle, 1998). However, the precise timing of forest expansions is still vague (Werneck, 2011).

The evolutionary history of a domain can be evaluated in studies of historical processes that drove phylogeographic patterns of specific taxonomic groups. However, studies focused on typical species from the Caatinga are unusual and the knowledge about the processes that drove the diversification of its biota is still scarce (e.g. Nascimento et al., 2013; Magalhães et al., 2014; Werneck et al., 2015; Thomé et al., 2016; Fonseca et al., manuscript submitted; São Pedro et al., *manuscript in prep*). Some studies on rainforests have evoked climatic variations during the Pleistocene as a key driver of the origin and maintenance of local biodiversity (Carnaval and Moritz, 2008; Damasceno et al., 2014). Most the studies, however, have shown that the diversification is older, and that the Pleistocene climatic fluctuations affected mostly the demographic history (Maciel et al., 2010; Prado et al., 2012; Werneck et al., 2012; Nascimento et al., 2013; Oliveira et al., 2015; Werneck et al., 2015; Gehara et al., manuscript submitted). Thus, study the taxonomic groups typical from the Caatinga is paramount to understand when and which processes were relevant to the diversification in this domain.

Proceratophrys cristiceps (Müller, 1983) is an anuran typical from the Caatinga and is widely distributed in this domain (Mângia et al., manuscript 1). This species has an explosive-breeding behavior and uses temporary or permanent sandy bottom streams as a reproductive site. During the dry season, individuals bury themselves, emerging after the first rains to reproduce. Herein, we evaluate the geographic structure and the genetic diversity of *P. cristiceps* within the Caatinga domain. Given that the Caatinga is predicted to have expanded and contracted in

response to Pleistocene climatic fluctuations (Wang et al., 2004), we examine historical demography of *P. cristiceps* in order to test for corresponding increases and decreases in effective population size.

Material and Methods

Sampling and data collection

We obtained a total of 109 tissues of *Proceratophrys cristiceps* from 28 localities in the Caatinga biome and adjacent areas (Figure 1). We extracted genomic DNA from muscle or liver samples using the phenol-chloroform protocol (Sam Brooks et al., 1989). We used polymerase chain reaction (PCR) to amplify four loci. Details about loci, primers and PCR protocols are listed in Table 1. PCR products were delivered to Macrogen (Seoul, Korea) for sequencing. First, we sequenced all individual for one mitochondrial DNA (mtDNA) gene (16S). For nuclear genes (nuDNA), we sequenced a subset of individuals (49 for POMC, 42 for CRYb, and 35 for rhodopsin), which we chosen to represent a wide geographic range within the Caatinga domain.

We checked and edited the resulting 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 gene and to avoid possible bias we removed them using Gblocks program (Castresana, 2000; Talavera and 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 nuclear genes, we used the PHASE algorithm (Stephens et al., 2001) implemented in the DnaSP 5.10 software (Librado and Rozas, 2009) using default options. We considered in the following analyses only samples with probability of pairs of alleles in heterozygosity higher than 80%. We deposited all sequences in GenBank (access numbers not available yet).

Population structure

We performed an exploratory analysis to investigate if *P. cristiceps* is composed by one or more populations along its distribution. We used GENELAND 4.0.3 program (Guillot et al., 2005a,b) implemented in R platform (R Development 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 set, one with just nuDNA data 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.

Genetic diversity and Haplotype networks

We calculated haplotype number (h), haplotype diversity (H_d), and nucleotide diversity (π) for each molecular marker using DnaSP 5.10 program (Librado and Rozas, 2009). In order to explore the relationship among haplotypes, we estimated haplotype networks for each marker in Haploviewer (Salzburger et al., 2011) using Bayesian gene trees constructed in BEAST v.1.8 software (Drummond et al., 2012). To generate gene trees, we selected the model of nucleotide substitution for each gene based on the Bayesian Information Criterion (BIC) with jModelTest (Darriba et al., 2012). The best-fit models were K80 for 16S and rhodopsin, JC for CRY b , and HKY + I for POMC. Then, we performed a run with 3×10^6 generations, sampling every 1,000 steps using a Yule Process 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 and Drummond, 2007). The first 20% of sampled genealogies were discarded as burn-in and the most credible clade was inferred 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/>).

Historical demography

If Pleistocene climatic fluctuations influenced the increases and/or decreases in effective population size of *Proceratophrys cristiceps*, we expected to see historical demographic responses dating to this period. In order to investigate the demographic process and estimate the effective population sizes (N_e) through time in *P. cristiceps*, we used three approaches. First, we calculated Tajima's D , which allows the rejection of a null hypothesis, and if a population has been stable through time, this statistic is expected to be zero. Negative values are expected if a population has experienced growth, as rare alleles are more numerous than expected, while positive values will be recovered if rare alleles have been eliminated following bottleneck (Tajima, 1989). We estimated Tajima's D for all genes with 1,000 simulations in DnaSP 5.10 software (Librado and Rozas, 2009). We also constructed a mismatch distribution analysis in DnaSP 5.10. Populations that have experienced recent and rapid expansion are expected to show unimodal distribution, whereas stationary populations will show multimodal distribution (Harpending et al., 1998).

Lastly, we examined the historical population dynamics of *P. cristiceps* using Bayesian Skyline Plot (BSP) (Drummond et al., 2005) implemented on BEAST v.1.8 software (Drummond et al., 2012). We used the same parameters that used to construct haplotype networks. Due to the lack of fossil calibration for this group, we used the substitution rate estimated for 16S mitochondrial gene of 2.8×10^{-9} substitutions/site/year suggested for some amphibian species (LyMBERAKIS et al., 2007; Moriarty-Lemmon et al., 2007). We evaluated the BSP with constant substitution model, two groups, 150 million of generations with 10% of *burn-in*. Using the

function *Bayesian Skyline Reconstruction* in Tracer software, we built the graphic that shows the dynamic of the historical demography of *P. cristiceps*.

Results

Population structure

We obtained a final nuclear DNA dataset of 238 bp for *CRYb*, 454 bp for *POMC*, and 351 bp for rhodopsin. The gene16S preserved around 93% (485 bp) of its original size, after gap exclusion in Gblock. Using two different haplotype datasets, one with just nuDNA data and another with both mtDNA and nuDNA data, GENELAND analyses detected only one cluster ($k = 1$; Fig. 2).

Genetic diversity and Haplotype networks

The 109 sequences of 16S mtDNA resulted in 18 haplotypes in a star-shaped network, presenting no geographic structure and extremely low levels of genetic diversity (Table 2 and Fig. 3). The central haplotype H1 is the most frequent in *P. cristiceps*, containing 84 individuals and occurring in all 27 localities (Table S1).

The three nuclear DNA showed higher levels of genetic diversity than the 16S mtDNA, however, the values are still considered low (Table 2). These genes also presented one haplotype more frequent (Table 2, Fig. 3, and Table S1).

Historical demography

The Tajima's D test recovered negative values for the 16S mtDNA, *CRYb*, *POMC*, and rhodopsin. However, it was significant only for 16S mtDNA (Table 2), which indicates a recent population expansion. The graphics of the mismatch distribution showed a unimodal distribution

for all genes (Fig. 4), which indicates additional evidence that the population has experienced recent and rapid expansion. The BSP analysis revealed an expansion around 220 thousands of years ago (Fig. 5), during the Pleistocene period.

Discussion

Proceratophrys cristiceps is a single population widely distributed across the Caatinga. We also observed that the most common haplotype in mtDNA for *P. cristiceps* is found all over the species distribution. These results contradict what is usually observed for widely distributed species, which face allopatric speciation and may be genetically structured in a geographic pattern. Geomorphological barriers, such as rivers (e.g. Pellegrino et al., 2005, Kaefer et al., 2013) and mountains ranges (e.g. Shepard and Burbrink, 2008; Firkowski et al., 2016; Mângia et al., manuscript 3), are usually responsible for such patterns of geographic structure.

Climatic oscillations or climatic gradients may also act in different latitudes, altitudes, and ecotone zones, which directly affect the genetic structure of some widely distributed species (e.g. *Hypsiboas albopunctatus* - Prado et al., 2012; *Cnemidophorus ocellifer* – Oliveira et al., 2015; *Pleurodema diplolister* - Thomé et al., 2016). For the Caatinga, the São Francisco River (Faria et al., 2013; Nascimento et al., 2013; Werneck et al., 2015), the Espinhaço mountain range (Thomé et al., 2016), the middle São Francisco Dunes region (Passoni et al. 2008; Siedschlag et al. 2010) and some refugee areas (Werneck et al., 2011), have been identified as barriers, which act promoting diversification and geographic structure of lineages of some organisms. However, none of these processes and events seems to have affected the genetic structure of *P. cristiceps*. Despite its widespread occurrence, we recovered one single population for *P. cristiceps* with no geographic genetic structure.

Population expansions following Pleistocene climatic fluctuations are known for several groups of organisms around the globe (e.g. Kokita and Nohara, 2011; Qu et al., 2011; Myers et al., 2013). Our results indicate that *Proceratophrys cristiceps* effective population size changed during this period, with a rapid and recent expansion during the upper Pleistocene. We also found low levels of haplotype diversity and a star-shape pattern in haplotype network for 16S, typical of populations that passed through a recent expansion (Avise, 2009). Our results are consistent with recent studies on widespread Caatinga lineages that have showed population expansions (e.g. Oliveira et al., 2015; Werneck et al., 2015; Thomé et al., 2016; São Pedro et al., *manuscript in prep*; Gehara et al., manuscript submitted), or short coalescent times/branch lengths during the Pleistocene (e.g. Werneck et al., 2012; Recoder et al., 2014). In contrast, some studies have reported population contractions (e.g. Magalhães et al., 2014) or stable population sizes during climatic fluctuations (e.g. Nascimento et al., 2011; Faria et al., 2013). Our results, along with the studies cited above, revealed that the Caatinga fauna, as well as the domain itself, were likely affected by climatic fluctuations during the Pleistocene.

The explosive breeding behavior of *P. cristiceps* might be a favorable natural history trait during drastic climatic changes. This type of reproduction is common in other species (e.g. *Dermatonotus muelleri*, *Physalaemus* spp., *Pleurodema diplolister*) typical from arid regions as the Caatinga (Nomura et al., 2009; Gally and Zina, 2013; Souza and Ávila, 2015). Examine the demographic histories and phylogeographic structures of these taxa, which shared the geographic distributions with *P. cristiceps*, would contribute with comparative studies that will assist to rewrite the historical climate change in Caatinga, as well as the effects of past events on the amphibian fauna in Caatinga.

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Table 1. Information on primers used in the present study.

Marker	Primers	Sequence 5'-3'	Reference
16S	16SAR	CGCCTGTTTATCAAAAACAT	Palumbi (1996)
	16SBR	CTCCGGTTTGAAGTCAGATCA	
CRY <i>b</i>	Cryb1Ls	CGCCTGATGATGTCTTTCCGCC	Dolman and Phillips (2004)
	Cryb2Ls	CCAATGAAGTTCTCTTTCTCAA	
POMC	POMC_DRV_F2	GGCRTTYTTGAAWAGAGTCATTAGWGG	Vieites et al. (2007)
	POMC_DRV_F1	ATATGTCATGASCCAYTTYCGCTGGAA	
Rhodopsin	Rhod1A	ACCATGAACGGAACAGAAGGYCC	Bossuyt and Milinkovitch (2000)
	Rhod1C	CCAAGGGTAGCGAAGAARCCTTC	

Table 2. Genetic statistics for each locus sequenced for *Proceratophrys cristiceps* from the Caatinga Biome in Northeastern Brazil.

Locus	<i>L</i> (bp)	<i>N</i>	<i>S</i>	<i>H</i>	<i>Hd</i>	π	Tajima's D	<i>P</i> -value
16S	485	109	19	19	0.392	0.00112	-2.41418	<0.01**
CRY <i>b</i>	238	84*	5	7	0.706	0.00420	-0.00098	>0.10
POMC	454	98*	6	10	0.708	0.00256	-0.00933	>0.10
Rhodopsin	351	70*	5	6	0.565	0.00247	-0.37092	>0.10

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

*Phased sequences.

**Significant.

Figure Captions

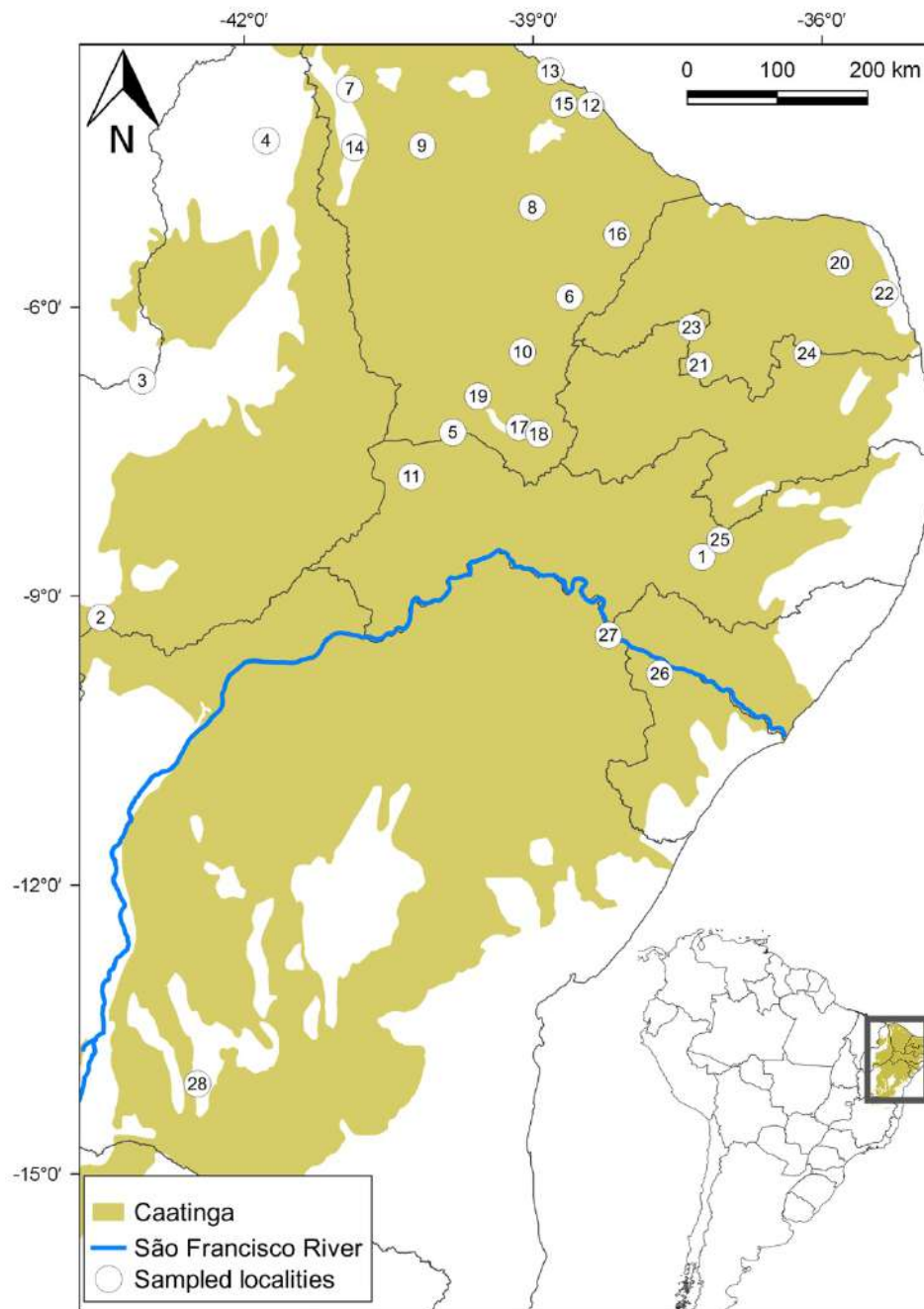


Figure 1. Distribution of sampled localities for *Proceratophrys cristiceps* in the Caatinga Biome in Northeastern Brazil. Number correspond to 28 localities' names in Table S1.

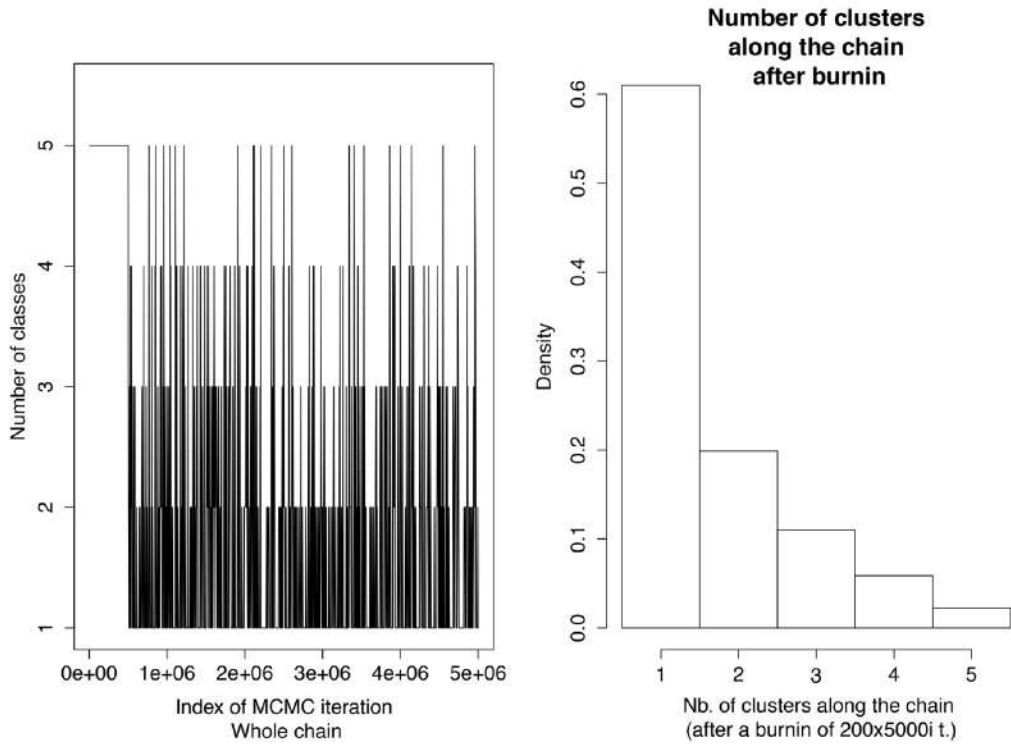


Figure 2. Plot of the number of populations simulated from the posterior distribution obtained with GENELAND for *Proceratophrys cristiceps* in the Caatinga Biome, Northeastern Brazil.

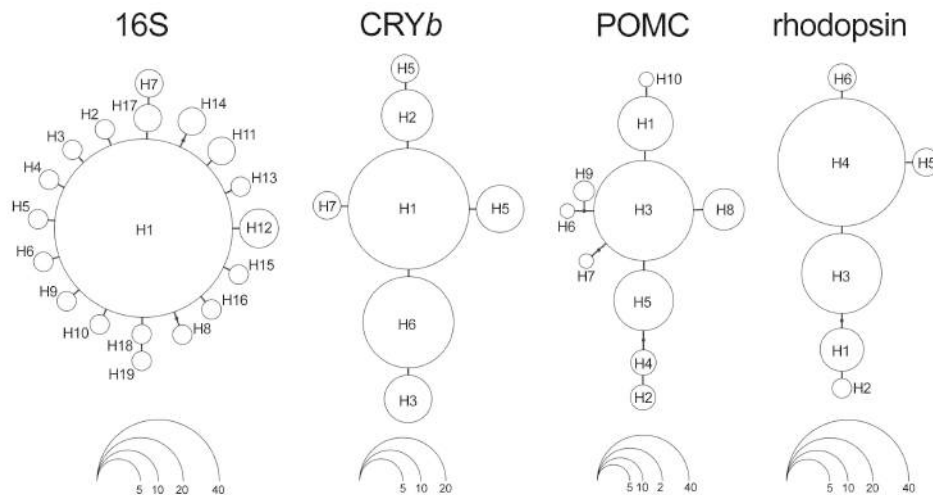


Figure 3. Bayesian haplotype network showing the genetic structure of *Proceratophrys cristiceps* in the Caatinga Biome, northeastern Brazil, according to each gene.

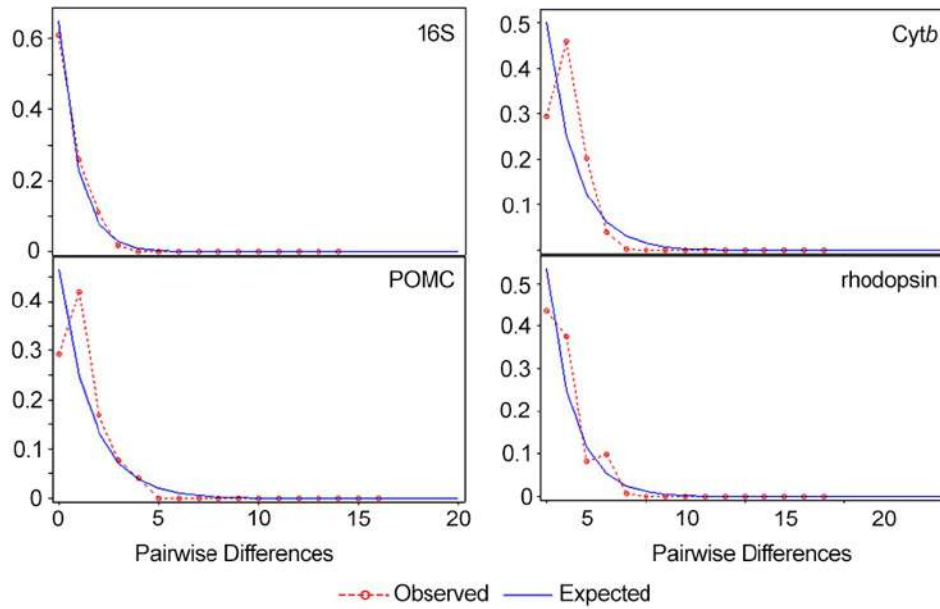


Figure 4. Mismatch distribution for each gene showing the demographic history of *Proceratophrys cristiceps* in the Caatinga Domain, northeastern Brazil.

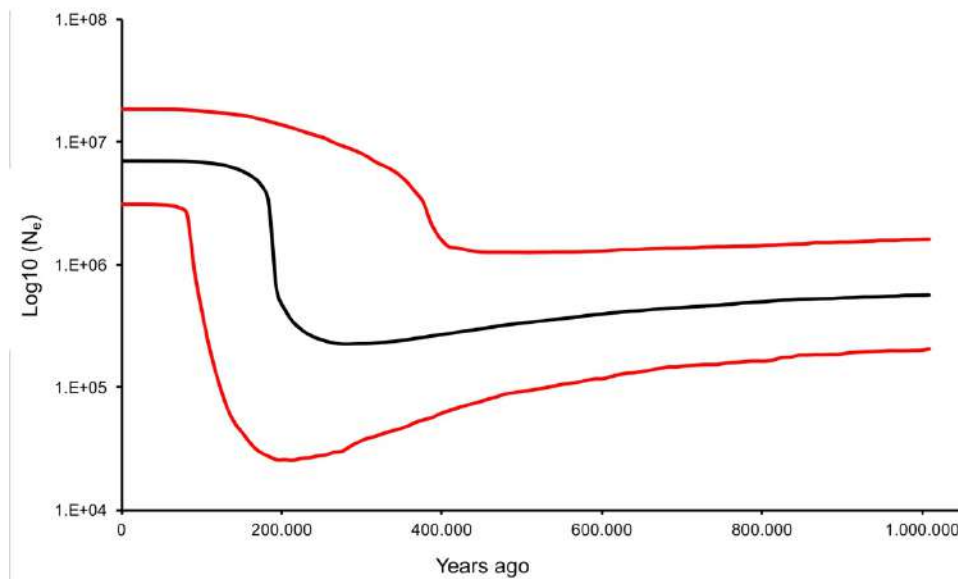


Figure 5. Demographic history through time (in years) of *Proceratophrys cristiceps* determined by the Bayesian skyline plot. The black line represents the median population size, and the grey lines represent 95% higher posterior probability.

Table S1. Samples of *Proceratophrys cristiceps* used in this study. For each sample is presented map code number (see Figure 1), locality, state, and mtDNA and nuDNA haplotypes (see Figure 3).

Map code	Specimen	Locality	State	Holotypes			
				16S	POMC	CRYb	RHO
1	AAGARDA_7747	PARNA Catimbau	PI	H4	H3 (2)	H1 (2)	H4 (2)
1	AAGARDA_7760	PARNA Catimbau	PI	H5	H10, H1	H6 (2)	H4 (2)
2	AAGARDA_11208	PARNA Confusões	PI	H11	H8 (2)	H1 (2)	*
2	AAGARDA_11209	PARNA Confusões	PI	H11	H8 (2)	H1 (2)	H4 (2)
2	AAGARDA_11212	PARNA Confusões	PI	H12	H8 (2)	H1 (2)	H4 (2)
2	AAGARDA_11485	PARNA Confusões	PI	H12	*	*	H4 (2)
2	AAGARDA_11486	PARNA Confusões	PI	H12	*	*	*
2	AAGARDA_11487	PARNA Confusões	PI	H12	*	*	*
3	CHUNFPI_1397	Floriano	PI	H14	*	H1 (2)	H4 (2)
3	CHUNFPI_1398	Floriano	PI	H14	H3 (2)	H1 (2)	H4 (2)
4	UnB_GRC_19531	Piripiri	PI	H17	*	*	*
4	UnB_GRC_19845	Piripiri	PI	H17	H1 (2)	H1 (2)	H4 (2)
2	AAGARDA_11207	PARNA Confusões	PI	H1	H3 (2)	*	H4 (2)
2	AAGARDA_11210	PARNA Confusões	PI	H1	H8 (2)	*	*
2	AAGARDA_11211	PARNA Confusões	PI	H1	H3 (2)	*	*
4	UnB_GRC_19659	Piripiri	PI	H1	H5, H1	*	*
4	UnB_GRC_19839	Piripiri	PI	H1	*	*	*
4	UnB_GRC_19844	Piripiri	PI	H1	*	*	*
4	UnB_GRC_20201	Piripiri	PI	*	H1 (2)	*	*
5	AAGARDA_2735	Crato	CE	H2	H3 (2)	H6, H3	H1 (2)
6	AAGARDA_10286	Jaguaribe	CE	H6	H3 (2)	H6 (2)	H4 (2)
7	AAGARDA_10694	PARNA Ubajara	CE	H7	H1 (2)	H1 (2)	H4 (2)
7	AAGARDA_10979	PARNA Ubajara	CE	H7	*	*	*
7	AAGARDA_10696	PARNA Ubajara	CE	H8	H1, H2	H1, H7	H3, H4
7	AAGARDA_10981	PARNA Ubajara	CE	H9	H5, H3	*	H4, H6
7	AAGARDA_10980	PARNA Ubajara	CE	H10	H3, H4	*	H4, H6
8	AAGARDA_11828	Quixadá	CE	H13	*	*	*
9	FSCHUFPB_843	Santa Quitéria	CE	H16	H3 (2)	H1 (2)	H4 (2)
5	UnB_GRC_22955	Crato	CE	H19	*	H6, H3	*
5	UnB_GRC_21987	Crato	CE	H18	*	*	H3, H4
5	AAGARDA_2739	Crato	CE	H1	H3 (2)	H2 (2)	H5 (2)
5	AAGARDA_2738	Crato	CE	H1	H3 (2)	*	*
5	UnB_GRC_22131	Crato	CE	H1	*	H1, H3	H3 (2)
6	AAGARDA_10176	Jaguaribe	CE	H1	H3 (2)	*	*
6	AAGARDA_10177	Jaguaribe	CE	H1	H3 (2)	*	*

6	AAGARDA_10178	Jaguaribe	CE	H1	*	*	*
6	AAGARDA_10179	Jaguaribe	CE	H1	*	*	*
6	AAGARDA_10399	Jaguaribe	CE	H1	*	*	*
6	AAGARDA_10400	Jaguaribe	CE	H1	*	H1, H2	H3, H4
6	AAGARDA_10401	Jaguaribe	CE	H1	H3 (2)	*	*
6	AAGARDA_10402	Jaguaribe	CE	H1	*	*	*
7	AAGARDA_10646	PARNA Ubajara	CE	H1	H1, H3	*	*
7	AAGARDA_10672	PARNA Ubajara	CE	H1	*	*	*
7	AAGARDA_10695	PARNA Ubajara	CE	H1	*	*	*
7	AAGARDA_10796	PARNA Ubajara	CE	H1	*	*	*
7	AAGARDA_10849	PARNA Ubajara	CE	H1	*	*	*
8	AAGARDA_11826	Quixadá	CE	H1	H3 (2)	H2 (2)	H4 (2)
8	AAGARDA_11829	Quixadá	CE	H1	*	*	*
7	AAGARDA_10678	PARNA Ubajara	CE	H1	*	*	*
10	FRD_681_UFPB	Várzea da Conceição	CE	H1	H3, H7	H1 (2)	*
11	FRD_959_UFPB	Trindade	CE	H1	*	*	*
9	FSCHUFPB_388	Santa Quitéria	CE	H1	*	*	*
9	FSCHUFPB_394	Santa Quitéria	CE	H1	H5, H3	H5 (2)	H4 (2)
9	FSCHUFPB_405	Santa Quitéria	CE	H1	*	*	*
12	PRC_002	Aquiraz	CE	H1	H5, H2	H1, H6	*
12	PRC_003	Aquiraz	CE	H1	*	*	*
13	SM_02	Pecém	CE	H1	H4 (2)	H1, H7	H3 (2)
14	TEC_1435_UFC	Betânea	CE	H1	*	H1, H6	*
15	TEC_1528_UFC	Maranguape	CE	H1	*	H3 (2)	*
16	TEC_1860_UFC	Tabuleiro do Norte	CE	H1	*	*	*
17	URCA_5416	Missão Velha	CE	H1	*	H1, H6	*
18	AAGARDA_11910	Milagres	CE	H1	H3 (2)	H1 (2)	H3, H4
5	AAGARDA_2740	Crato	CE	*	H3 (2)	H1, H6	H1 (2)
5	AAGARDA_2737	Crato	CE	*	H3 (2)	H1 (2)	*
19	URCA_4949_FB	Farias Brito	CE	*	*	*	H3 (2)
20	AAGARDA_5688	João Câmara	RN	H3	*	*	*
21	AAGARDA_5583	ESEC Seridó	RN	H1	H5 (2)	H6 (2)	H1, H2
21	AAGARDA_5605	ESEC Seridó	RN	H1	*	*	*
21	AAGARDA_5607	ESEC Seridó	RN	H1	*	*	*
21	AAGARDA_5679	ESEC Seridó	RN	H1	*	*	*
21	AAGARDA_5689	ESEC Seridó	RN	H1	*	*	*
21	AAGARDA_5820	ESEC Seridó	RN	H1	*	*	H3, H4
21	AAGARDA_5858	ESEC Seridó	RN	H1	*	*	H3, H4
21	AAGARDA_5859	ESEC Seridó	RN	H1	*	*	*
21	AAGARDA_5977	ESEC Seridó	RN	H1	H9 (2)	*	*

22	AAGARDA_1754	Macaíba	RN	H1	*	H1, H6	H3, H4
22	AAGARDA_1755	Macaíba	RN	H1	*	*	*
22	AAGARDA_1758	Macaíba	RN	H1	H3, H6	*	*
22	AAGARDA_8867	Macaíba	RN	H1	H5 (2)	*	*
22	AAGARDA_8868	Macaíba	RN	H1	*	*	*
20	AAGARDA_9811	João Câmara	RN	H1	H3 (2)	*	*
20	AAGARDA_5553	João Câmara	RN	H1	H6 (2)	*	H3, H4
23	SM_12	São José do Brejo da Cruz	PB	H1	*	*	*
24	SM_01	Cuité	PB	H1	H5, H3	H4 (2)	H3, H4
23	SM_04	São José do Brejo da Cruz	PB	H1	*	H4 (2)	*
23	SM_05	São José do Brejo da Cruz	PB	H1	*	*	*
23	SM_06	São José do Brejo da Cruz	PB	H1	*	*	*
23	SM_07	São José do Brejo da Cruz	PB	H1	*	*	*
23	SM_08	São José do Brejo da Cruz	PB	H1	*	*	*
23	SM_09	São José do Brejo da Cruz	PB	H1	*	*	*
23	SM_10	São José do Brejo da Cruz	PB	H1	H1, H2	H6 (2)	H3, H4
23	SM_11	São José do Brejo da Cruz	PB	H1	*	*	*
23	SM_12	São José do Brejo da Cruz	PB	H1	*	*	*
23	SM_13	São José do Brejo da Cruz	PB	H1	*	*	*
25	FRD_1011_UFPB	Arcoverde	PE	H15	H5, H3	H1, H6	H4 (2)
1	AAGARDA_7706	PARNA Catimbau	PE	H1	H5 (2)	H1, H6	*
1	AAGARDA_7707	PARNA Catimbau	PE	H1	*	*	*
1	AAGARDA_7710	PARNA Catimbau	PE	H1	H1 (2)	*	*
1	AAGARDA_7711	PARNA Catimbau	PE	H1	H1 (2)	H1 (2)	*
1	AAGARDA_7712	PARNA Catimbau	PE	H1	*	*	*
25	FRD_499_UFPB	Arcoverde	PE	H1	*	*	*
25	FRD_500_UFPB	Arcoverde	PE	H1	*	*	*
25	FRD_679_UFPB	Arcoverde	PE	H1	*	*	*
25	FRD_1009_UFPB	Arcoverde	PE	H1	H5 (2)	H1, H3	H3, H4
25	FRD_1010_UFPB	Arcoverde	PE	H1	*	*	*
25	FRD_1021_UFPB	Arcoverde	PE	H1	*	*	*
25	FRD_1038_UFPB	Arcoverde	PE	H1	*	*	*
26	FSCHUFPB_318	Poço Redondo	SE	H1	*	*	*
26	FSCHUFPB_319	Poço Redondo	SE	H1	*	*	*
26	FSCHUFPB_338	Poço Redondo	SE	H1	H5, H3	H4 (2)	*
27	FSCHUFPB_311	Paulo Afonso	BA	H1	H5 (2)	H1, H6	*
27	FSCHUFPB_312	Paulo Afonso	BA	H1	*	*	*
27	FSCHUFPB_313	Paulo Afonso	BA	H1	*	*	*
27	FSCHUFPB_314	Paulo Afonso	BA	H1	*	*	*
27	FSCHUFPB_324	Paulo Afonso	BA	H1	*	*	*

28	UFMG_254	Caetité	BA	H1	H3 (2)	H6 (2)	*
29	SM_03	Catolé do Rocha	PB	*	H1, H3	H2 (2)	*

*samples that gene amplification failed; Locality abbreviations: National Park (PARNA), Ecological Station (ESEC); State abbreviations: Piauí (PI), Ceará (CE), Rio Grande do Norte (RN), Paraíba (PB), Pernambuco (PE), Sergipe (SE), and Bahia (BA).