UNIVERSIDADE ESTADUAL DE CAMPINAS
INSTITUTO DE BIOLOGIA
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AVALIAÇÃO DA POSIÇÃO TAXONÔMICA DE POPULAÇÕES DE *Pseudopaludicola* MIRANDA-RIBEIRO (ANURA: LEPTODACTYLIDAE: LEIUPERINAE)

ASSESSMENT OF TAXONOMIC STATUS OF Pseudopaludicola *MIRANDA-RIBEIRO POPULATIONS (ANURA: LEPTODACTYLIDAE: LEIUPERINAE)*

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Dissertação apresentada ao Instituto de Biologia da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Mestre em Biologia Animal, na área de Biodiversidade Animal

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ORIENTADOR: PROF. DR. ARIOVALDO ANTONIO GIARETTA

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RESUMO

Pseudopaludicola é um gênero neotropical de anfíbio anuro que abriga 18 espécies e constitui um grupo natural, com populações distribuídas ao longo da América do Sul. A real diversidade de espécies desse grupo ainda se encontra subestimada e estudos taxonômicos integrados (*integrative taxonomy*) ainda são escassos para o gênero. O presente trabalho obteve em campo espécimes vivos, dados acústicos, e tecidos de populações já conhecidas e ainda não estudadas de *Pseudopaludicola* visando atribuí-las uma identificação taxonômica apropriada. A partir de uma abordagem integrativa, propomos a ocorrência de uma espécie inédita do grupo de *P. saltica* no nordeste brasileiro. Nossa hipótese é sustentada por evidências morfológicas, acústicas, moleculares, citogenéticas e filogenéticas. Além disso, avaliamos o status taxonômico de populações de perto relacionadas à *P. ternetzi*. Com base em dados acústicos, morfológicos e moleculares atribuímos a elas o nome *P. ternetzi*, e propomos uma diagnose acústica desta última espécie em relação a todas as suas congêneres, inclusive para sua espécie-irmã, *P. ameghini*. Adicionalmente, detectamos uma variação morfológica e acústica intraespecífica da população do Pontal do Araguaia (MT) em relação às outras populações de *P. ternetzi*.

ABSTRACT

The dwarf swamp frog, genus *Pseudopaludicola*, includes 18 species, which occur throughout South America. Integrated taxonomic studies are insufficient to describe the underestimated diversity of this monophyletic frog group. The major aim of this study is to improve the *Pseudopaludicola* taxonomic knowledge from a integrative approach. We propose the occurrence of a new species from *P. saltica* species group in northeastern Brazil, hypothesis supported by morphological, bioacoustics, molecular, cytogenetic and phylogenetic evidences. In addition, we evaluated the taxonomic status of populations closely related to *P. ternetzi*. Based on acoustic, morphometric and molecular data we attribute to them the name *P. ternetzi*, and propose an acoustic diagnosis for this species in relation to all its congeners, including its sister species, *P. ameghini*. Additionally, we evaluate a morphometric and acoustic intraspecific variation of *P. ternetzi* from Pontal do Araguaia population (MT) in relation to others studied populations.

SUMÁRIO

1 INTRODUÇÃO

1.1 Introdução geral

Os anuros (Lissamphibia, Anura) são popularmente conhecidos como sapos, rãs e pererecas. Diversas espécies desse grupo têm servido como modelo biológico no entendimento de processos evolutivos e em tempos recentes também tem chamado a atenção de áreas aplicadas da biologia pelas propriedades farmacológicas de diversos produtos presentes em sua pele (Clarke, 1997). A maior riqueza em espécies do grupo está nos trópicos, particularmente na região Neotropical (Duellman & Trueb, 1986) e, em especial, no Brasil (Segalla *et al*., 2014). Atualmente se reconhecem no Brasil 1026 espécies de anfíbios, sendo 988 pertencentes à ordem Anura (Segalla *et al.*, 2014), o que representa cerca de 15 % da diversidade mundial de Anura (Frost, 2015). Grande parte dessa biodiversidade pode ser explicada pelos diversos ecossistemas tropicais e subtropicais do país, principalmente em função da Amazônia, Cerrado, Mata Atlântica e Pantanal.

Entretanto, o desmatamento, o avanço desenfreado da fronteira agrícola, a mineração, o fogo e os projetos de desenvolvimento (e.g. barragens, estradas, indústrias e empreendimentos imobiliários) são apontados como as principais causas de destruição de habitats no Brasil (Silvano & Segalla, 2005). De alguma forma, todos os biomas brasileiros estão sendo severamente afetados, especialmente a Mata Atlântica (Fundação SOS Mata Atlântica, 2002) e o Cerrado, sendo ambos reconhecidos como *hot spots* mundiais; que são vinte e cinco centros de alta diversidade biológica que estão sob forte ameaça (Myers *et al*., 2000). Dentre os tetrápodes modernos, os anuros são considerados o grupo cujas espécies estão sob maior ameaça de extinção nas próximas décadas (Wells, 2007). Stuart *et al*. (2004) reportaram que 43% das espécies de anfíbios estão sofrendo alguma forma de declínio populacional, com cerca de 33% das espécies estando globalmente ameaçadas e que 122 espécies já se encontram possivelmente extintas. Além disso, Stuart *et al*. (2004) ainda apontam que o cenário é tão preocupante que a maioria dessas perdas de diversidade de espécies do grupo teria ocorrido recentemente, a partir dos anos 1980.

1.2 A taxonomia e definições de espécies

Taxonomia é o processo científico em que os grupos naturais são identificados, descritos, nomeados e classificados. É uma pesquisa excitante, não só porque produz uma indispensável contribuição para a ciência, proporcionando o entendimento da biodiversidade, mas, em um nível mais básico, porque satisfaz a necessidade humana da descoberta (Kaiser *et al*., 2013).

Vários cientistas têm apontado que a biodiversidade está enfrentando uma crise (e. g. Wilson, 2003; Wheeler *et al*., 2004) e dado que se estima em milhões o número de espécies que ainda estão para serem descobertas e descritas (Dayrat, 2005; Wilson, 2004) é importante enfatizar a relevância dos taxonomistas na ciência moderna. A maioria das perguntas na biologia evolutiva (e.g. especiação), ecologia (e.g. o desenvolvimento dos ecossistemas), biologia da conservação (e.g. as prioridades de conservação) e a biogeografia (e.g. processos de diversificação) dependem dos inventários de espécies e do conhecimento taxonômico sobre elas (Dayrat, 2005). A taxonomia tradicional baseada unicamente na morfologia tem algumas limitações e uma abordagem integrativa em taxonomia é necessária quando a morfologia pura se revela incapaz de trazer à tona toda a diversidade de determinados grupos, tal abordagem envolve diferentes tipos de dados (e.g. acústicos, moleculares, citogenéticos e comportamentais) (Dayrat, 2005).

Muitos autores reconhecem que a espécie representa uma das unidades mais fundamentais na biologia, equiparando-a a genes, células e organismos (Mayr, 1982; de Queiroz, 2005; de Queiroz, 2007). Atualmente existem diferentes conceitos de espécie (Mayden, 1997; de Queiroz, 1998; de Queiroz, 2007) e, numa revisão, Mayden (1997) listou pelo menos 24 conceitos diferentes de espécie, dentre eles o ecológico, o evolutivo, o filogenético e o tradicional conceito biológico. Todos esses conceitos possuem defensores contemporâneos e ainda se busca um consenso (Mayden, 1997; de Queiroz, 2007).

De Queiroz (2007) propõe um conceito unificado de espécie baseando-se no que ele considera como elemento comum a todos os conceitos e o chama de "unidade conceitual subjacente". Para ele espécies são linhagens metapopulacionais (populações que incluem subpopulações conectadas) que evoluem separadamente. Ainda segundo de Queiroz (2007), em termos práticos, a delimitação de espécies pelos taxonomistas depende do reconhecimento dessas linhagens as quais normalmente, mas não necessariamente, divergiram a ponto de serem feneticamente distinguíveis. Dessa forma, muitas vezes essas linhagens tornam-se diagnosticáveis em termos de estados de caracteres fixos (e.g. genitália, gametas, sistemas de desenvolvimento e comunicação sonora). Nesses casos, os mecanismos de identificação de parceiros coespecíficos dessas linhagens divergiram ao ponto em que os organismos pertencentes a diferentes linhagens já não se reconhecem mais como potenciais parceiros (de Queiroz, 2007).

1.3 Acústica de Anura

A comunicação animal é amplamente conhecida para diferentes táxons animais (e.g. insetos, aves, mamíferos) especialmente dentro de contextos reprodutivos e territoriais (Duellman & Trueb, 1986; Searcy & Anderson, 1986; Gerhardt & Ruber, 2002). O sinal acústico mais comum emitido pelos machos anuros durante a estação reprodutiva é o canto de anúncio que possui duas importantes finalidades: a atração de fêmeas coespecíficas sexualmente receptivas (Blair, 1958; Wells, 1977; Duellman & Trueb, 1986) e a sinalização da ocupação de um determinado sítio de vocalização, ajudando a manter o espaçamento entre os emissores (Wells, 1977; Narins *et al*., 2007). Além disso, o canto de anúncio também pode indicar informações relacionadas à qualidade genética do seu emissor (e.g. tamanho corporal) (Searcy & Anderson, 1986; Gerhardt, 1991; Gerhardt & Ruber, 2002), e em alguns casos, a identidade individual dos machos coespecíficos em um coro (conjunto de indivíduos vocalizando) (Narins *et al*., 2007).

As vocalizações de anúncio de anuros em geral são espécie-específico, tendo uma fundamental importância no reconhecimento específico das espécies do grupo, portanto, têm sido importante na elucidação de questões taxonômicas, como o reconhecimento de novas espécies dentro de um complexo de espécies crípticas (Salthe & Mecham, 1974; Wells, 1977). A utilização dessa ferramenta já é extensivamente utilizada para o reconhecimento da diversidade ainda subestimada de Anura, bem como para subsidiar a avaliação do status taxonômico de espécies mal caracterizadas e/ou com amplas distribuições geográficas (e.g. Wynn & Heyer, 2001; Andrade & Carvalho, 2013; Pansonato *et al*., 2014a). Os caracteres acústicos dos machos estão sujeitos à seleção sexual e atuam como barreira pré-zigótica de isolamento reprodutivo entre as espécies, tendo, portanto, papel importante em eventos de especiação (Blair, 1958; Martof, 1961; Duellman & Trueb, 1986; Searcy & Anderson, 1986; Ryan *et al*., 1990; Ryan & Rand, 1993; Boul *et al*., 2007). Sendo assim, diversas espécies de Anura têm servido como modelo biológico no entendimento de padrões e processos evolutivos (Cocroft & Ryan, 1995; Robillard *et al.*, 2006; Wells, 2007; Erdtmann & Amézquita, 2009). Inclusive, o canto também pode servir como indicador *a posteriori* da robustez de topologias

(Maddison & Slatkin, 1991; Winkler, 2006) ou contribuir com dados dentro de uma analise filogenética de "evidencia total" (Kluge, 1998).

1.4 Caracterização do táxon de interesse

Até o presente momento, o gênero *Pseudopaludicola* Miranda-Ribeiro, 1926, abriga 18 espécies (Frost, 2015) que ocorrem principalmente em ambientes abertos da América do Sul, da Colômbia até a Argentina, nas Guianas e Suriname e no Brasil, no Uruguai e Argentina (Lobo, 1992). *Pseudopaludicola* é considerado um gênero monofilético (Lynch, 1989; Lobo, 1995; Veiga-Menoncello *et al*., 2014) e é suportado por caracteres morfológicos/osteológicos, como a presença de tubérculos antebraquiais (sinapomorfia facilmente reconhecível) (Lynch, 1989; Lobo, 1995) e hioides e processos alares formando uma só placa (Cannatella & Duellman, 1984), além dos caracteres genéticos recém obtidos a partir de análises moleculares de fragmentos mitocondriais (Veiga-Menoncello *et al*., 2014). De acordo com Trewavas (1933) essa placa hioidea alargada seria o produto da fusão dos processos alares, bem como do ramo posterolateral. Trewavas (1933) considera como outra autapomorfia a cintura escapular do tipo arcífero modificada, apresentando as cartilagens epicoracoídeas muito estreitas e sobrepostas entre si. Segundo Lynch (1989) o gênero é dividido em grupos de espécies: o de *Pseudopaludicola falcipes* (Hensel, 1867) e o de *P. pusilla* **(**Ruthven, 1916). Com base em caracteres osteológicos (falanges terminais em forma de "T"), Lynch (1989) sugeriu que o grupo de *P. pusilla* (que incluía *P. boliviana* Parker, 1927, *P. pusilla*, *P. llanera* Lynch, 1989 e *P. ceratophyes* Rivero & Serna, 1985) é monofilético; enquanto o grupo de *P. falcipes* (que inclui as demais espécies até então descritas) não pôde ser definido em termos de derivações compartilhadas e seria parafilético. Fávero *et al*. (2011) apoiou esta proposta de parafiletismo deste grupo de espécies baseando-se no número cromossômico de alguns táxons atribuídos ao grupo de *P. falcipes* que variam de 2N = 16 a 2N = 22, não apresentando resultados conclusivos para considerar esse grupo monofilético. Em uma análise molecular posterior, Veiga-Menoncello *et al*. (2014) corroboraram a natureza monofilética do grupo de espécies de *P. pusilla* e o parafiletismo do grupo de *P. falcipes*. *Pseudopaludicola canga* Giaretta & Kokubum, 2003 em sua descrição original foi atribuída ao grupo de espécies de *P. pusilla* baseado na presença de falanges terminais em forma de 'T'. Entretanto, Cardozo & Suárez (2012) indicou com base em um estudo osteológico em *P. canga* que este caráter é ausente na espécie, portanto, não seria apropriado alocar esta espécie no grupo de *P. pusilla*.

Lobo (1995) analisou as relações filogenéticas de nove espécies então conhecidas e/ou aceitas de *Pseudopaludicola* com base em caracteres osteológicos e de morfologia externa, utilizando como grupo externo oito espécies de *Physalaemus* e duas de *Leptodactylus*. Lobo (1995) confirmou o monofiletismo de *Pseudopaludicola,* o qual corroborou também o monofiletismo do grupo de *P. pusilla*, mas com um arranjo interno diferente para as relações entre as espécies. A análise de Lobo (1995) revelou ainda *P. saltica* como irmã de *P. falcipes*, e possivelmente também de *P. ternetzi*. *Pseudopaludicola mineira* Lobo, 1994 apresentou uma relação incerta com as demais espécies e *P. pusilla* mostrou-se irmã das espécies pertencentes ao seu grupo. Veiga-Menoncello *et al*. (2014) forneceu uma filogenia com base em dados moleculares mitocondriais de onze das dezoito espécies conhecidas até aquele momento e forneceu um bom suporte para o monofiletismo do gênero e do grupo de *P. pusilla*, bem como corroborou o parafiletismo do grupo de espécies que possuem falanges terminais simples. Segundo os mesmos autores, é possível reconhecer com base na filogenia proposta um grupo natural que é composto por espécies que compartilham a distinta característica morfológica de possuírem pernas extremamente longas: o grupo de espécies de *P. saltica* (ver Toledo 2010). Além disso, uma espécie adicional, irmã de todas as espécies até então reconhecidas no grupo de *P. saltica* (*P. saltica* + *P. murundu*), foi revelada como um táxon ainda não descrito na filogenia publicada por Veiga-Menoncello *et al*. (2014).

Espécies de *Pseudopaludicola* apresentam os menores tamanhos da sub-família Leiuperinae (Haddad & Cardoso, 1987), geralmente não ultrapassando os 20 mm de comprimento total, apresentando morfologia e coloração muito semelhantes entre si. Lobo (1992) relata que as maiores espécies da fauna argentina alcançam 25 mm.

Historicamente o gênero *Pseudopaludicola* apresentou vários problemas taxonômicos dos quais alguns ainda perduram. Milstead (1963) sinonimizou *P. ameghini* (Cope, 1887), *P. mystacalis* (Cope, 1887) e *P. saltica* (Cope, 1887) com *P. falcipes*. Bokermann (1966), porém, reconheceu as quatro espécies no oeste e sudeste do Brasil e considerou *P. ternetzi* Miranda-Ribeiro, 1937 como sinônimo de *P. ameghini*. Em seguida, Lynch (1971) listou cinco espécies para o sul e sudeste da América do Sul, *P. falcipes, P. ameghini*, *P. mystacalis*, *P. saltica* e *P. ternetzi*. Lynch (1989) relata que os trabalhos anteriores não fornecem suporte para as sinonimizações e revalidação de *P. ternetzi*. Haddad e Cardoso (1987) avaliando as formas encontradas na Chapada dos Guimarães (MT) com base em dados morfométricos e acústicos colocaram *P. ameghini* como sinônimo de *P. mystacalis* e consideraram *P. saltica* e *P. mystacalis* como espécies plenas. Lobo (1996) corroborou essa proposta de sinonimização e revalidou também as espécies *P. mystacalis* e *P. ternetzi*.

Recentemente, diversos trabalhos atenuaram alguns dos problemas taxonômicos do gênero. Fávero *et al*. (2011) sugere a revalidação de *P. ameghini* baseado no número de cromossomos no seu cariótipo (2N = 20), diferenciando-se de *P. mystacalis* (2N = 16). Pansonato *et al*. (2013) revalidaram de *P. ameghini* com base em dados acústicos e morfológicos. Cardozo & Toledo (2013) baseados na falta de diferenciação acústica e morfológica rejeitou o status de espécie válida de *P. riopiedadensis* Mercadal de Barrio e Barrio, 1994 e a colocou como um sinônimo-júnior de *P. ternetzi*. Pansonato *et al*. (2014a) acessou a variação geográfica de *P. mystacalis* baseado em dados acústicos e morfométricos. Neste mesmo trabalho, os autores reavaliaram a distribuição e o status taxonômico de *P. serrana* Toledo, 2010 e, a partir de uma abordagem acústica, morfológica e molecular a consideraram como um sinônimo-júnior de *P. murundu* Toledo, Siqueira, Duarte, Veiga-Menoncello, Recco-Pimentel e Haddad, 2010. Carvalho *et al.* (in press) revisitou os caracteres diagnósticos das espécies com o padrão trinado nos seus cantos de anúncio (*P. canga*; *P. hyleaustralis* Pansonato, Morais, Ávila, Kawashita-Ribeiro, Strussmann & Martins, 2012; *P. facureae* Andrade & Carvalho, 2013; and *P. parnaiba* Roberto, Cardozo & Ávila, 2013) e propôs que *P. canga* e *P. parnaiba* não podem ser diferenciadas uma da outra com base em caracteres morfológicos/morfométricos e acústicos.

As relações filogenéticas entre *Pseudopaludicola* e outros gêneros de Leptodactylidae já foram acessadas por meio de dados morfológicos, osteológicos, miológicos, reprodutivos, e citogenéticos. Esses estudos indicaram uma relação estreita de *Pseudopaludicola* com *Physalaemus*, *Pleurodema* ou *Edalorhina* (Lynch, 1971; Heyer 1974 e 1975; Cannatella & Duellman, 1984). Cannatella & Duellman (1984) sugeriram uma origem de *Pseudopaludicola* a partir de *Physalaemus,* hipótese que considerava *Pseudopaludicola* parafilético. Porém, Lynch (1989) questionou esta hipótese e revisou *Pseudopaludicola* confirmando o seu monofiletismo, com base na presença de um tubérculo na face posterior do antebraço.

Análises filogenéticas com base em dados moleculares também têm produzido resultados no que diz respeito às relações de *Pseudopaludicola* com outros gêneros leptodactilídeos. Frost *et al*. (2006) indicaram *Pseudopaludicola* como sendo um táxon irmão de um clado formado pelos gêneros *Scythrophrys* + *Paratelmatobius* + *Adenomera* + *Lithodytes* + *Leptodactylus*. Posteriormente, Grant *et al*. (2006) apontaram que *Pseudopaludicola* seria de perto relacionada a *Pleurodema*, *Edalorhina* e *Physalaemus*, todas essas espécies formariam a família Leiuperidae que também incluía *Somuncuria*. Entretanto, *Pseudopaludicola*, *Pleurodema*, *Edalorhina*, *Engystomops* e *Physalaemus* foram colocados na subfamília Leiuperinae por Pyron & Wiens (2011), clado este recuperado dentro da família Leptodactylidae. Uma análise filogenética do gênero *Pleurodema* (Faivovich *et al*., 2012) recuperou *Pseudopaludicola* como um táxon irmão de *Paratelmatobius* (Paratelmatobiinae) o que acarretaria uma natureza parafilética a subfamília Leiuperinae, no entanto, este estudo incluiu uma única espécie de *Pseudopaludicola*. Fouquet *et al*. (2013) recentemente recuperaram com alto suporte *Pseudopaludicola* como sendo grupo-irmão de todos outros gêneros que compõem Leiuperinae, sendo essa subfamília um grupo natural dentro do clado Leptodactilydae.

Giaretta & Facure (2009) com base na morfologia de larvas e comportamento reprodutivo de quatro espécies de *Pseudopaludicola* (*P. saltica, P. mystacalis, P. ternetzi e P. facureae* Andrade e Carvalho, 2013) fornecem evidências contra a hipótese de perda de desova em ninho de espuma em *Pseudopaludicola* (caráter presente em vários outros Leiuperinae). Recentemente, Fouquet *et al.* (2013), com base nas relações de parentesco de Leptodactylidae, sugerem que a construção de ninho de espuma pode ter surgido de forma independente em Leptodactylinae e Leiuperinae. Além disso, Giaretta & Facure (2009) também não descartam com base nos seus dados a natureza parafilética de *Physalaemus* em relação à *Pseudopaludicola*. Uma vez que *P. saltica*, *P. ternetzi* e *P. mystacalis* apresentam duas lacunas nas papilas marginais ao longo dos lábios inferiores e duas fileiras posteriores de dentes labiais e *P. facureae* apresenta o mesmo padrão visto em *Physalaemus cuvieri* e *Physalaemus centralis*: três lacunas nas papilas marginais ao longo dos lábios e três fileiras posteriores de dentes labiais (Giaretta & Facure, 2009).

Ainda deve haver várias espécies não descritas para este gênero de lissanfíbios neotropicais (Toledo, 2010; Veiga-Menoncello *et al*., 2014). Para o Cerrado são conhecidas onze espécies (Frost, 2014): *P. mineira* descrita da Serra do Cipó (MG); *P. mystacalis* descrita da Chapada dos Guimarães (MT) e conhecida nos municípios de Primavera (PA), Itapipoca (CE); Taíba (CE), Balsas (MA), Brejo do Piauí (PI), Uruaçu (GO), Uberlândia (MG), Cuiabá (MT), Nossa Senhora do Livramento (MT); Poconé (MT), Cáceres (MT) e Icém (SP) (Giaretta *et al*., 2008; Giaretta & Facure, 2009; Pansonato *et al*., 2014); *P. saltica* descrita da Chapada dos Veadeiros (GO) e conhecida em Uberlândia (MG); *P. ternetzi* descrita de Uruaçu (GO) e com distribuição conhecida para Uberlândia (MG) (Giaretta *et al*., 2008; Giaretta & Facure, 2009; Fávero *et al*., 2011, Cardoso & Toledo, 2013); *P. giarettai* descrita originalmente de Curvelo (MG) (Carvalho, 2012); *P. murundu* descrita de Rio Claro (SP) (Toledo *et al*., 2010) e conhecida dos municípios mineiros de Santana do Riacho, Brumadinho, São João Del Rey, Itabirito, Lavras Novas, Mariana, Ouro Branco, Botumirim (Toledo, 2010; Pansonato *et al*., 2014); *P. hyleaustralis* descrita de Alta Floresta (MT) e conhecida em outros quatro municípios do norte do Mato Grosso (Pansonato *et al*., 2012); *P. facureae* descrita de Uberlândia e conhecida no município de Limeira do Oeste (MG); *P. parnaiba* conhecida somente da sua localidade-tipo, município de Ribeiro Gonçalves (PI) (Roberto *et al*., 2013); *P. pocoto* Magalhães, Loebmann, Kokubum, Haddad, e Garda, 2014 descrita do município de Santa Quitéria (CE) e conhecida em dez municípios de três estados localizados no nordeste brasileiro: Macaíba e Serra Negra, ambos do Estado do Rio Grande do Norte; Aguiar e Patos, ambos do Estado da Paraíba; Fortaleza, Cratéus, Nova Russas, Morada Nova e Missão Velha, todos do Estado do Ceará; e Betânia do Estado de Pernambuco (Magalhães *et al*., 2014); *P. atragula* Pansonato, Mudrek, Veiga-Menoncello, Rossa-Feres, Martins, e Strüssmann, 2014 descrita do município de Icém (SP) e conhecida do município de Nova Aliança (SP) (Pansonato *et al*., 2014b).

Dentre as 18 espécies de *Pseudopaludicola* quinze possuem cantos descritos: *Pseudopaludicola boliviana* (Duré *et al*., 2004); *P. canga* (Giaretta & Kokubum, 2003); *P. falcipes* (Haddad & Cardoso, 1987; Silva *et al*., 2008); *P. giarettai* (Carvalho, 2012); *P. hyleaustralis* (Pansonato *et al*., 2012); *P. facureae* (Andrade & Carvalho, 2013); *P. mystacalis* (Haddad & Cardoso, 1987; Silva *et al*., 2008); *P. saltica* (Toledo, 2010; Haddad & Cardoso, 1987); *P. murundu* (Toledo *et al*., 2010); *P. mineira* (Pereira & Nascimento, 2004); *P. parnaiba* (Roberto *et al*., 2013); *P. pocoto* (Magalhães *et al*., 2014); *P. ternetzi* (Cardozo & Toledo, 2013), *P. ameghini* (Pansonato *et al*., 2013), e *P. atragula* (Pansonato *et al*., 2014b). As outras três espécies de *Pseudopaludicola* não possuem canto descrito até o presente momento.

2 OBJETIVOS

2.1 Objetivo geral

Contribuir para o conhecimento da biodiversidade, ainda subestimada, dos anuros brasileiros, a partir da avaliação da posição taxonômica de populações e espécies de *Pseudopaludicola*.

2.2 Objetivos específicos

No presente trabalho avaliamos a posição taxonômica de duas populações de espécies que pertencem a dois distintos clados do gênero, o clado 2N = 20 e o clado do grupo de espécies de *P. saltica* (Veiga-Menoncello *et al*., 2014). Para tal, obteve-se por meio dos trabalhos de campo espécimes em vida, amostras teciduais e cantos de anúncios dessas populações-problema e de outras espécies de perto relacionadas para a caracterização e comparações entre estas populações e espécies, visando: i) caracterizar acusticamente essas populações e espécies já conhecidas e reconhecer espécies potencialmente novas; ii) analisar e comparar padrões de distribuição das populações e espécies

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4 RESULTADOS

4.1 Manuscrito I (formatado essencialmente nas normas do periódico Herpetologica)

A New Species of Long-legged *Pseudopaludicola* from Northeastern Brazil (Anura, Leptodactylidae, Leiuperinae)

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RRH: NEW SPECIES OF *PSEUDOPALUDICOLA*

ABSTRACT: A recently published phylogeny corroborated the monophyly of the genus *Pseudopaludicola* and revealed several potential undescribed taxa. In the present study we describe the sister clade to the remaining long-legged species (*P. saltica* + *P. murundu*), the third recognized species of the monophyletic *P. saltica* clade, as a new species from northeastern Brazil. The new species is included in the *P. saltica* species group based on morphological (the presence of long hind limbs) and molecular evidences (mitochondrial genes) and is diagnosed by single, dark, and subgular vocal sac with dark longitudinal folds in males, presence of 11 pairs of chromosomes, and by an advertisement call composed of notes with up to 7 nonconcatenated pulses separated by regular interpulse intervals. We also describe the karyotype and tadpoles of the new species and compare them with the other long-legged species. Our populations were supported as an undescribed and independently evolving species within the *P. saltica* clade based on the generalized mixed Yule-coalescent (GMYC) species delimitation method. Although almost morphologically cryptic to *P. saltica* and *P. murundu*, this new species is easily distinguishable by means of acoustical and genetic traits.

Key words: Advertisement call; GMYC; Integrative taxonomy; Karyotype; *Pseudopaludicola saltica* clade; Species delimitation

AT PRESENT, the dwarf swamp frogs, *Pseudopaludicola* Miranda-Ribeiro, 1926 includes 18 species (Frost, 2015) occurring throughout South America, east of the Andes (Lynch, 1989). *Pseudopaludicola* is recognized as monophyletic based on osteological (Lobo, 1995) and morphological features, such as the presence of hypertrophied antebrachial tubercle (Lynch, 1989; Lobo, 1995), as well as by molecular analyses based on mitochondrial DNA (Veiga-Menoncello et al., 2014). Lynch (1989) proposed the *P. pusilla* (Ruthven, 1916) species group, including *P. boliviana* Parker, 1927, *P. ceratophyes* Rivero and Serna, 1985, *P. llanera* Lynch, 1989, and *P. pusilla*, all sharing the presence of T-shaped terminal phalanges. In subsequent analyses, Lobo (1995) and Veiga-Menoncello et al. (2014) corroborated the monophyly of the *P. pusilla* group. Cardozo and Suárez (2012) withdrew *P. canga* Giaretta and Kokubum, 2003 of the *P. pusilla* (Hensel, 1867) group. Cardozo and Toledo (2013) based on the lack of differentiation in both advertisement call and morphology, placed *P. riopiedadensis* as a junior synonym of *P. ternetzi* Miranda-Ribeiro, 1937. Pansonato et al. (2014a) considered *P. serrana* Toledo, 2010 a junior synonym of *P. murundu* Toledo, Siqueira, Duarte, Veiga-Menoncello, Recco-Pimentel and Haddad, 2010. Carvalho et al. (in press) revisited the diagnoses of the species with trilled advertisement calls (*P. canga*; *P. hyleaustralis* Pansonato, Morais, Ávila, Kawashita-Ribeiro, Strussmann, and Martins, 2012; *P. facureae* Andrade and Carvalho, 2013; and *P. parnaiba* Roberto, Cardozo, and Ávila, 2013) and proposed that *P. canga* and *P. parnaiba* could not be distinguished from each other based in morphology/morphometric, color pattern and bioacoustics traits.

Although advertisement call features have provided relevant information for taxonomy within *Pseudopaludicola* (as mentioned above), integrative taxonomic studies including molecular evidences are still scarce for the genus, but are equally important for describing biological diversity (Glaw et al., 2010; Padial et al., 2010). Despite the considerable rising number of described species in the past five years (six species; Andrade and Carvalho, 2013; Magalhães et al., 2014; Pansonato et al., 2014b, and references therein), a recent molecular phylogenetic approach of *Pseudopaludicola* revealed several populations that may be considered undescribed species (Veiga-Menoncello et al., 2014), suggesting that the diversity within the genus is still underestimated. Such complex taxonomy and recent findings confirm the need of a more thorough taxonomic study of the genus, including multiple sources of evidence.

Within Clade I (2N=22), a sub-group of long-legged species can be recognized, the *P. saltica* (Cope, 1887) group (see Toledo, 2010; Veiga-Menoncello et al., 2014), which includes *P. saltica*, *P. murundu* and an additional species (*P.* aff. *saltica*, described herein). This species (sister of *P. saltica* + *P. murundu*) was considered undescribed by Veiga-Menoncello et al. (2014), and is restricted to northeastern Brazil, while *P. saltica* and *P. murundu* are distributed in central and southeastern Brazil (Toledo, 2010; Toledo et al., 2010). Based on adult and larval morphology, advertisement call, molecular data, and chromosome morphology, we herein describe the third species of the *P. saltica* group from northeastern Brazil.

MATERIAL AND METHODS

Reference specimens.—Specimens (adults and tadpoles) from type-series of the new species were collected and recorded in the Serra das Flores, municipality of Viçosa do Ceará, Ceará State (CE), Brazil (03°23’07” S, 41°09’29” W; 700 m above sea level; *datum* = WGS84) by D. Loebmann. Additional specimens (ZUEC 21858–72) were collected and recorded in the Floresta Nacional (FLONA) de Nísia Floresta, municipality of Nísia Floresta, Rio Grande do Norte State (RN), Brazil (06°04’47.92” S, 35°10’57.22” W; 51 m above sea level; *datum* = WGS84) by F.M. de Magalhães, D.J. Santana, and A.A. Garda. Reference specimens and tadpoles are deposited in the Célio F. B. Haddad amphibian collection (CFBH) at the Universidade Estadual Paulista Júlio de Mesquita Filho (UNESP), municipality of Rio Claro, São Paulo State, Brazil, and in the amphibian collection of the “prof. Adão José Cardoso” zoology museum (ZUEC), Universidade Estadual de Campinas (UNICAMP), municipality of Campinas, São Paulo State, Brazil. Specimens analyzes from the Universidade Federal de Uberlandia frog collection are referred to as AAG-UFU.

Morphometric measurements.—We measured morphometric characters of 19 adult males and 2 adult females from type-locality and 15 adult males and 4 adult females of the new species from FLONA de Nísia Floresta under a stereomicroscope Zeiss Stemi 2000 coupled to an ocular micrometer. Eight measurements follow Duellman (2001): snout-vent length (SVL), head length (HL), head width (HW), internarial distance (IND), snout-eye distance (SED) (= snout length), eye diameter (ED), tibia length (SL) (= shank length), and foot length (FL); three measurements follow Heyer et al. (1990): forearm length (FAL), hand length (HAL), and thigh length (TL). We take SVL of the adults with a Mitutoyo Absolute digital caliper (to the nearest 0.1 mm) under a stereomicroscope. For morphologic/morphometric comparisons, we also measured twenty adult males of *P. saltica* from Municipality of Chapada dos Guimarães (type-locality); Mato grosso State and nine adult males of *P. murundu* from district of Itapé (type-locality), Municipality of Rio Claro, São Paulo State and eleven adult males from Serra da Moeda, Municipality of Brumadinho, Minas Gerais State (see appendix 1).

Advertisement call.—We recorded eight males and analyzed a total of twenty-four advertisement calls and two hundred and twenty-four notes for the new species. We recorded calls (one male) from Viçosa do Ceará (CE) with a Sony cassette tape recorder (TCM-150) coupled to a directional microphone Yoga® (HT 81 Boom) positioned ca. 2 m from the calling male. Recordings were made between 19:00–21:00h and digitized at 44.1 kHz and 16-bit resolution. We recorded vocalizations (seven males) from FLONA with a Tascam DR-40 digital recorder coupled to a Sennheiser ME66/K6 directional microphone. We analyzed calls using Raven Pro 1.5, 64-bit version (Bioacoustics Research Program, 2014) with the following settings: window type = Hann; window size = 256 samples; 3 dB filter bandwidth = 248 Hz; brightness = 50%; contrast = 50%; overlap = 85% (locked); DFT size = 1024 samples (locked); grid spacing (spectral resolution) = 43.1 Hz. Temporal traits were measured on the oscillogram and spectral traits were measured on the spectrogram. Raven Pro 1.5 obtained the dominant and others frequencies bands automatically through its "Peak Frequency (Hz)" function. We generated call figures using Seewave v.1.6 package (Sueur et al., 2008) on R version 3.0.2 (R Core Team, 2014). Seewave settings: Hanning window, 85% overlap and 516 points resolution (FFT). Call duration is the time that males spend emitting a single series of pulsed notes. Note and pulse terminologies followed Magalhães et al. (2014), and overall acoustic terminology followed Duellman and Trueb (1994). We calculated means and standard deviations considering mean values of individual males, whereas the range encompassed the minimum and maximum values for all call sample variation. For each advertisement call, we analyzed ten notes and all pulses/interpulse intervals that comprise these notes. Pulse rate was calculated as pulses per second. We noticed that within each note, the variability of interpulse interval was higher in *P. saltica* and more constant in the other two species. Hence, we quantified this variability through the Coefficient of Variation [CV = (SD/mean) x 100], and used the mean and variance in subsequent analyses (e.g. Discriminant Analysis).

Additionally, for acoustic comparisons, we recorded six topotypic males of *P. murundu* on 08 March 2015 and nine males of *P. saltica* from Uberlândia, Minas Gerais State, Brazil. We also reanalyzed the original recordings from the species description of *P. murundu*, (FNJV 12876; Toledo et al., 2010), one recording from the type locality (LH 676; Pansonato et al., 2014a), and four recordings from São João Del Rei, Minas Gerais State (FNJV 12877–80; Toledo, 2010); from *P. saltica*, we reanalyzed three recordings (13A-01, 42A-06, LH-13) of topotypes described by Pansonato et al. (2013).

Voucher specimens for call recordings: *Pseudopaludicola* sp. nov.: ZUEC 21858–61; *P. murundu*: AAG-UFU 5126; and *P. saltica*: AAG-UFU 2308, 2612.

Tadpole morphology.—We assigned tadpoles to *Pseudopaludicola* species because in the pond they were found the only other anuran species that reproduces is *Pleurodema diplolister* (Peters, 1870), which have very distinct larvae. Furthermore, we collected tadpoles in different stages, including metamorphic, confirming the correct assignment. We measured eight specimens at Gosner's (1960) stages 27 to 29, and nine specimens at stages 35 and 37, following Altig and McDiarmid (1999): body height, body width, tail length, maximum tail height, tail muscle height, tail muscle width, total length, oral disc width, internarial distance, interorbital distance, eye diameter, nostril diameter, eye-nostril distance, nostril-snout distance. Labial tooth row formula was described as in Altig and McDiarmid (1999). Terminology for gap configuration in oral discs marginal papillae followed Candioti et al. (2011). Measurements of tadpoles were made with a micrometric ocular (to the nearest 0.01 mm) coupled to an Olympus SZ40 stereomicroscope.

Statistical analysis.—Considering the (multivariate) morphological and acoustic datasets, we seek to discriminate between populations/species by applying two functions: 1) "randomforest" (RF) (radomForest package, Liaw and Wiener, 2002), and 2) "dapc" (adegenet package, see Jombart, 2008 and Jombart et al., 2010). RF algorithm constructs many (e.g. 500) classification trees using bootstrap samples from the original dataset and then generates classifiers and aggregates results by voting to classes (Breiman, 2001). When the training set for the current tree is drawn by sampling with replacement, about one-third of the cases are left out of the sample. This oob (out-of-bag) data is used to get a running unbiased estimate of the classification error as trees are added to the forest. After each tree is built, all of the data are run down the tree, and proximities are computed for each pair of cases. If two cases occupy the same terminal node, their proximity is increased by one. At the end of the run, proximities are normalized by dividing them by the total number of trees. Proximities are used to replacing missing data, locate outliers, and produce illuminating low-dimensional views of the data (Breiman, 2001).

The classic Discriminant Analysis (DA) depends on multivariate normality (Pohar et al., 2004) and on a larger number of objects than variables. The multivariate normality assumption was tested through the function "mardiaTest" (MVN package; Korkmaz et al., 2014) and was met only for the acoustic data. The application of DA on a few axes (preserving about 95% of the variance) of a Principal Component Analysis, as performed by the "dapc", improves the imbalance between objects and variables (Jombart et al., 2010). For the call analyses, the plotted points in the scatter plot figure correspond to the mean values obtained from each individual recorded. The DA ellipses are centered on means, their width and height are given by variances, and covariance sets their slopes (Dray and Dufour, 2007). Despite the lack of normality in our morphometric datasets, the results of "dapc" are evaluated for both datasets within an exploratory context and to assess their congruence in relation to "randomForest" results. The directly or indirectly packages related the application of both Discriminant Functions were done in R (R Core Team, 2014).

We used the following morphological variables for both Discriminant Analyses and statistical tests*:* SVL, HL, HW, ED, SED, IND, FAL, HAL, TL, SL and FL; for acoustic were: pulse duration, interpulse interval, interpulse interval variance, pulses/second, note duration, internote interval, notes/second, pulses/note, minimum of dominant frequency, maximum of dominant frequency and peak of dominant frequency. Considering that both analyses were highly concordant in species discrimination (see results), we present the RF results in tables and DAPC in scatterplots.

We tested all morphometric and acoustic variables for statistical significance of the differences among population/species through the Exact Wilcoxon Mann Whitney Rank Sum Test using the package coin (Resampling Statistics, Hothorn et al., 2008; function “wilcox_test”) in R. We tested the significance of the found differences between the variances of the interpulse interval of the three species through the Permutational Bartlett’s test of homogeneity of variances (function “perm.bartlett.test”) using package RVAideMemoire (Hervé, 2015). This function performs a permutational Bartlett’s test of homogeneity of k variances. As these tests were done between species pairs, we adjusted the significance levels (“*P*”) considering the number of pairings through the method of Holm (p.adust function in R). We assumed significance when $P \leq 0.05$.

Chromosomal morphology.—We transported live specimens from the type locality to the chromosome studies laboratory at UNICAMP, São Paulo State, Brazil: one female (ZUEC 21004) and four males (ZUEC 20999; 21001; 21003; 21006). We obtained mitotic metaphases from epithelium intestinal cell suspensions, according to King and Rofe (1976), after treatment *in vivo* with 2% colchicine solution for at least four hours. Conventionally, we stained the slides with Giemsa 10% and examined using a photomicroscope Olympus BX60. We based our morphometric analyses on at least three metaphases of each individual and the chromosomal classification relative to centromeric position was done according to the criteria proposed by Green and Sessions (1991).

Genetic distance.—We obtained sequences of all specimens of the *P. saltica* group (Veiga-Menoncello et al., 2014) from GenBank and considered for evaluation of mean pair-wise sequence divergence using MEGA, version 6 (Tamura et al., 2013) with default settings.

Phylogenetic tree estimation and species delimitation.—We wanted to assess the evolutionary independence of lineages between populations from CE and RN States in comparison to other *Pseudopaludicola* species, and confirm that these two populations correspond to the same species by means of molecular evidences. We sequenced partial fragments of 12S and 16S ribosomal RNA mitochondrial genes from four individuals from the RN population (deposited in GenBank, accession numbers: XXX), while sequences from two specimens from CE population were obtained from GenBank (accession numbers KJ147033; KJ147034). Genomic DNA was extracted from liver tissue using the Phenol-Chloroform protocol (Sambrook and Russell, 2006). We used primers MVZ50 of Graybeal (1997) and 16Sa/16Sb of Palumbi et al. (1996) using the following PCR conditions: 1× buffer, dNTP at 0.2 mM, each primer at 0.2 μM, $MgCl_2$ at 2mM, 1U Taq polymerase and 2 μl of template DNA, in a total reaction volume of 25 μl. We used the following cycling program: 94°C for 2 min, followed by 35 cycles of 94°C for 45 s, 50°C for 30 s, and 72°C for 1 min, and concluding with a 5 min extension at 72°C. PCR products were purified with Ethanol/ Sodium Acetate and sequenced at Macrogen Inc. (Seoul, South Korea). We also included all 12S and 16S sequences from *Pseudopaludicola* specimens available in GenBank, plus three additional outgroups (*Leptodactylus pendatactylus*, *Pleurodema diplolister* and *Physalaemus nattereri*; see Supplementary Figure1 for specimen's accession numbers). Sequence alignment was performed using the MUSCLE algorithm (Edgar, 2004) as implemented in the software MEGA version 6.0.6 (Tamura et al., 2013). The final alignment contains partial sequences of 12S (599bp) and 16S (512bp) mitochondrial genes (totaling a 1.115bp concatenated dataset) from 87 specimens, representing 13 of the 18 currently recognized *Pseudopaludicola* species, three undescribed taxa (referred as *Pseudopaludicola* sp.), and three outgroups previously mentioned.

We then estimated a Bayesian ultrametric mitochondrial gene tree with BEAST v1.7.5

software (Drummond et al., 2012) implementing a GTR+I+G model, as suggested by the Akaike Information Criterion (Akaike, 1974) in jModeltest version 2.1.6 (Darriba et al., 2012). We performed five independent runs with 30 million generations each sampling every 3.000 steps using a Yule tree prior with a lognormal uncorrelated relaxed clock model. We checked for stationary posterior distributions, effective sample sizes (ESS) above 200, and convergence between runs by examining parameter traces with the program Tracer v1.6 (Rambaut et al., 2014). We combined runs and trees after removing a 10% burn-in with Log Combiner v1.7.5 (Drummond et al., 2012), and annotated tree files and computed the maximum clade credibility (MCC) tree with TreeAnnotator v1.7.5 (Drummond et al., 2012).

To objectively delimit species based on the mitochondrial dataset, we used both the maximum likelihood (ML) and the Bayesian implementation of the generalized mixed Yule-coalescent (GMYC) model, which delimit independently evolving species using single-locus data (Ponts et al., 2006). The ML method implements a model-based analysis to locate threshold points (or nodes) on the genealogy where there are transitions in branching rates reflecting either inter- or intra-specific evolutionary processes using an ultrametric gene tree as guide (Pons et al., 2006). We used the entire dataset (containing all sequenced *Pseudopaludicola* specimens, excluding outgroups) to perform this analysis because the GMYC is expected to perform best and provide robust results when more species and sampling of individuals per species are included (Papadopoulou et al., 2009; Fujisawa and Barraclough, 2013). We used the single-threshold version of the ML method implemented in the R v3.0.2 package ‘splits’. We then applied the Bayesian implementation of the GMYC model to account for uncertainty in genealogy estimation (Reid and Carstens, 2012) with the R v3.0.2 package ‘bGMYC’, which calculates the marginal posterior probabilities of species limits from the posterior distribution of ultrametric trees reconstructed with BEAST. For the bGMYC analysis, a post-burn-in sample of 100 trees was used to calculate the posterior distribution of the GMYC model. The vector of starting parameters for the model was set to c(1,1.2,35), while scaling parameters was set to c(5,15,0.5). Priors on parameters t1, t2 and py2 were set to 18, 84 and 1.2, respectively. Remaining priors were set as default. We ran the bGMYC analysis for 100,000 generations, with a burn-in of 90,000 generations and a thinning interval of 100 samples.

SPECIES DESCRIPTION

Pseudopaludicola sp. nov.

(Figs. 1–3, Tables 1, 2)

Pseudopaludicola sp. (aff. *saltica*): Loebmann and Haddad (2010)

Pseudopaludicola sp. (aff. *saltica*): Veiga-Menoncello et al. (2014)

Holotype.—Adult male (CFBH 32609; Figs. 1, 2) collected in Serra das Flores, municipality of Viçosa do Ceará, Ceará State (03°23’07” S, 41°09’29” W, 700 m above sea level) by D. Loebmann on 3 February 2009.

Paratopotypes.—Eighteen males: ZUEC 20475, ZUEC 20477–84, CFBH 32617–25; and two females: ZUEC 20476 and CFBH 32614. All adult specimens were collected on 3 February 2009 by D. Loebmann. Nine tadpoles (CFBH 32626) were collected on 14 May 2008, and twenty tadpoles (ZUEC 20485) on 20 April 2009.

List of additional specimens.—BRAZIL: Rio Grande do Norte State, municipality of Nísia Floresta: Floresta Nacional de Nísia Floresta (FLONA): ZUEC 21858–72.

Diagnosis.—*Pseudopaludicola* sp. nov. is assigned to the genus *Pseudopaludicola* by its tiny size and the presence of hypertrophied antebrachial tubercles (see Lynch, 1989; Lobo, 1995) and to the *P. saltica* species group by having its tibio-tarsal articulation reaching beyond the end of the snout when legs are adpressed to body. The new species is characterized by the following combination of characters: (1) long hind limbs, (2) single, dark, and subgular vocal sac with dark longitudinal folds in males; (3) white to light brown nuptial pads in males, which cover the external part of thumb; (4) 11 pairs of chromosomes; (5) advertisement call composed of notes with up to 7 nonconcatenated pulses separated by regular interpulse intervals.

Comparison with other species.—*Pseudopaludicola* sp. nov. is promptly diagnosed from the *P. pusilla* species group by the absence of either T-shaped terminal phalanges or expanded toe tips (disks or pads) (Lynch, 1989; Lobo, 1995; Cardozo and Suarez, 2012). The new species has phalanges shaped similarly to those of *P. facilpes* (see Fig. 2B in Cardozo and Suárez, 2012). The new species can also be distinguished from *P. boliviana*, *P. ceratophyes*, and *P. llanera* by the absence of enlarged palpebral tubercle (Lynch 1989) and from *P. boliviana*, *P. ceratophyes* by the absence of an enlarged, conical tubercles on the heels. *Pseudopaludicola* sp. nov. is distinguished from all species of the genus, except from *P. saltica* and *P. murundu* by having long hindlimbs, with tibio-tarsal articulation reaching beyond the tip of snout when the leg is adpressed to body. The new species distinguishes from *P. saltica* by having dark vocal sacs in males, smaller thigh ($P = 0.007$), shank ($P < 0.001$) and foot ($P = 0.007$) lengths, and smaller eye diameter ($P = 0.015$); from *P. murundu* by its larger shank length ($P = 0.023$) and head width ($P < 0.001$), and longer internarial ($P = 0.002$) and eye-snout distances ($P < 0.001$).

Tadpoles of the *P. saltica* species group are similar in external morphology, indistinguishable based on most of the measured characters (see Giaretta and Facure, 2009; Toledo, 2010; Toledo et al., 2010).

Pseudopaludicola sp. nov. is diagnosed from *P. canga* (Giaretta and Kokubum, 2003; Pansonato et al., 2012), *P. giarettai* Carvalho, 2012 (Carvalho, 2012), *P. hyleaustralis* (Pansonato et al., 2012), *P. facureae* (Andrade and Carvalho, 2013), and *P. parnaiba* (Roberto et al., 2013) by emitting pulsed notes, whereas all the five abovementioned species emits non-pulsed notes. Note structure (2–7 nonconcatenated pulses) distinguishes the calls of *P.* sp. nov. from those of *P. mystacalis* (Cope, 1887) (12–14 concatenated pulses [= lack of interpulse interval]; Pansonato et al., 2014a); and *P. boliviana* (calls with five notes and 3–6 concatenated pulses each one; Duré et al., 2004).

The new species differs from other congeners [values within square brackets] with which it shares notes with nonconcatenated pulses by the following acoustic traits: *P. ternetzi* has shorter internote interval (84–184 vs. [18–61 ms]) and lower dominant frequency (5.0–5.9 vs. [3.6–3.8 kHz]; Cardozo and Toledo, 2013); *P. ameghini* (Cope, 1887) has a lower dominant frequency [3.2–4.4 kHz] (Pansonato et al., 2013); *P. mineira* Lobo, 1994 has shorter note duration (56–178 ms vs. [mean 40 ± 4 ms]), less pulses per note (2–7 vs. [2–3]), and lower dominant frequency [4.3–4.8 kHz] (Pereira and Nascimento, 2004); *P. falcipes* has shorter note duration [mean 40 ms] and internote interval [mean 70 ms] (Haddad and Cardoso, 1987); *P. pocoto* Magalhães, Loebmann, Kokubum, Haddad and Garda, 2014 has longer note duration [126–290 ms], longer interpulse interval [49–166 ms] and lower pulse rate (17–26 vs. [10–18 pulses/second], Magalhães et al., 2014); and *P. atragula* Pansonato, Mudrek, Veiga-Menoncello, Rossa-Feres, Martins and Strüssmann, 2014 has longer note duration [300–700 ms], higher number of pulses per note [9–36 pulses/note] and lower dominant frequency [3.6–4.2 kHz] (Pansonato et al., 2014b).

Pseudopaludicola sp. nov. is distinguished from *P. murundu* by its lower dominant frequency ($P = 0.003$), longer note duration ($P = 0.006$), lower variance in interpulse intervals ($P = 0.008$) and higher number of pulses per note ($P = 0.002$); and from *P. saltica* by having regular (low variance) interpulse intervals (CV = 43.3 ± 7.8 [33.5–56.2] in the new species and CV =

90.6 ± 17.2 [53.0–111.8] in *P. saltica*, *P* = 0.006), higher dominant frequency (*P* = 0.003), and shorter note duration (*P* < 0.001). In addition, the new species can be distinguished from *P. murundu* and *P. saltica* by emitting notes with up to seven pulses (up to six pulses/note in these species).

In both multivariate approaches (randomForest and dapc) based on morphology there was no noticeable discrimination between the three species (see Table 4 and Fig. 4A). For those multivariate analyses based on acoustic traits, we found a considerable discrimination of the new species from *P. saltica* and *P. murundu* (Table 5), with only one male of new species being classified as *P. murundu* due to its higher dominant frequency. Accordingly, the dapc (Fig. 4B) resulted in total discrimination of the three species. Interpulse interval variance, peak of dominant frequency and note duration were the main sources of variation (ca. 80%), enabling to distinguish *P.* sp. nov. from *P. saltica* and *P. murundu*.

Pseudopaludicola sp. nov. (2n = 22 chromosomes) is also diagnosed from *P. mystacalis* (2n = 16), *P. canga*, *P. facureae*, *P. atragula* (2n = 18), *P. ameghini* and *P. ternetzi* (2n = 20) in chromosome number (Duarte et al., 2010; Fávero et al., 2011; Veiga-Menoncello et al., 2014).

Description of the holotype.—Body elliptic and broad (Fig. 1A,B) (Table 1). Head triangular, slightly longer than wide. Snout sub-elliptical in dorsal view and rounded in profile (sensu Heyer et al., 1990; Fig. 2A,B). Eyes protuberant, their diameter larger than the interorbital distance; interorbital area flat; pupil rounded; upper eyelids with 3–4 discrete tubercles. Nostrils not protuberant and closer to the snout tip than to the eyes. *Canthus rostralis* rounded, smooth; loreal region slightly concave. Single subgular vocal sac, externally expanded, large, and with longitudinal folds; choanae well-separated from each other; vocal slits present. Tympanum indistinct; a dermal fold extending from the back of eyes to the insertion of the arm.

Mouth opening ventral. Vomerine teeth absent (unnoticeable also to touch). Tongue elliptical, free posteriorly; without pigmentation on its base. Flanks with discrete granules. One ovoid antebrachial tubercle present in the first quarter of the forearm. Fingers and toes tips not expanded. Outer and inner metacarpal tubercles well-defined, rounded. Finger subarticular tubercles single and conical. Few (about three) rounded supernumerary tubercles in the hand. Only one subarticular tubercle, at the basis of each finger; finger III with an extra subarticular tubercle between first and second phalanges. Thumb with keratinized light brown nuptial pad, extending from the basis of the hand to the proximal limit of the terminal phalanx, covering almost all the external portion of the finger. No finger webbing. Fingers slightly fringed at sides. Finger lengths (when adpressed to one another) I<IV<II<III (Fig. 2C). Outer and inner metatarsal tubercles well-defined, conical; internal larger than the external; external more protuberant than the internal. Toes subarticular tubercles well-defined, single, enlarged and conical (Figs. 1B, 2D). Supernumerary tubercles absent on the foot. Toes webbed basally and extensively fringed at side to almost their tips. A well-developed fold from the internal metatarsal tubercle to the mid-ventral tarsus, continuing towards the toe tip as fringes. Toe length (when adpressed to one another) I<II<III=V<IV (Fig. 2D). Hindlimbs robust and long with tibio-tarsal articulation reaching beyond the end of the snout. Thigh shorter than tibia; foot longer than thigh and slightly shorter than tibia. Transverse stripes on thighs (2–3), shanks (3–4), feet (3–4), and forearms (2–3). Calcaneus appendices absent. Belly skin smooth. Skin of ventral thighs (sit pad) granulate. Dorsal surfaces of head, body, and limbs smooth, with some tubercles; the skin on the scapular region has two arc-shaped granular folds. One gland on each side of the cloaca, cloacal region smooth (Fig. 1B).

Measurements of holotype (mm).—SVL 15.1, HL 4.6, HW 5.2, ED 1.8, IND 1.4, SED 2.9, HAL 4.5, TL 8.5, SL 10.6, FL 10.1.

Color of the holotype in preservative.—Back brown with gray spots; light beige belly. Back darker than the dorsal surface of limbs; coloration of the plantar surface similar to that of dorsal legs; palmar surface almost without pigmentation. Region between mouth and eyes with alternated vertical dark brown and light beige stripes. Ventral surface of arms and legs light beige. Dorsal surface of arms light beige with brown spots; dorsal surface of legs beige with brown transversal descontinuos strips. Thin dark vertebral line from the snout tip to cloacal region. Light beige nuptial pads with brown margins (Fig. 1).

Variation among types.—Back color varies from gray to brown, having dark gray or dark brown irregular spots. In adult males, vocal sac can be dark as well as the ventral surfaces of arms and legs. The belly is consistently beige. Females differ by having white throat and are slightly larger than males (Table 1). When present (5 out 18 paratopotypes) the vertebral line can be red, orange, or yellow. When the vertebral line is present a spot with the same color of it is visible on upper arms (Fig. 3A,C). Conversely, the specimens collected at Nísia Floresta municipality do not exhibit the vertebral line, and such feature was not observed in other specimens in the field (FMM, personal observation).

Distribution.—*Pseudopaludicola* sp. nov. is known from type-locality “Serra das Flores”, Municipality of Viçosa do Ceará, Ceará State, 03°23’07” S, 41°09’29” W, 700 m above sea level, and Floresta Nacional (FLONA) de Nísia Floresta, municipality of Nísia Floresta, Rio Grande do Norte State, Brazil. The FLONA de Nísia Floresta is about 700 km southeast of the type-locality.

Natural history notes.—Loebmann and Haddad (2010) conducted a 24-month period field work in Planalto of Ibiapaba and only one population of *P*. sp. nov. was found there. Besides *P*. sp. nov., two other congeneric species were registered at Planalto of Ibiapaba: *P*. *mystacalis* and *P*. *pocoto*. These species are not syntopic with new species, which in turn cooccurs with at least other six frog species: *Leptodactylus vastus* Lutz, *Physalaemus cuvieri* Fitzinger, *Pleurodema diplolister* Peters, *Proceratophrys caramaschii* Cruz, Nunes and Juncá, and *Scinax* sp. (gr. *ruber*). At Nísia Floresta municipality, *Elachistocleis cesarii* (Miranda-Ribeiro), *Leptodactylus troglodytes* Lutz, *Pleurodema diplolister, Scinax fuscomarginatus* (Lutz) and the congeneric *P. mystacalis* occur in sympatry with *P.* sp. nov. *Pseudopaludicola* sp. nov. is nocturnal and breeds during the rainy season (February to May in Ceará State, and from June to August in Rio Grande do Norte State), mainly during the first rains. Males call in chorus during breeding season on grasslands flooded with a thin layer of water (up to 1 cm depth). Satellite behavior was observed for populations from Ceará State (Fig. 3D). Amplectant pairs lay their eggs in the water where exotrophic tadpoles develop, i.e., reproductive mode 1 (Haddad and Prado, 2005).

Advertisement call.—Quantitative variables are summarized in Table 2. The advertisement call of the new species consists of a long (7.0–108.5 s) series of pulsed notes (18–574 notes/call). Notes vary from 56–178 ms in duration separated by intervals of 84–184 ms; notes has a slightly ascendant frequency modulation throughout their duration, and are emitted at a rate of 4.0–6.9 notes/second. Notes composed by 2−7 nonconcatenated pulses. Pulses last from 6 to 18 ms separated by intervals of 1–46 ms (variance = 5.9 ± 1.3 (0.2–39.2) and released at a rate of 17 to 26 pulses/second. Dominant frequency peaks from 5082 to 5986 Hz; minimum frequency from 4318 to 5057 Hz and the maximum from 6000 to 6676 Hz. Other emphasized frequency band can be assessed at a higher frequency (Fig. 5A), peaking from 9862 to 12102 Hz (10922 ± 369). Air temperature of recorded calls varied from 22.2 to 24.0°C.

Tadpole description.—Table 3 and Fig. 6A,D. Body flattened ventrally (body height/body width = 0.78; 0.69–0.96), elliptical in dorsal, ventral views. Body length about 30%

(27–36%) of total length. Snout oval in dorsal view and sloped in lateral view. Nostrils rounded, dorsolaterally directed, closer to the eyes (0.48 ± 0.12; 0.29–0.7) than to the snout (1.37 ± 0.22; 0.96–1.74). Small dorsolaterally oriented eyes (eye diameter/body width = 0.22; 0.19–0.28). Spiracle single and sinistral, inner wall free from body. Tail muscle about 56% of the total tail height. Dorsal and ventral fins originate near the tail/body junction. Oral disc anteroventral. Papillae on the lateral region of the mouth. Posterior margin with two ventrolateral gaps. One broader gap in dorsal marginal papillae. Labial tooth row formula 2(2)/2–3[1]. Narrow jaw sheaths with triangular serration; lower jaw U-shaped and upper jaw sheath arc-shaped with long lateral processes. In preservative dorsal surface dark brown, tail beige, and fins transparent with few scattered brown spots; internal organs visible in ventral view.

Karyotype description.—*Pseudopaludicola* sp. nov. karyotype consisted of 2n=22 chromosomes organized in seven pairs of metacentric chromosomes (1, 2, 5, 7, 9, 10, and 11), three submetacentric pairs (3, 4, and 6), and one telocentric pair (8). A remarkable size heteromorphism was observed on the long arm of pair 8 (ZUEC 20999; 21001; 21003; 21006), due to secondary constriction present in one of the homologous, which resulted in the description of morphs 8 and 8’ (Fig. 7; Table 6).

Genetic divergence analyses.—The divergences between *Pseudopaludicola* sp. nov. and other species of the *P. saltica* species group ranged from 2.5 to 2.9 % (Table 7).

Phylogenetic tree estimation and species delimitation.—The mtDNA gene tree recovered with BEAST is generally congruent with the Bayesian tree topology inferred by Veiga-Menoncello et al. (2014). Also, the mitochondrial gene tree confirmed (with high posterior probability) that *P.* sp. nov. populations from CE and RN States are reciprocally monophyletic in respect to the sister clade formed by *P. saltica* + *P. murundu* (recovered with lower posterior probability). The GMYC ML analysis identified 18 evolutionary entities (confidence interval 16–

21), including three evolutionary entities within the *Pseudopaludicola saltica* clade, and 17 genetic clusters (confidence interval 15–19) with a significant model of species delimitation (x^2=15.21, $P < 0.0005$). The mean number of evolutionary entities delimited by the bGMYC analysis was 18 (conspecificity probability threshold = 0.5), and 23 genetic clusters with 95% HPD probability interval, including 3 singletons. Most of the ML entities match those coalescent units with the highest marginal probabilities (which corresponds to currently recognized species in this genus or candidate species). Specifically, the delimitation of the three species within the *P. saltica* clade is supported by high posterior probability (>95%), providing additional evidence that *P*. sp. nov. is an independently evolving species within the *P. saltica* clade, which also includes *P. saltica* and *P. murundu* (see Fig. 8).

DISCUSSION

By adding more calls to former comparisons, we were able to improve the diagnosis between *P. murundu* of *P. saltica* (Table 5 and Fig. 4B). *Pseudopaludicola murundu* has pulsed notes with pulses separated by regular interpulse intervals (low variance) and higher dominant frequency (Fig. 5B), whereas *P. saltica* calls have pulses separated by irregular interpulse intervals ($P = 0.05$) and lower dominant frequency ($P < 0.001$, Fig. 5C; further details in Table 2). Moreover, our statistical morphometric analysis also showed that *P. saltica* has a larger head ($P = 0.005$), thigh ($P = 0.004$), and shank ($P = 0.002$) lengths than *P. murundu*.

The diploid number 2n=22 described herein for *P*. sp. nov. has also been reported for other species within the *P. saltica* group (Duarte et al., 2010; Toledo et al., 2010). A comparison among the karyotypes of *P. saltica* (Duarte et al., 2010), *P. murundu* (Toledo et al., 2010), and *P*. sp. nov. revealed interspecific differences in the morphology of pair 8. In *P*. sp. nov. and *P. saltica* females both pairs of chromosome 8 are telocentric (Duarte et al., 2010). Conversely, in males of *P*. sp. nov. and *P. murundu* (Toledo et al., 2010) this same pair is also composed of telocentric chromosomes, but with remarkable size heteromorphism (see Fig. 7). In *P. saltica* males, the NOR-bearing pair 8 is heteromorphic with telocentric and submetacentric homologous, characterizing a XX/XY sex-determination system for this species with telocentric X and submetacentric Y (Duarte et al., 2010). Sexual dimorphism in chromosomal morphology was not mentioned by Toledo et al. (2010) in the description of *P. murundu*. Unfortunately, only males were analyzed by these authors (a juvenile male was misinterpreted as a female). Therefore, further *P. murundu* female samples will certify if the size heteromorphism observed between the homologous of pair 8 (as observed in new species) is a male-specific chromosome, indicating a morphological differentiation of XY/XX sex chromosomes, and if *P. murundu* also shares this putative sex-specific difference, which could be considered a synapomorphy of the *P. saltica* species group.

This is the first integrative taxonomic study to assess morphological (adult and larval), acoustic, chromosomal, and molecular evidences in a species description within the genus *Pseudopaludicola*. Given that almost all distinct lines of evidences have yielded congruent results (except for the external morphology of both adults and tadpoles), we have unequivocally confirmed that *P*. sp. nov. is a independently evolving species within the *P. saltica* clade. Moreover, our results reinforced that *P. serrana* is a junior synonym of *P. murundu* (Pansonato et al., 2014a) because all sequenced individuals (including three sequences from *P. serrana* type locality) were recovered as a single species in the delimitation analysis. Besides recovering almost all current recognized species as independently evolving units (corroborating the current taxonomy of the genus), both ML and Bayesian GMYC analysis pointed that some widely distributed species may also correspond to more than one taxon. For instance, BEAST gene tree recovered two main lineages within *P. mystacalis* with high posterior probability, and such lineages have less than 0.05% of marginal posterior probability of being conspecific, according to the bGMYC analysis (see Fig. 8). Additionally, these analyses also recovered as independently evolving entities three unidentified taxa (*Pseudopaludicola* sp.) that are still not attributed to any known species or are in description process (sensu Veiga-Menoncello et al., 2014). Such results reinforce that the species richness within *Pseudopaludicola* is likely underestimated, as highlighted by the first molecular phylogenetic assessment of this genus made by Veiga-Menoncello et al. (2014). Finally, integrative taxonomic studies (such as ours) can significantly improve species descriptions and delimitation, especially when dealing with a rich and morphologically cryptic group, as is the case of the species in the genus *Pseudopaludicola*.

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APPENDIX I

Specimens Examined

Pseudopaludicola ameghini—BRAZIL: MATO GROSSO: Chapada dos Guimarães (ZUEC 14138–14139; 14141–14145).

Pseudopaludicola canga—BRAZIL: PARÁ: Marabá: Serra dos Carajás (ZUEC 9990; 10034; 14370; 14372–14374; 14378).

Pseudopaludicola facureae—BRAZIL: MINAS GERAIS: Uberlândia (AAG-UFU 0853–0855; ZUEC 13651–13652; 14215; 14218–14219; 14221; 14224).

Pseudopaludicola falcipes—BRAZIL: RIO GRANDE DO SUL: Porto Alegre (ZUEC 14008; 14022; 14162–14166; 14168).

Pseudopaludicola mineira—BRAZIL: MINAS GERAIS: Jaboticatubas (ZUEC 1574; 1582–1583; 1586–1587; 1590; 1593–1595; 1600; 1604; 1617).

Pseudopaludicola murundu—BRAZIL: SÃO PAULO: Águas de Santa Bárbara (ZUEC 20507–20508), Rio Claro (AAG-UFU 5125–5126; CFBH 8235–8242; ZUEC 14284–14290). BRAZIL: MINAS GERAIS: Brumadinho (ZUEC 16396–16398; 16442–16443; 19549; 19551; 19555; 19557–19578; 19560), Santana do Riacho (ZUEC 2323), São João del Rei (ZUEC 16447–16452; 16455–16456).

Pseudopaludicola mystacalis—BRAZIL: GOIÁS: Itapirapuã (ZUEC 10222). BRAZIL: MATO GROSSO: Cáceres (ZUEC 10286), Chapada dos Guimarães (ZUEC 5115; 5117; 5119; 5121; 10685). BRAZIL: MATO GROSSO DO SUL: Três Lagoas (16720; 16949). BRAZIL: TOCANTINS: Formoso do Araguaia (ZUEC 10154).

Pseudopaludicola saltica—BRAZIL: MATO GROSSO: Chapada dos Guimarães (ZUEC 14228; 14230–14233; 14235; 14239–14240; 14244; 14247; 14272; 5134–5146; 5854–5855). BRAZIL: MINAS GERAIS: Andradas (ZUEC 10888–10889), Nova Lima (ZUEC 10828–

10832), Poços de Caldas (ZUEC 5256), São Roque de Minas (ZUEC 4352), Uberlândia (AAG-UFU 2308; 2630; 4598; 4631; 4735; 4707–4711).

Pseudopaludicola ternetzi—BRAZIL: MINAS GERAIS: Uberlândia (ZUEC 14036–14039; 14170–14171). BRAZIL: TOCANTINS: Formoso do Araguaia (ZUEC 10140–10143; 10145; 10147; 10150; 10153).

TABLES

TABLE 1.—Morphometric characters of *Pseudopaludicola* sp. nov. type series (including holotype) from the municipality of Viçosa do Ceará, Ceará State, Brazil; and adult males from Floresta Nacional (FLONA) de Nísia Floresta, Rio Grande do Norte State, Brazil. Values presented in millimeters as mean ± standard deviation (minimum–maximum); *n* = number of measured specimens.

	Type-series		FLONA
Characters	Males (*n*=19)	Females (*n*=2)	Males (*n*=14)
Snout-vent-length (SVL)	15.4 ± 0.9 (13.9–16.6)	16.1–17.0	15.0 ± 0.6 (14.3–16.2)
Head length (HL)	4.7 ± 0.4 (3.9–5.6)	5.0–5.1	4.6 ± 0.3 (4.2–5.0)
Head width (HW)	5.3 ± 0.2 (5.0–5.9)	6.1–6.2	5.5 ± 0.2 (5.2–5.9)
Internarial distance (IND)	1.5 ± 0.1 (1.3–1.7)	1.6–1.7	1.5 ± 0.1 (1.3–1.6)
Eye diameter (ED)	1.9 ± 0.2 (1.6–2.4)	2.1	2.0 ± 0.1 (1.8–2.1)
Snout-eye distance (SED)	2.7 ± 0.2 (2.4–3.0)	2.8–3.0	2.7 ± 0.1 (2.5–2.9)
Hand length (HL)	4.2 ± 0.1 (4.0–4.5)	4.3–4.4	4.1 ± 0.2 (3.8–4.6)
Thigh Length (THL)	8.6 ± 0.3 (8.1–9.3)	9.8–10.2	9.1 ± 0.4 (8.5–9.8)
Shank length (TBL)	10.1 ± 0.6 (9.1–11.0)	10.4–11.2	10.1 ± 0.3 (9.5–10.5)
Foot length (FL)	9.7 ± 0.3 (9.1–10.3)	10.5–10.6	9.7 ± 0.4 (9.0–10.4)

TABLE 2.—Advertisement call traits of the *Pseudopaludicola saltica* species group: *P*. sp. nov. from the municipalities of Viçosa do Ceará, Ceará State (type-locality), and Nísia Floresta, Rio Grande do Norte State; *P. saltica* from the municipalities of Chapada dos Guimarães, Mato Grosso State (type-locality) and Uberlândia, Minas Gerais State; and *P. murundu* from the municipalities of Rio Claro (type-locality), São Paulo State, and São João Del Rei, Minas Gerais State. Mean + SD (minimum–maximum). *n* = number of specimens recorded (number of analyzed notes). CV = Coefficient of variation.

	P. sp. nov.	*P. murundu*	*P. saltica*
Variables	*n* = 8 (224)	*n* = 12 (120)	*n* = 12 (120)
Call duration (s)	33.6 ± 35.1 (7.0–108.5)	11.3 ± 5.3 (5.7–19.5)	45.4 ± 18.0 (28.8–74.6)
Note duration (s)	0.115 ± 0.019 (0.056–0.178)	0.080 ± 0.020 (0.027–0.126)	0.076 ± 0.015 (0.030–0.108)
Internote interval (s)	0.125 ± 0.013 (0.084–0.184)	0.117 ± 0.016 (0.079–0.184)	0.122 ± 0.016 (0.080–0.198)
Notes/second	5.3 ± 1.0 (4.0–6.9)	5.2 ± 0.9 (4.0–7.0)	5.6 ± 1.2 (4.0–8.0)
Pulse duration (s)	0.010 ± 0.002 (0.006–0.018)	0.011 ± 0.002 (0.002–0.018)	0.009 ± 0.002 (0.003–0.015)
Interpulse interval (s)	0.018 ± 0.003 (0.001–0.046)	0.015 ± 0.006 (0.001–0.039)	0.017 ± 0.009 (0.001–0.048)
CV interpulse interval	43.3 ± 7.8 (33.5–56.2)	46.9 ± 18.7 (25.9–78.3)	90.6 ± 17.2 (53.0–111.8)
Pulses/second	21.1 ± 2.6 (17.1–25.7)	18.3 ± 4.0 (10.8–25.0)	20.9 ± 5.1 (13.0–28.0)
Pulses/note	4.9 ± 0.7 (2.0–7.0)	3.7 ± 0.5 (2.0–6.0)	4.0 ± 1.2 (2.0–6.0)

Dominant frequency. (Hz)	5429.7 ± 222.3 (5081.8–5986.2)	5827.9 ± 230.7 (5081.8–6375.0)	5032.5 ± 272.4 (4478.9–5531.2)
Min. dominant freq. reached (Hz)	4541.0 ± 525.4 (4317.7–5057.1)	4730.5 ± 354.8 (3852.2–5398.5)	3531.7 ± 766.3 (2553.4–4718.0)
Max. dominant freq. reached (Hz)	6447.9 ± 436.8 (6000.2–6676.1)	7091.2 ± 294.0 (5952.2–8015.1)	6686.8 ± 384.4 (5916.1–7830.0)

TABLE 3.—Measurements of 17 tadpoles of *Pseudopaludicola* sp. nov. at Gosner's (1960) stages 35–37 (*n* = 9) and at stages 27–29 (*n* = 8). Values presented in millimeters as mean ± SD (minimum–maximum).

Characteristic	Stages 27 to 29	Stages 35 and 37
Body length (BL)	6.66 ± 0.30 (6.34 – 7.28)	6.66 ± 0.30 (6.34 – 7.28)
Body height (BH)	2.77 ± 0.27 (2.53 – 3.43)	2.76 ± 0.27 (2.53 – 3.43)
Body width (BW)	3.44 ± 0.34 (2.79 – 3.94)	3.43 ± 0.34 (2.79 – 3.94)
Tail length (TAL)	15.03 ± 0.87 (13.82 – 16.03)	15.03 ± 0.87 (13.82 – 16.03)
Maximum tail height (MTH)	2.47 ± 0.30 (2.10 – 3.14)	2.47 ± 0.30 (2.10 – 3.14)
Tail muscle height	1.57 ± 0.23 (1.26 – 1.89)	1.56 ± 0.23 (1.26 – 1.89)
Tail muscle width (TMH)	1.37 ± 0.14 (1.12 – 1.58)	1.37 ± 0.14 (1.13 – 1.58)
Total length (TL)	21.69 ± 0.97 (20.30 – 22.86)	21.69 ± 0.97 (20.30 – 22.86)
Oral disc width (ODW)	1.22 ± 0.13 (0.98 – 1.44)	1.22 ± 0.13 (0.98 – 1.44)
Internarial distance (IND)	1.04 ± 0.09 (0.95 – 1.19)	1.04 ± 0.09 (0.95 – 1.19)
Interorbital distance (IOD)	0.88 ± 0.13 (0.72 – 1.07)	0.88 ± 0.13 (0.72 – 1.07)
Eye diameter (ED)	0.86 ± 0.09 (0.75 – 1.01)	0.86 ± 0.09 (0.74 – 1.01)
Nostril diameter (ND)	0.14 ± 0.03 (0.09 – 0.19)	0.13 ± 0.03 (0.09 – 0.19)
Eye-nostril distance (END)	0.46 ± 0.11 (0.32 – 0.62)	0.46 ± 0.11 (0.32 – 0.62)
Nostril-snout distance (NSD)	1.44 ± 0.20 (1.05 – 1.74)	1.44 ± 0.20 (1.05 – 1.74)

TABLE 4.—Confusion matrix for species of *Pseudopaludicola saltica* group based on morphometric data through Random Forests model. Settings: number of tree permutations = 500; number of variables tried at each split = 3.0; error rate = 36.99%.

	P. sp. nov.	*P. murundu*	*P. saltica*	Classification error
P. sp. nov.	26	4	3	0.21
P. murundu	5	11	4	0.45
P. saltica	5	6	9	0.55

TABLE 5.—Confusion matrix for species of *Pseudopaludicola saltica* group based on acoustic data through Random Forests model. Settings: number of tree permutations = 500; number of variables tried at each split = 3.0; error rate = 6.25%.

	P. sp. nov.	*P. murundu*	*P. saltica*	Classification error
P. sp. nov.	7	1	0	0.12
P. murundu	0	12	0	0.00
P. saltica	1	0	11	0.08

TABLE 6.—Morphometry and classification of chromosomes of *Pseudopaludicola* sp. nov. The chromosomal classification relative to centromeric position follows Green and Session (1991): M: metacentric; SM: submetacentric; T: telocentric.

Chromosome number												
	1	2	3	4	5	6	7	8	8’	9	10	11
Relative size	15.72	13.25	12.41	11.12	9.79	8.39	8.05	6.74	3.83	6.09	4.64	4.43
Arm ratio	1.03	1.23	2.03	1.99	1.13	1.70	1.26	16.5	8.4	1.14	1.08	1.01
Classification	M	M	SM	SM	M	SM	M	T	T	M	M	M

TABLE 7.—Uncorrected *p*-distances among species of the *Pseudopaludicola saltica* group using the final portion of the mitochondrial 16S rRNA gene (566 bp).

Species	New species	*P. saltica*	**GenBank accession number**
New species	0	-	KJ147033; KJ147034
P. saltica	0.029	0	KJ147002
P. murundu	0.025	0.027	KJ147031; KJ147053

FIGURES

FIGURE CAPTIONS

FIG. 1.—*Pseudopaludicola* sp. nov., adult male, holotype (CFBH 32609). Dorsal (A) and ventral (B) views. SVL = 15.3 mm.

FIG. 2.—*Pseudopaludicola* sp. nov., adult male, holotype (CFBH 32609). (A) Dorsal and (B) lateral views of head; (C) ventral views of hand and (D) foot. Scale bar = 5 mm.

FIG. 3.—Specimens of *Pseudopaludicola* sp. nov. in life from Viçosa do Ceará Municipality, Ceará State, Brazil. (A) Male vertebral line absent; (B) Male with red vertebral line; (C) Male with red vertebral line; male vocalizing in the presence of a satellite male (D); (E) Couple in axilar amplexus; (F) Male and female highlighting the sexual dimorphism by the presence of the dark-colored vocal sac in males.

FIG. 4.—(A) Scatterplot with the two first axes of the Discriminant Analysis (DAPC) on the first seven Principal Components on the morphometric dataset of *Pseudopaludicola saltica* species group, and (B) two first axes of the DAPC scatterplot on the first six Principal Components from call traits.

FIG. 5.—Audiospectrograms (above) and corresponding oscillograms (below) detailing three pulsed notes of advertisement calls of species from *Pseudopaludicola saltica* group (note that pulses are nonconcatenated). (A) *Pseudopaludicola* sp. nov. from Floresta Nacional de Nísia Floresta, municipality of Nísia Floresta, Rio Grande do Norte State. Sound file: ASUFRN236;

approximately 20:00 h, 03 July 2013; air 22ºC. Vouchered recording (ZUEC 21860). (B) *Pseudopaludicola murundu* from municipality of Rio Claro, São Paulo State. Sound file: Pseudop_murunduRioClaroSP3aAAGm671; 22:34 h, 08 March 2015; air 24ºC, water 25ºC. Unvouchered recording. (C) *Pseudopaludicola saltica* from Clube de Caça e Pesca Itororó de Uberlândia, municipality of Uberlândia, Minas Gerais State. Sound file: Pseudop_salticUberlMG4bAAGm; 20:44 h, 19 March 2011; air 23ºC, water 25.8ºC. Vouchered recording (AAG-UFU 2308).

FIG. 6.—Tadpole (stage 37) of *Pseudopaludicola* sp. nov. from the municipality of Viçosa do Ceará, Ceará State, Brazil. (A) Dorsal, (B) ventral, and (C) lateral views, and the (D) oral disc.

FIG. 7.—Karyotypes of *Pseudopaludicola* sp. nov. showing conventional Giemsa staining. (A) One female (ZUEC 21004) and (B) and one male (ZUEC 21006).

FIG. 8.—Summary of species delimitation analyses using maximum likelihood and Bayesian implementations of the Generalized Mixed Yule Coalescent model for *Pseudopaludicola* genus, with focus on *P. saltica* clade (depicted with dashed lines). The topology represents the maximum clade credibility tree from BEAST with the respective node posterior probability (values indicated by the circles). The ML entities identified by the GMYC method are outlined with continuous contours. Numbers are the posterior probability of species identities sampled from a posterior distribution of 100 trees generated in BEAST. The gray scale plot is a sequence-by-sequence matrix colored by pair-wise posterior probabilities of conspecificity, where off-diagonal patterns indicate uncertainty of species limits owing to topological variation of phylogenetic tree.

FIGURE 1

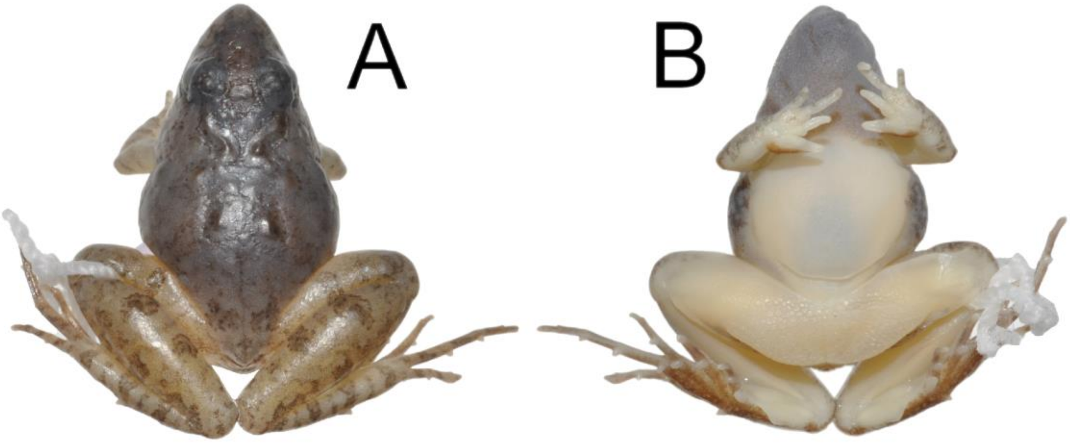


FIGURE 2

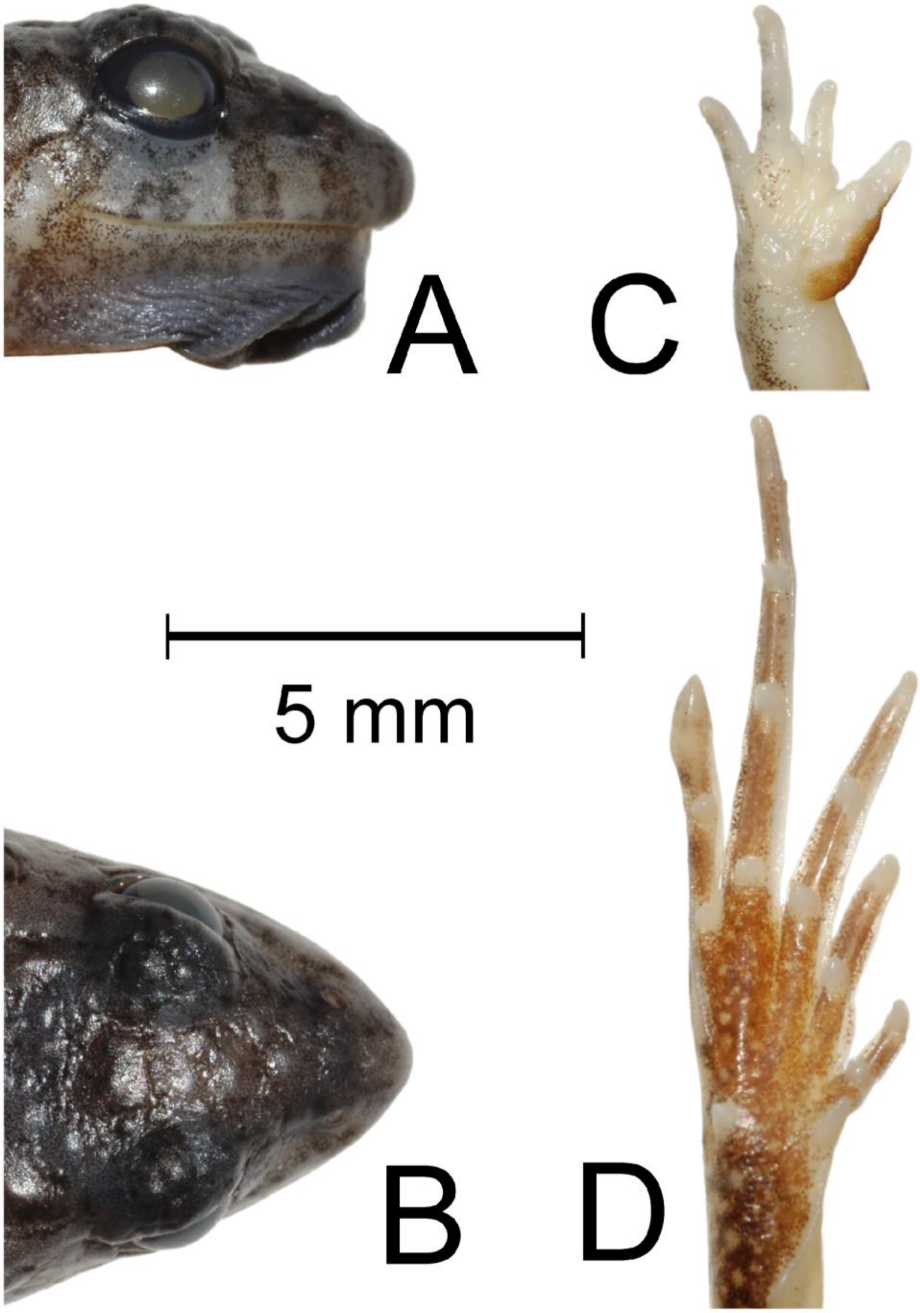


FIGURE 3

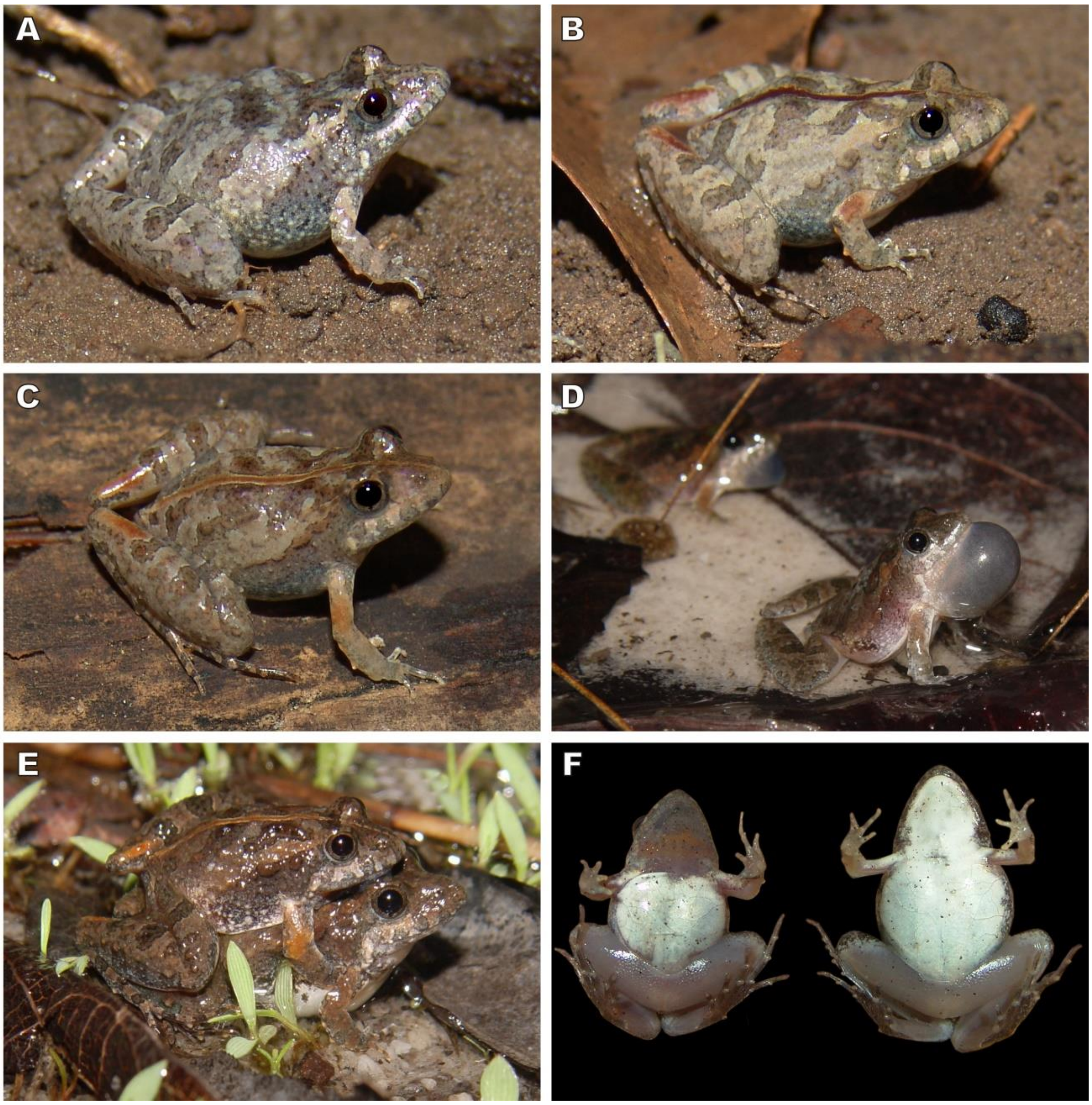


FIGURE 4

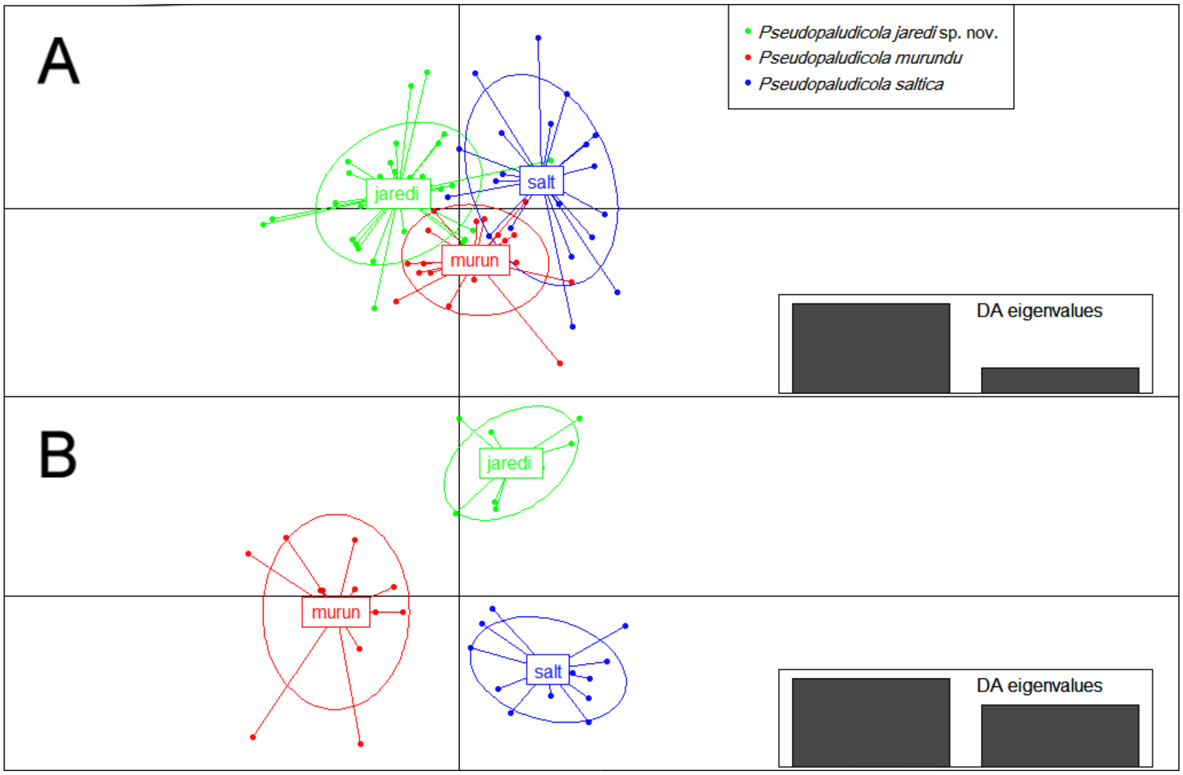

A
● *Pseudopaludicola jaredi* sp. nov.
● *Pseudopaludicola murundu*
● *Pseudopaludicola saltica*
jaredi
salt
murun
DA eigenvalues
B
jaredi
murun
salt
DA eigenvalues

FIGURE 5

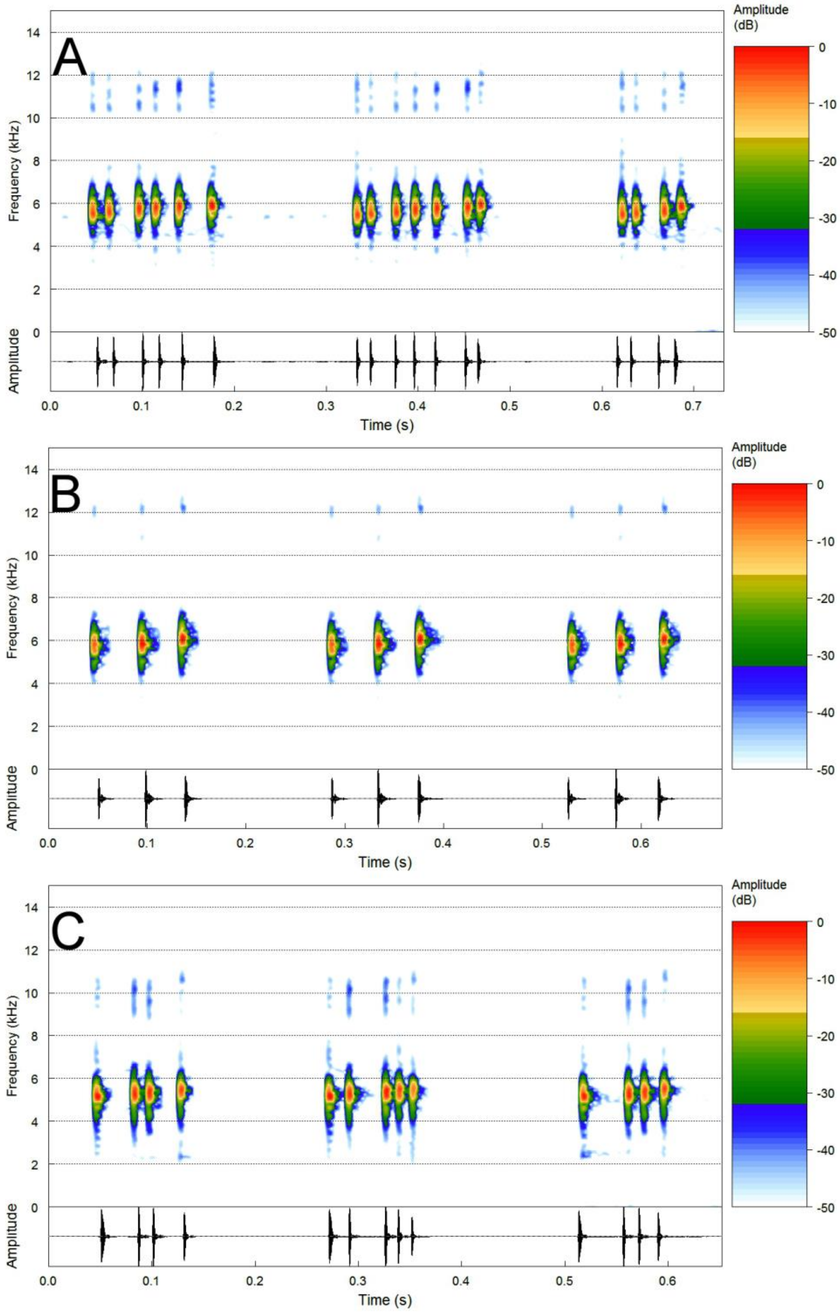

A
B
C
Frequency (kHz)
14
12
10
8
6
4
2
0
Amplitude
Time (s)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Amplitude (dB)
0
-10
-20
-30
-40
-50

FIGURE 6

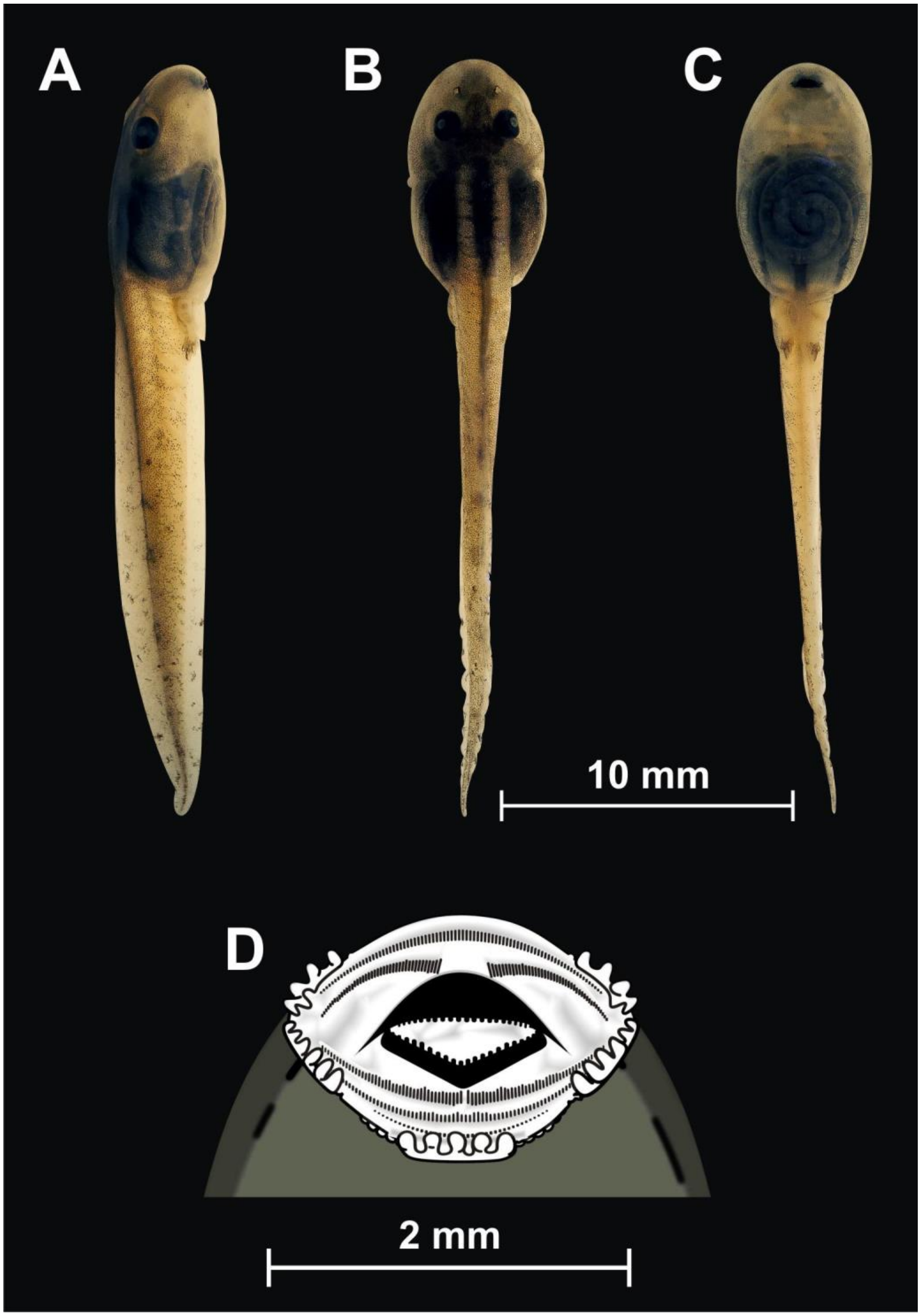


FIGURE 7

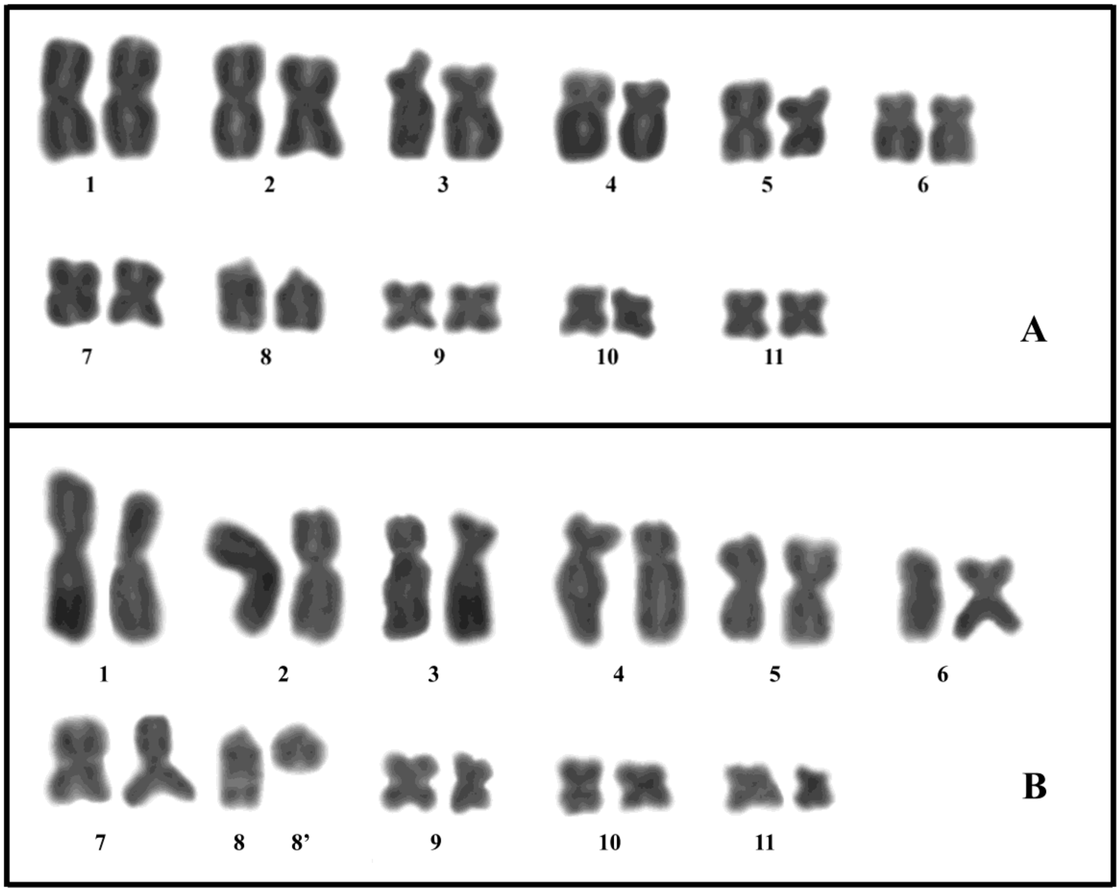
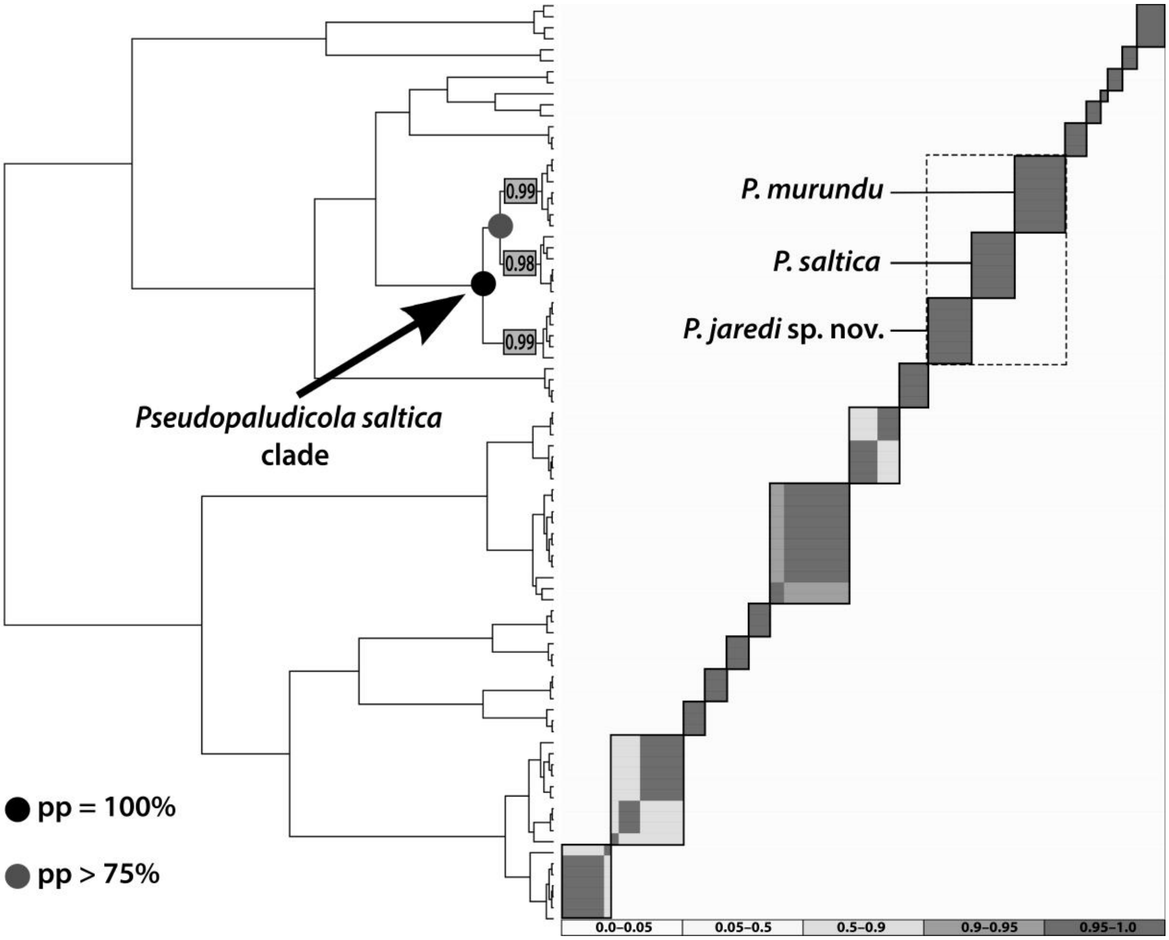


FIGURE 8


0.99
0.98
0.99
Pseudopaludicola saltica clade
P. murundu
P. saltica
P. jaredi sp. nov.
● pp = 100%
● pp > 75%
0.0–0.05
0.05–0.5
0.5–0.9
0.9–0.95
0.95–1.0

4.2 Manuscrito II (formatado essencialmente de acordo com o periódico Herpetologica)

Assessment of the species identity of a *Pseudopaludicola* population from Mato Grosso, Brazilian Cerrado: divergent results from phenotypic and genetic evidence

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RRH: Taxonomic assessment of a *Pseudopaludicola* population

4.2.1 ABSTRACT

The dwarf swamp frogs, genus *Pseudopaludicola*, include 18 species, which occur throughout South America. *Pseudopaludicola ternetzi* has already had several taxonomic problems. This species is known in Brazilian states of São Paulo, Bahia, Goiás, Tocantins and Minas Gerais, and eastern Paraguay. In this paper, we redescribe the morphology and advertisement calls of *P. ternetzi*, reevaluating its diagnostic characters, and try to determine the specific status of a population previously assigned to *P*. cf. *ternetzi* based on acoustic, morphological, and genetic data. Based on our results, we could detect a divergence of phenotypic and genetic characters of a population of *P. ternetzi*, in addition to the strong temperature influence on the temporal traits of the advertisement call from this same population. Additionally, we found acoustic diagnostic characters for *P. ternetzi* that could distinguish it from all congeners, including its sibling species, *P. ameghini*.

KEY-WORDS: *Pseudopaludicola ternetzi*, *Pseudopaludicola ameghini*, Advertisement call, Cerrado, Taxonomy

4.2.2 INTRODUCTION

Taxonomic studies are insufficient for many taxa from Tropical regions and it is particularly noticeable for Neotropical frogs (Dubois, 2003; Toledo, 2010). The dwarf swamp frogs, genus *Pseudopaludicola* Miranda-Ribeiro, 1926, include 18 species (Frost, 2015), which occur throughout South America (Lynch, 1989; Toledo, 2010). The integrative taxonomy (Dayrat, 2005) has been proved a relevant approach to unravel actual *Pseudopaludicola* species diversity (Toledo et al., 2010; Carvalho, 2012; Andrade and Carvalho, 2013; Magalhães et al., 2014; Pansonato et al., 2014). *Pseudopaludicola* is recognized as monophyletic, supported by a distinctive set of osteological (Lobo, 1995) and morphological features, such as hypertrophied antebrachial tubercles (Lynch, 1989; Lobo, 1995). Molecular analyses based on mitochondrial fragments (Veiga-Menoncello et al., 2014) also supported its monophyly. Lynch (1989) proposed the monophyletic *P. pusilla* (Ruthven, 1916) group, which includes four species at present, all sharing the presence of T-shaped terminal phalanges (Cardozo and Suárez, 2012; Lobo, 1995; Veiga-Menoncello et al., 2014); all remaining species have been assigned to the paraphyletic *P. falcipes* group (Veiga-Menoncello et al., 2014).

Miranda-Ribeiro (1937) described *P. ternetzi* from “Goyaz (= state of Goiás)”, Brazil. Caramaschi and Pombal (2011) restricted the type-locality to the municipality of Uruaçu, state of Goiás, Brazil". *P. ternetzi* is thought to occur in Brazilian states of São Paulo, Bahia, Goiás, Tocantins, Mato Grosso and Minas Gerais, eastern and southern Paraguay (Lobo, 1996; Frost, 2015). Bokermann (1966) included *P. ternetzi* as a junior synonym of *P. ameghini* (Cope, 1887). Lobo (1996) redescribed and revalidated *P. ternetzi* based on morphological and osteological data. Recently, Cardozo and Toledo (2013), based on the lack of differentiation in both acoustic and morphological features, rejected the status of *P. riopiedadensis* Mercadal Barrio and Barrio, 1994 as a valid species, and regarded it as a junior synonym of *P. ternetzi*.

In a recent molecular phylogeny, Veiga-Menoncello et al. (2014) pointed out to several populations that could actually be considered as undescribed species of *Pseudopaludicola* and suggested that taxonomic studies with this genus were still incomplete. In addition, the same authors proposed a group that included two closely related species that shared a chromosome number of 20: *P. ameghini* and *P. ternetzi*.

Herein we describe the morphology and advertisement call of *P. ternetzi*, reevaluating its diagnostic characters, and try to determine the specific status of a population of *Pseudopaludicola* sp. from municipality of Pontal do Araguaia (Mato Grosso State) based on call, morphology and genetic data.

4.2.3 MATERIALS AND METHODS

All individuals of *Pseudopaludicola ternetzi* were collected and/or recorded in the following municipalities: Ituiutaba, state of Minas Gerais (= MG) (19°00'05.52"S, 49°27'24.97"W, 600 m above sea level); Minaçu, state of Goiás (= GO) (13°41'56.98"S, 48°16'43.14"W, approximately 860 m above sea level; 120 km northeast of Uruaçu, type-locality); Uberlândia (MG) (19°04'39.24"S, 48°39'38.54"W, 675 m above sea level) and Monte Alegre de Minas (MG) (19°02'36.23"S, 49°01'16.37"W, 635 m above sea level), and Caldas Novas (GO) (17°48'31.41"S, 48°41'58.22"W, 990 m above sea level), Brazil.

The studied population came from the municipality of Pontal do Araguaia, state of Mato Grosso (= MT), Brazil (15°58'13.68"S, 52°17'48.44"W, 320 m above sea level); adult specimens are housed in the collection of frogs (AAG-UFU) of the Universidade Federal de Uberlândia, municipality of Uberlândia, state of Minas Gerais, Brazil, and in the amphibian collection of the Museu de Zoologia (ZUEC) at the Universidade Estadual de Campinas, municipality of Campinas, state of São Paulo, Brazil.

Morphometric analyses.—Morphometric characters of 27 adult males of *P. ternetzi*, and 16 adult males and six adult females from Pontal do Araguaia population (= PA population) (Appendix 1) were measured under a stereomicroscope Zeiss Stemi 2000 coupled to an ocular micrometer. Eight measurements follow Duellman (2001): snout-vent length (SVL), head length (HL), head width (HW), internarial distance (IND), snout-eye distance (SED) (= snout length), eye diameter (ED), tibia length (SL) (= shank length), and foot length (FL); three measurements follow Heyer et al. (1990): forearm lenght (FAL), hand length (HAL), and thigh length (TL). The SVL of the adults were taken with a Mitutoyo Absolute digital caliper (to the nearest 0.1 mm) under a stereomicroscope.

Acoustic analysis.—Calls were recorded with a Marantz PMD 671, a Boss 864 (both coupled to Sennheiser ME67/K6 microphones) and a M-audio Microtrack II (Sennheiser ME66/K6) digital recorders. Recorders were set at 44.1 or 48.0 kHz sampling rates, and a 16-bit resolution. Twenty six males of *P. ternetzi* were recorded: eighteen males from Ituiutaba (MG) and eight from Minaçu (GO). Eighteen males of the studied population were recorded from Pontal do Araguaia (MT). All recordings were taken from 16:40–20:59 h and between 13 September 2011 and 26 November 2014. Voucher specimens for *P. ternetzi* recordings: Ituiutaba (MG): AAG-UFU 5009–5010; and Minaçu (GO): AAG-UFU 5021–5022; Pontal do Araguaia population (MT): AAG-UFU 1086, 1304–1307, and 3453–3455.

Four males of *P. ameghini* were recorded on 14 December 2008 by B. F. V. Teixeira in the municipality of Chapada dos Guimarães (MT) (type-locality) (15°21'23.64"S, 55°49'33.93"W, approximately 369 m above sea level) and on 21 November 2012 in the district of São Vicente, municipality of Santo Antonio do Laverger (MT) by T. R. de Carvalho (15°49'26.35"S, 55°30'48.48"W, approximately 620 m above sea level), Brazil. Recordings were made between 18:02–20:00 h (voucher AAG-UFU 1443).

Call, note and pulse terminology followed Magalhães et al. (2014), other call terminologies followed Duellman and Trueb (1994). Means and standard deviations were calculated considering mean values of individual males, whereas the range encompassed the minimum and maximum values for all call sample variation. For each recorded call, ten notes and all pulses that comprised these notes were analyzed. Air temperature was taken for each recorded male. Calls were analyzed using Raven Pro 1.5, 64-bit version (Bioacoustics Research Program, 2014) with the following settings: window type = Hann, window size = 256 samples, 3 dB filter bandwidth = 248 or 270 Hz, brightness = 50%, contrast = 50%, overlap = 85% (locked), DFT size = 1024 samples (locked), and a grid spacing (spectral resolution) = 43.1 or 46.9 Hz, color map = Cool. Temporal traits were analyzed in oscillograms and spectral traits in spectrograms. Figures were generated using Seewave v.1.6 package (Sueur et al., 2008) on the R (version 3.0.2) platform (R Core Team, 2014). Seewave settings were Hanning window, 85% overlap and 256 points resolution (FFT).

Within-male call variation was assessed through the coefficients of variation [CV = (SD/mean) x 100]. To CV calculation, all individual call samples were taken into account, except for the traits of pulses per second and notes per second, which were obtained from each call registered of each male (*n* = 11 males from PA and 13 males from other populations of *P. ternetzi*). The average was calculated for each trait from individual male CV values. Acoustic traits with low within-male coefficients of variation (usually less than 5%) were classified as static and traits with higher coefficients of variation (usually greater than 12%) as dynamic (*sensu* Gerhardt, 1991).

Pansonato et al. (2013) described the advertisement call of *P. ameghini* based on calls of five males from the type-locality (municipality of Chapada dos Guimarães) and surrounding localities. Given that we found some inconsistencies in Pansonato et al.'s (2013) paper (e.g. note duration 0.01±0.001 s (range 0.002–0.008)), we mostly based the acoustic comparisons between *P. ternetzi* and *P. ameghini* on our own data.

Molecular analysis.—The protocols of extraction, amplification and sequencing of the genetic material were the same followed by Veiga-Menoncello et al. (2014). Sequences of topotypic specimens of *P. ternetzi* and *P. ameghini* (Veiga-Menoncello et al., 2014) were obtained from GenBank and considered for evaluation of mean pair-wise distance using MEGA, version 5 (Tamura et al., 2011) with default settings.

Statistical analyses.—Considering the acoustic and morphometric datasets, we seek for discrimination between PA population, *P. ternetzi* and *P. ameghini* by application of function "randomforest" (RF) [randomForest package (Liaw and Wiener, 2002)]. RF algorithm constructs many (*e.g.* 500) classification trees using bootstrap samples from data (each split using the best predictors randomly chosen at each node), then generating classifiers and aggregating results by voting to classes (Liaw and Wiener, 2002). The RF results include an estimate of distances among the objects, which can be subject to a Multidimensional Scaling Analysis (MDS; Jaworska and A. Chupetlovska-Anastasova, 2009) with the "proximity.plot" function (rfPermute package (Archer, 2014)), which also allows display the results graphically. The directly or indirectly packages related to the application of the discriminant functions were done in R (R Core Team, 2014). The function “importance” (RF package) is the extractor function for variable importance measures as produced by randomForest. This function calculates the importance variable through two measures. The first measure (Mean Decrease Accuracy) is computed from permuting OOB data: for each tree, the prediction error on the out-of-bag portion of the data is recorded (error rate for classification, MSE for regression). Then, the same is done after permuting each predictor variable. The difference between the two are then averaged over all trees, and normalized by the standard deviation of the differences. If the standard deviation of the differences is equal to 0 for a variable, the division is not done (but the average is almost always equal to 0 in that case). The second measure (Mean Decrease Gini) is the total decrease in node impurities from splitting on the variable, averaged over all trees. For classification, the node impurity is measured by the Gini index (Liaw and Wiener, 2002). The results of these measures are presented graphically in the dotchart form, which is a matrix of importance measure, one row for each predictor variable; the columns are different importance measures (Liaw and Wiener, 2002).

For the multidimensional analysis/plots and statistical tests, we used the following morphometric variables: SVL, HL, HW, ED, SED, IND, FAL, HAL, TL, SL, FL; and acoustic variables: pulse duration, interpulse interval, pulses/second, note duration, internote interval, notes/second, pulses/note, minimum of dominant frequency, maximum of dominant frequency, and peak of dominant frequency. All morphometric and acoustic traits were tested for statistical significance of the differences population-population and species-species through the Exact Wilcoxon Mann Whitney Rank Sum Test using the package coin (Resampling Statistics model) (Hothorn et al., 2008; function wilcox_test) in R. As these tests were done between species pairs, the significance level ("*P*") was adjusted considering the number of pairings through the method of "Holm" (p.adust function in R). Significance was considered when $P < 0.01$.

4.2.4 RESULTS

Color, morphology and morphometry

Adult males of *P. ternetzi* ranged in SVL from 15.3 to 18.1 mm; those from PA ranged from 14.0 to 16.7 mm, while adult females ranged from 16.5 to 20.2 mm (further details in Table 1). Preserved specimens of *P. ternetzi* populations had brown surfaces in dorsal view, with darker brown and black blotches and few small clear spots scattered on the dorsum and limbs; light beige venter (belly unpigmented). Ventral surface of hands and feet dark brown, mottled. A discrete lateral stripe that begins behind the eyes and goes up to half of the axilla-groin distance. Dorsal surface of arms light brown with brown spots; dorsal surface of legs brown with dark brown spots. Live specimens had a dorsum dark brown; flanks brown; throat and belly cream; pale cream blotches; ventral surface of hands and feet dark brown, mottled; a discrete whitish lateral stripe that begins behind the eyes and goes up to half of the axilla-groin distance.; transverse dark brown stripes on thighs, shanks, feet and forearms (see Fig. 1).

Advertisement call

Pseudopaludicola ternetzi advertisement call.—Quantitative traits are summarized in Table 2. *Pseudopaludicola ternetzi* (Fig. 2C, D) releases a long advertisement call, lasting 7.8–88.5 s, consisting of a sequence of pulsed notes series (1–11 series of notes/call) which lasts 0.298–72.1 s, at intervals of 0.126–1.1 s. The notes last from 26 to 80 ms, at intervals of 25 to 88 ms, and released at a rate of 10 to 12 notes/second. Each note is composed of 3 to 5 nonconcatenated pulses. The pulses last from 5 to 16 ms, separated by intervals of 1−11 ms, and released at a rate of 30 to 39 pulses/second. The amplitude is ascendant at the beginning and descendent at the end of the call, both increase and decrease in sound energy is gradual and discrete. The dominant frequency peak (= fundamental) varies from 3515.6 to 4078.1 Hz. Each note can possess up to two harmonics; being the second harmonic from 6801.6 to 7429.7 Hz. Frequency modulation is unnoticeable along the call. The air temperature varied from 21.5 to 29.0°C and water temperature from 25.1 to 28°C.

Pseudopaludicola ameghini advertisement call.—*Pseudopaludicola ameghini* emits a long call (Fig. 2E, F) lasting 10.5–41.6 s, consisting of a sequence of pulsed notes series (4–6 series of notes/call) with duration of 0.433 to 17.9 s, separated by intervals of 0.31 to 2.5 s. The notes last from 72 to 114 ms, separated by intervals of 46 to 109 ms, and released at a rate of 6 to 8 notes/second. Each note is composed of 4 to 5 nonconcatenated pulses. The pulses last from 7 to 16 ms, separated by intervals of 1–23 ms, and released at a rate of 29–32 pulses/second. The peak of dominant frequency (=fundamental) varies around 3661 to 4312 Hz. Each note can possess a second harmonics that varies around 7020 to 8719 Hz. The air temperature varied from 23 to 26.3° C and water temperature from 26.6 to 28.2° C.

PA population advertisement call.—Males from this population emits its notes and pulses at rates of 12.0 to 16.0 notes/second and 41.0 to 54.0 pulses/second; while males from Goiás and Minas Gerais emit at rates of 9.7 to 12.3 notes/second and 29.7 to 39.0 pulses/second (further details in Table 2) of the values of the acoustic traits from PA. High emission rates of notes and pulses are striking temporal acoustic traits of this population, being a relevant intraspecific acoustic variation of *P. ternetzi*.

Genetic divergence analyses

The uncorrected p-distance in DNA sequences in 16S of PA and topotypical *P. ternetzi* is 0.2%, whereas among PA and *P. ameghini* is 1.5%. The genetic divergence between PA population and *P. ternetzi* is remarkably low. The lowest genetic distance between two valid species of *Pseudopaludicola* is between *P. ternetzi* and *P. ameghini*, 1.7% (see Table 3 and Pansonato et al., 2014).

Morphometric and acoustic comparisons between Pontal do Araguaia population, *P. ternetzi* and *P. ameghini*

Pontal do Araguaia population (=PA) differed from *P. ameghini* [values within brackets] (Pansonato et al., 2013) by having a smaller SVL ([16.3–18.2] vs. 14.0–16.7 mm), shorter thigh ([7.3–8.7] vs. 5.4–6.6 mm), shorter shank ([8.4–9.5] vs. 6.2–7.4 mm) and shorter foot ([8.7–9.7] vs. 6.4–7.5 mm). Males from this same population differed from *P. ternetzi* [values within brackets] by shorter head ([3.7–4.6] vs. 3.2–3.6 mm), narrower head ([4.9–5.8] vs. 4.4–4.9 mm), and shorter thigh length ([6.8–7.7] vs. 5.4–6.6 mm) (see also Table 1). We also found significant differences ($P < 0.01$) between PA and *P. ternetzi* in all morphometric variables, except for internarial distance ($P = 0.02$). Adult males from PA also differed from *P. ternetzi* by their smaller SVL ([17.1 ± 0.8; 16.0–18.6 mm, $n = 13$; Lobo, 1996] vs. 15.5 ± 0.9; 13.8–16.9 mm; $P < 0.01$).

The calls from PA differed from those of *P. ameghini* by their higher pulse rate ([29–32] x 41–54 pulses/second), higher note rate ([6–8] vs. 12–16 notes/second), and shorter note duration ([72–114] vs. 32–73 ms). We also found significant differences (with $P < 0.01$) between PA population and *P. ameghini* in the following temporal variables: pulses per note, pulse duration, interpulse interval, and internote interval. In addition, PA could also be diagnosed from *P. ameghini* by having notes predominantly with 3 pulses; while *P. ameghini* with 4 pulses (see also Table 2 and Fig. 2A–F).

The calls from PA differed from those of *P. ternetzi* by their higher pulse ([30–39] vs. 41–54 pulses/second) and note ([10–12] vs. 12–16) rates (Table 2). We also found significant differences (with $P < 0.01$) in the following acoustic variables: pulse duration, interpulse interval, internote interval, peak of dominant frequency, and maximum dominant frequency reached.

Pseudopaludicola ternetzi is easily distinguished from *P. ameghini* [values within brackets] by its higher note rate ([6.0–8.0] vs. 10.0–12.0 notes/second). In addition, *P. ternetzi* also differs from *P. ameghini* by having longer note duration, larger pulses per note, pulse duration and interpulse interval (with $P < 0.01$) (see also Table 2). *Pseudopaludicola ternetzi* could also be diagnosed from *P. ameghini* by having notes predominantly with 3 pulses; while *P. ameghini* with 4 pulses (see Fig. 2C–E)

A multidimensional approach (randomForest) on morphometric data highlighted the complete segregation between PA population and *P. ternetzi* (Fig. 3A). Distinct clusterings were promoted due to thigh length, length and width of the head, which were the main sources of variation (Fig. 3B). In the case of the multidimensional approach (randomForest) on bioacoustic data, it also highlighted the segregation between *P. ternetzi*, *P. ameghini*, and PA population (Fig. 4A), being the pulse and note rates, and internote interval as the main sources of variation (Fig. 4B). Additionally, the distinction between PA population and *P. ternetzi* was obtained from internote interval (Fig. 4B). Even though there was no overlap in mean values of each male for this variable, we did not use it as an important character due to overlap in range considering the whole sample (Table 2). We emphasize that there are no overlaps of the points in the morphometric and acoustic multidimensional graphs that represent the adult males of *P. ameghini, P. ternetzi,* and PA population. In addition, two classification matrices using Random Forests model corroborated a total morphometric discrimination (0.0 % error) in the class among PA population and *P. ternetzi* (*n* = 16 adult males from PA population and 27 adult males from *P. ternetzi*); and a total acoustic discrimination among PA population, *P. ternetzi* and *P. ameghini* (*n* = 18 adult males from PA population, 26 adult males from *P. ternetzi* and 4 adult males from *P. ameghini*).

Acoustic comparisons of *P. ternetzi* with other species

Pseudopaludicola ternetzi is also promptly diagnosed from *P. canga* Giaretta and Kokubum, 2003 (Giaretta and Kokubum, 2003; Pansonato et al., 2012), *P. giarettai* Carvalho, 2012 (Carvalho, 2012), *P. hyleaustralis* Pansonato, Morais, Ávila, Kawashita-Ribeiro, Strüssmann and Martins, 2012 (Pansonato et al., 2012), *P. facureae* Andrade and Carvalho, 2013 (Andrade and Carvalho, 2013) and *P. parnaiba* Roberto, Cardozo and Ávila, 2013 (Roberto et al., 2013) by possessing a pulsed note structure (Fig. 2C, D), whereas these five species have calls composed of non-pulsed notes.

The advertisement call of *P. ternetzi* distinguishes it from other congeners (except from *P. ameghini*) that also has notes with non-concatenated pulses (with lack of interpulse interval, *sensu* Magalhães et al., 2014) by the following quantitative traits [values characters of comparative species within brackets]*:* *P. falcipes* (Hensel, 1867) has longer interpulse interval ([mean = 30] vs. 1–14 ms) and smaller number of pulses per note ([mean = 2] vs. 3–6 pulses per note) (Haddad and Cardoso, 1987); *P. mineira* Lobo, 1994 has smaller number of pulses per note [2 pulses per note], longer interpulse interval [mean 20±2 ms], longer internote interval ([mean 100 ± 40] vs. 41 ± 7 ms) (Pereira and Nascimento, 2004); *P. pocoto* has longer note duration ([126–290] vs. 26–80 ms), longer interpulse interval [43–166 ms], higher peak dominant frequency ([5.2–6.4] vs. 3.5–4.1 kHz) (Magalhães et al., 2014); *P. atragula* has a larger number of pulses per note [9–36 pulses per note] and note duration [300–700 ms] (Pansonato et al., 2014); *P. saltica* has smaller number of pulses per note [1–4 pulses per note], shorter pulse duration ([2–5] vs. 4–16 ms), longer interpulse interval [3–60 ms] (Pansonato et al., 2013); *P. murundu* has a longer note duration [90–110 ms], higher dominant frequency [5.2–6.4 kHz] (Toledo et al., 2010). Note structure (3–6 non-concatenated pulses per note) distinguishes the calls of the *P. ternetzi* from those of *P. mystacalis* (Cope, 1887) [12–14 concatenated pulses/note;

Pansonato et al., 2013] and *P. boliviana* [calls with sets of five notes each one formed by 3–6 concatenated pulses; Duré et al., 2004]. Besides all these acoustic differences, *P. ternetzi* is also distinguished from all species abovementioned by having a long advertisement call consisting of a series of pulsed notes emitted at higher rates in both notes/second and pulses/second (Fig 2C, D and Table 2).

Specimens of *P. ternetzi* were found in a highly disturbed (cattle farming) Cerrado phytophysiognomy (Oliveira-Filho and Ratter, 2002) area in open grassy sites at the border of a Vereda, a habitat established over hydromorphic terrain with scattered Buriti palm (*Mauritia flexuosa*). *Pseudopaludicola ternetzi* begins its calling activity in the late afternoon (ca. 16:30 h) and decreases its activity as the air temperature decreases. For Pontal do Araguaia's population, we found *P. mystacalis* calling syntopically, within less than 100 meters and *P. saltica* (AAG-UFU 2832) in the neighboring municipality of Barra do Garças. Additionally, Lobo (1996) reports that *P. mystacalis* occurs sympatrically with *P. ternetzi* in the municipalities of Chapada dos Guimarães, state of Mato Grosso, and Rio Verde, state of Goiás.

4.2.5 ADDITIONAL REMARKS

It is expected that temperature affects more the fine-scale temporal properties of anuran signals (e.g. the pulse rate) than the spectral ones (Zweifel 1968; Schneider 1977; Gerhardt & Huber 2002; Wells 2007; Rodriguez *et al*., 2010). In this study, air temperature registered during field records of the populations of *P. ternetzi* broadly overlapped, therefore, we found a significant difference between PA population [values within brackets] and *P. ternetzi* populations ([air: 26.3 ± 0.4; 26.0–27.5° C] vs. 25.7 ± 2.5; 21.5–29.0° C; *P* = 0.027). On the other hand, water temperatures not overlapped ([29.0–31.0° C] vs. 25.1–28° C). Differentially, the males from PA emit calls with higher pulse and note rates than other two studied populations of *P.*

ternetzi. From within-male call variation of the all three populations (PA, Minaçu and Ituiutaba), the traits that were classified as static (CV < 5%) were the pulse and note rates, besides the spectral traits. Therefore, we attribute part of the found differences in the pulse and note rates to *P. ternetzi* (Table 2) to physiological response to air and water temperatures.

Sampled populations of this study are distributed in six municipalities from three Brazilian states. The municipality of Minaçu is ca. 140 km northeast from type-locality of *P. ternetzi* (municipality of Uruaçu, state of Goiás), while municipality of Pontal do Araguaia is ca. 360 km southwest (see Fig. 5). The occurrence of *P. ternetzi* in municipality of Uberlândia and surrounding region (Triângulo Mineiro region, state of Minas Gerais) has been supported by cytogenetic, molecular, reproduction and acoustic data (Giaretta and Facure, 2009; Duarte et al., 2010; Fávero et al., 2011; Cardoso and Toledo, 2013; Veiga-Menoncello et al., 2014) and now for the present study. The occurrence of *P. ternetzi* in municipality of Minaçu (GO) also has already been reported by Cardozo and Toledo (2013). Lobo (1996) has already reported the occurrence of *P. ternetzi* in the state of Mato Grosso, municipalities of Buriri, Alto Araguaia, Chapada dos Guimarães, Porto Esperidião and Aldeia Velha. Therefore, based on *P. ternetzi* distributional records, it is possible to recognize that this species has a wide distribution throughout central eastern region of South America.

Our acoustic analysis also revealed important results for the differentiation of *P. ternetzi* of *P. ameghini*; therefore, specific identity of these two species is supported by diagnostic and significant differences in its temporal acoustic traits, corroborating recent papers which proposed the recognition of *P. ameghini* as a valid species (Fávero et al., 2011; Pansonato et al., 2013).

4.2.6 CONCLUSIONS

Based on our morphometric analysis, it can be recognized that the PA population is smaller in SVL than all known populations of *P. ternetzi*. In addition, males from PA emit advertisement calls with higher pulses and notes rates, but part of these differences can be attributed to physiological response to temperature. However, the very low divergence (0.2%) of the mitochondrial sequence of DNA of this population regarding the sequence of topotypical *P. ternetzi* revealed a low genetic differentiation between these two populations. Therefore, considering the conflict between genetic and phenotypic characters, we attribute the found phenotypic differences (acoustic and morphometric) to an intraspecific acoustic and morphometric variation in *P. ternetzi*. Lastly, acoustic traits of *P. ternetzi* allow to diagnose it from all other species of the genus, including *P. ameghini*, its sister species.

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4.2.8 APPENDIX 1. Examined specimens

Pseudopaludicola ternetzi – BRAZIL: GOIÁS: Caldas Novas: (AAG-UFU 4322–3, 4348, 4353); MATO GROSSO: Pontal do Araguaia: (AAG-UFU 1074–7, 1301–07, 3453–5, 3460–1, 3463–5, 1069, 1073, 3456; MINAS GERAIS: Ituiutaba: (AAG-UFU 0944, 3512–5, 5011, 5014, 5016–7, 5021–2); Monte Alegre de Minas: (AAG-UFU 4607); Uberlândia: (AAG-UFU 2624–5, 2627, 4670–1; ZUEC 14037–8, 14169–72). *Pseudopaludicola ameghini* – BRAZIL: MATO GROSSO: Chapada dos Guimarães: (AAG-UFU 1443; ZUEC 14138–9, 14141–5). *Pseudopaludicola canga* – BRAZIL: PARÁ: Serra dos Carajás: (ZUEC 9990, 10034, 14370, 14372–74, 14378). *Pseudopaludicola facureae* – BRAZIL: MINAS GERAIS: Uberlândia: (AAG-UFU 0853–55; ZUEC 13651–52, 14215, 14218–19, 14221, 14224). *Pseudopaludicola falcipes* – BRAZIL: RIO GRANDE DO SUL: Porto Alegre: (ZUEC 14008, 14022, 14162–66, 14168). *Pseudopaludicola mineira* – BRAZIL: MINAS GERAIS: Jaboticatubas: (ZUEC 1574, 1582–83, 1586–87, 1590, 1593–95, 1600, 1604, 1617). *Pseudopaludicola murundu* – BRAZIL: SÃO PAULO: Águas de Santa Bárbara: (ZUEC 20507–08); Rio Claro: (CFBH 8235–8242; ZUEC 14284–90). *Pseudopaludicola mystacalis* – BRAZIL: GOIÁS: Itapirapuã: (ZUEC 10222); MATO GROSSO: Cáceres: (ZUEC 10286); Chapada dos Guimarães: (ZUEC 5115, 5117, 5119, 5121, 10685); MATO GROSSO DO SUL: Três Lagoas: (16720, 16949); TOCANTINS: Formoso do Araguaia: (ZUEC 10154). *Pseudopaludicola saltica* – BRAZIL: MATO GROSSO: Chapada dos Guimarães (ZUEC 5134–46, 5854–5855); MINAS GERAIS: Uberlândia: (AAG-UFU 2308, 2630, 4598, 4631, 4735, 4707–11);

4.2.9 TABLES

TABLE 1.—Morphometry (in millimeters) of specimens from Pontal do Araguaia population and a pooled sample of *Pseudopaludicola ternetzi* from the states of Minas Gerais and Goiás. Mean±SD (minimum–maximum). *n* = number of specimens analyzed.

Characters	Pontal do Araguaia population		*P. ternetzi**
	Males (*n* = 16)	Females (*n* = 6)	Males (*n* = 27)
Snout vent length	15.3 ± 0.7 (14.0–16.7)	17.9 ± 1.5 (16.5–20.2)	16.8 ± 0.7 (15.3–18.1)
Head length	3.5 ± 0.1 (3.2–3.6)	3.9 ±0.4 (3.4–4.4)	4.1 ± 0.2 (3.7–4.6)
Head width	4.7 ± 0.1 (4.4–4.9)	5.2 ± 0.6 (4.2–5.8)	5.3 ± 0.3 (4.9.0–5.8)
Internarial distance	0.9 ± 0.1 (0.7–1.1)	1.1 ± 0.1 (1.0–1.2)	1.0 ± 0.1 (0.9–1.2)
Eye diameter	1.5 ± 0.1 (1.4–1.6)	1.6 ± 0.1 (1.5–1.8)	1.6 ± 0.1 (1.3–1.8)
Snout eye distance	2.0 ± 0.1 (1.8–2.1)	2.2 ± 0.2 (1.9–2.4)	2.3 ± 0.2 (2.0–2.7)
Forearm length	2.4 ± 0.1 (2.3–2.6)	2.8 ± 0.3 (2.4–3.1)	2.7 ± 0.1 (2.4–2.9)
Hand length	3.4 ± 0.1 (3.2–3.7)	3.8 ± 0.3 (3.4–4.1)	3.9 ± 0.2 (3.5–4.2)
Thigh length	6.3 ± 0.3 (5.4–6.6)	6.9 ± 0.7 (5.6–7.6)	7.2 ± 0.2 (6.8–7.7)
Shank length	7.1 ± 0.4 (6.2–7.4)	7.6 ± 0.6 (6.6–8.4)	7.8 ± 0.5 (7.0–8.8)
Foot length	7.0 ± 0.3 (6.4–7.5)	7.8 ± 0.7 (6.7–8.6)	7.8 ± 0.4 (7.2–8.5)

* (MG: Ituiutaba, Monte Alegre de Minas and Uberlândia; GO: Caldas Novas and Minaçu)

TABLE 2.—Acoustic traits of Pontal do Araguaia population, *Pseudopaludicola ternetzi* from the municipalities of Ituiutaba, state of Minas Gerais, and Minaçu, state of Goiás; and *P. ameghini* from municipalities of Chapada dos Guimarães (type-locality) and Santo Antonio do Leverger, both state of Mato Grosso, Brazil. Mean ± standard deviation (minimum–maximum). *n* = number of males recorded (number of analyzed pulsed notes).

Traits	Pontal do Araguaia *n* = 18 (240)	*P. ternetzi* *n* = 26 (260)	*P. ameghini* *n* = 4 (90)
Call duration (s)	57.8 ± 62.8 (4.8–222.9)	39.5 ± 26.7 (7.8–88.5)	24.5 ± 14.1 (10.5–41.6)
Series of notes duration (s)	30.1 ± 46.0 (0.473–192.5)	17.4 ± 10.4 (0.298–72.1)	4.4 ± 3.9 (0.433–17.9)
Interseries interval	0.348 ± 0.186 (0.093–1.427)	0.3 ± 0.2 (0.126–1.1)	0.923 ± 0.459 (0.31–2.5)
Series per call	3.1 ± 2.1 (1.0–8.0)	4.0 ± 2.7 (1.0–11.0)	4.8 ± 1.0 (4.0–6.0)
Note duration (s)	0.042 ± 0.004 (0.032–0.073)	0.044 ± 0.003 (0.026–0.08)	0.094 ± 0.009 (0.072–0.114)
Internote interval (s)	0.033 ± 0.004 (0.019–0.073)	0.046 ± 0.003 (0.025–0.088)	0.059 ± 0.010 (0.046–0.109)
Pulse duration (s)	0.009 ± 0.001 (0.005–0.016)	0.008 ± 0.001 (0.004–0.012)	0.010 ± 0.002 (0.007–0.016)
Interpulse interval (s)	0.005 ± 0.001 (0.001–0.011)	0.008 ± 0.001 (0.001–0.014)	0.013 ± 0.004 (0.001–0.023)
Notes/second	13.6 ± 1.0 (12.0–16.0)	11.1 ± 0.8 (9.7–12.3)	6.8 ± 1.0 (6.0–8.0)
Pulses/second	45.6 ± 3.4 (41.0–54.0)	36.0 ± 1.9 (29.7–39.0)	30.8 ± 1.3 (29.0–32.0)
Pulses/note	3.4 ± 0.3 (3.0–6.0)	3.3 ± 0.2 (3–5)	4.5 ± 0.2 (4.0–5.0)

Peak of dom. freq. (Hz)	3998.6 ± 348.1 (3609.4–4500.0)	3736.8 ± 127.7 (3515.6–4078.1)	3888.9 ± 257.1 (3660.6–4312.5)
Min. reached dom. freq. (Hz)	2383.6 ± 154.9 (1563.1–2826.9)	2546.5 ± 269.3 (1946.5–2200.9)	2490.0 ± 320.0 (2112.5–3017.4)
Max. reached dom. freq. (Hz)	5213.9 ± 441.8 (4544.3–6100.0)	4735.3 ± 258.0 (4242.9–5450.6)	5263.1 ± 219.3 (4990.3–5700.3)
Air temperature (˚C)	26–27.5	21.5–29.0	23.0–26.3
Water temperature (˚C)	29.0–31.0	25.1–28.0	26.6–28.2

TABLE 3.—Uncorrected *p*-distances among specimens from Pontal do Araguaia population; *P. ternetzi* (type-locality), and *P. ameghini* (type-locality) using the final portion of the mitochondrial 16S rRNA gene (566 bp).

Population/Species	Pontal do Araguaia	*P. ternetzi*
Pontal do Araguaia	0	-
P. ternetzi	0.002	0
P. ameghini	0.015	0.017

4.2.10 FIGURES

FIGURE CAPTIONS

FIG. 1.—Specimens of *Pseudopaludicola* sp. in life. **A**—Adult male (AAG-UFU 1304; SVL = 15.7 mm); **B**—adult male (AAG-UFU 1306; SVL = 15.5 mm); **C**—adult male (AAG-UFU 1075; SVL 15.2 mm); **D**— adult male (AAG-UFU 1307; SVL = 15.7 mm). All specimens from municipality of Pontal do Araguaia, state of Mato Grosso, Brazil.

FIG. 2.—Oscillogram of the entire advertisement call with series of pulsed notes; and audiospectrogram (above) and corresponding oscillogram (below) detailing three pulsed notes from our studied populations: Pontal do Araguaia population **(A, B)**, *Pseudopaludicola ternetzi* from municipality of Minaçu, state of Goiás **(C, D)** and *P. ameghini* from municipality of Chapada dos Guimarães, state of Mato Grosso (type-locality) **(E, F)**.

FIG. 3.—First and second dimensions of the Multidimensional scaling of the proximity scores from Random Forest of the morphometric data comparing sixteen adult males of *Pseudopaludicola* sp. from Pontal do Araguaia (green circles), and twenty seven adult males of *P. ternetzi* from municipalities of Uberlândia, Ituiutaba and Monte Alegre de Minas, all three of state of Minas Gerais, and Caldas Novas and Minaçu, both state of Goiás (red circles) **(A)**. Dotchart of variable importance as measured by a Random Forest **(B)**. Abbreviations: araguaia (Pontal do Araguaia population), ternetzi (*P. ternetzi*).

FIG. 4.—First and second dimensions of the Multidimensional scaling of the proximity scores from Random Forest of the acoustic data comparing eighteen adult males of *Pseudopaludicola* sp. from Pontal do Araguaia, state of Mato Grosso (green circles); twenty six adult males of *P. ternetzi* from states of Goiás and Minas Gerais (red circles); and four

adult males of *P. ameghini* from state of Mato Grosso (blue circles) **(A)**. Dotchart of variable importance as measured by a Random Forest **(B)**. Abbreviations: araguaia (Pontal do Araguaia population), ternetzi (*P. ternetzi*), ameghini (*P. ameghini*).

FIG. 5.—Distribution of our sampled municipalities of *Pseudopaludicola ternetzi* and Pontal do Araguaia population on a topographic map of the region orange highlighted in Brazil (top left). White circles: (1) Pontal do Araguaia, state of Mato Grosso; (2) Uberlândia, (3) Monte Alegre de Minas, and (4) Ituiutaba, all three state of Minas Gerais; (5) Minaçu and (6) Caldas Novas, both state of Goiás. Additionally, black star (7): Uruaçu, state of Goiás, type-locality of *P. ternetzi*.

FIGURE 1

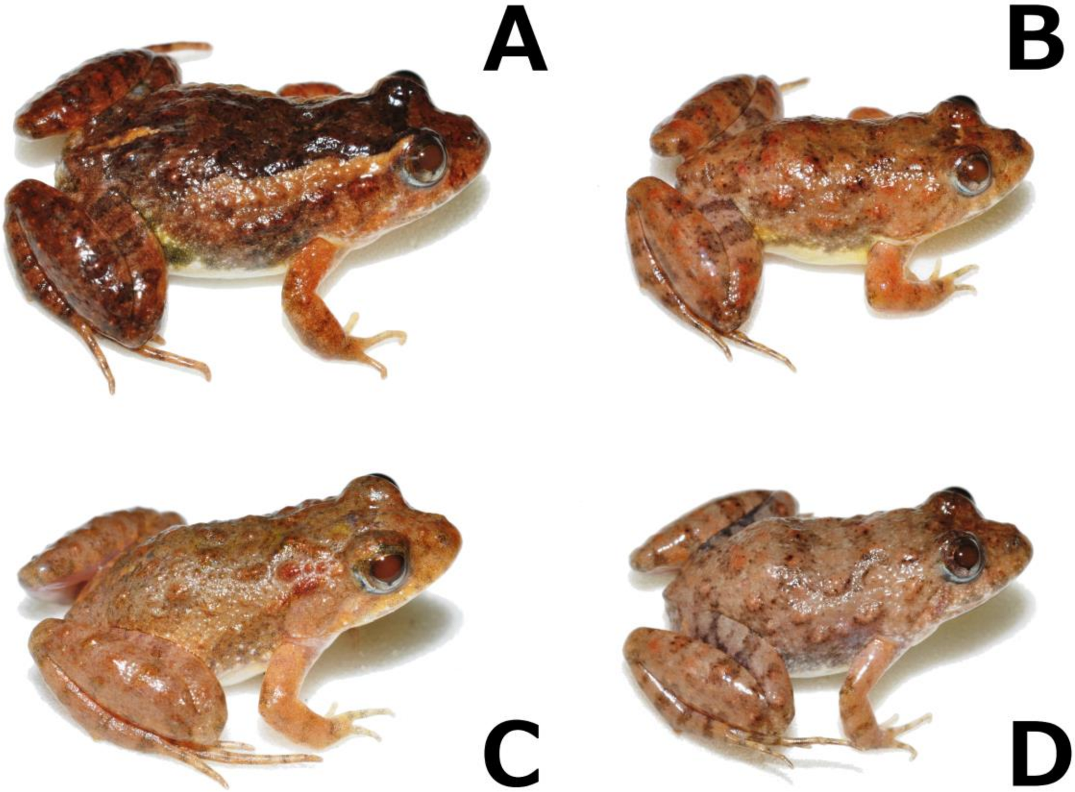
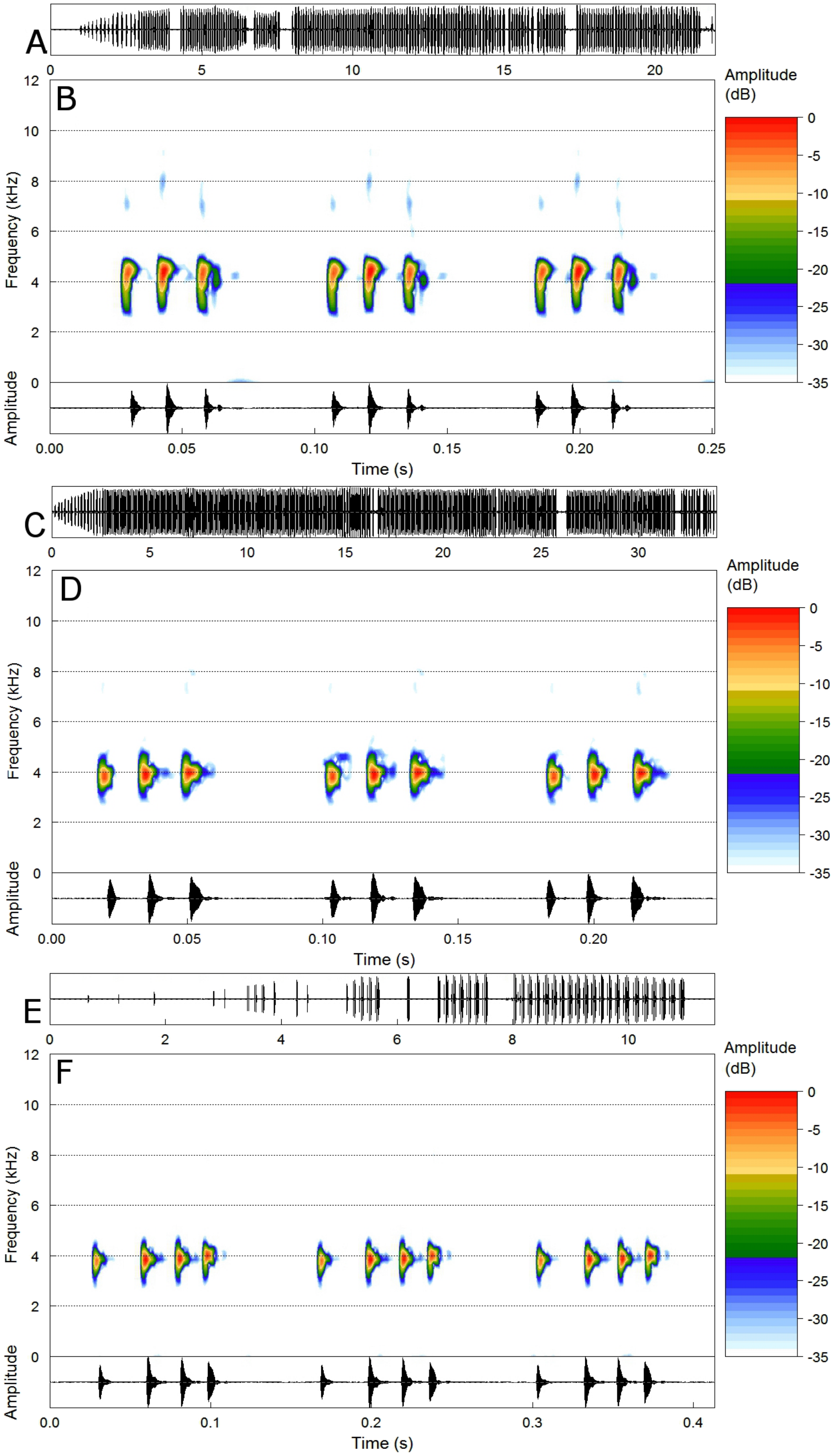


FIGURE 2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0
5
10
15
20
B
12
10
8
6
4
2
0
Frequency (kHz)
Amplitude
0.00
0.05
0.10
0.15
0.20
0.25
Time (s)
Amplitude
(dB)
0
-5
-10
-15
-20
-25
-30
-35
C
0
5
10
15
20
25
30
D
12
10
8
6
4
2
0
Frequency (kHz)
Amplitude
0.00
0.05
0.10
0.15
0.20
Time (s)
Amplitude
(dB)
0
-5
-10
-15
-20
-25
-30
-35
E
0
2
4
6
8
10
F
12
10
8
6
4
2
0
Frequency (kHz)
Amplitude
0.0
0.1
0.2
0.3
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Time (s)
Amplitude
(dB)
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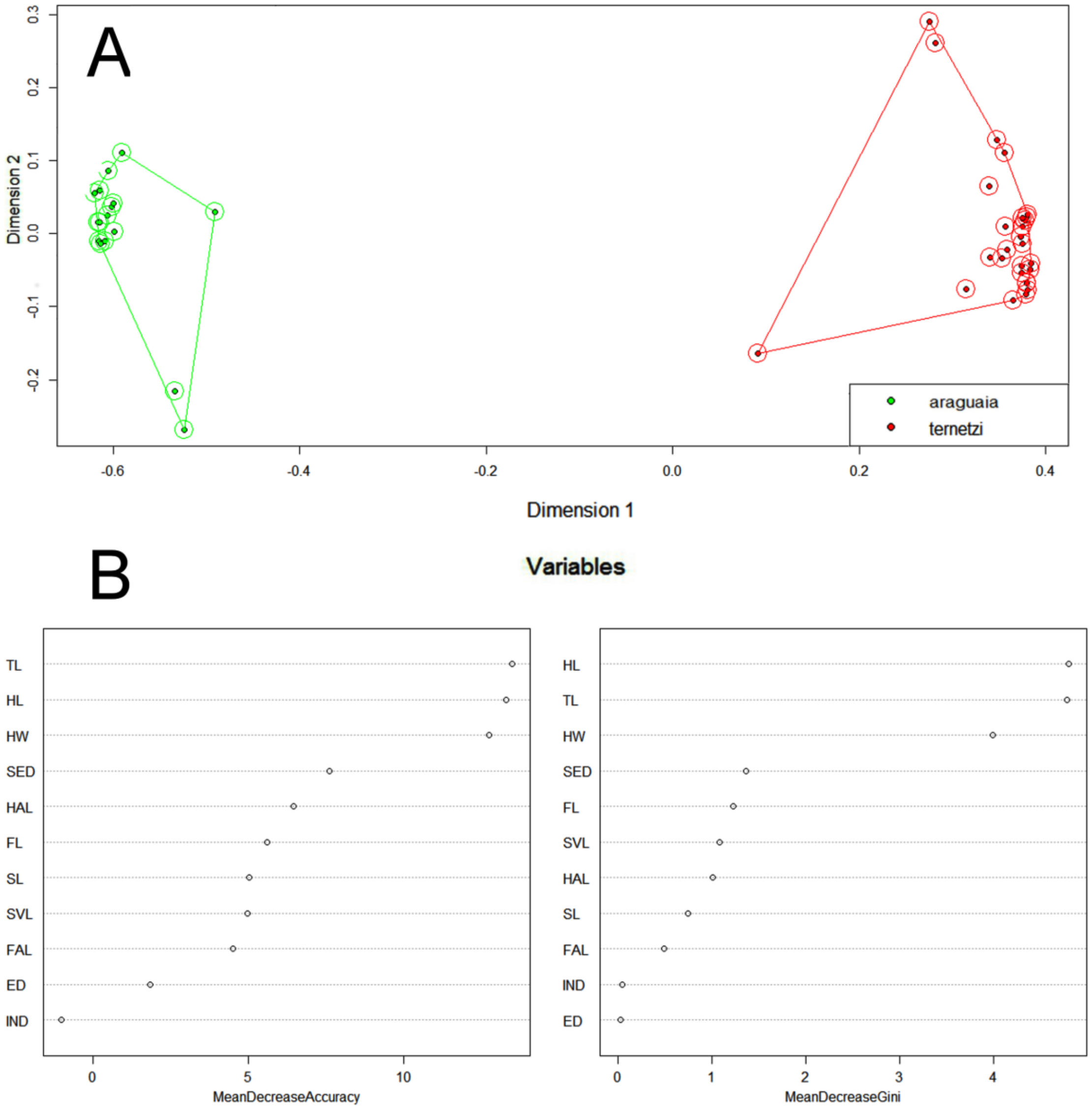
-20
-25
-30
-35

FIGURE 3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Dimension 2
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0.2
0.1
0.0
-0.1
-0.2
-0.6
-0.4
-0.2
0.0
0.2
0.4
Dimension 1
araguaia
ternetzi
B
Variables
TL
HL
HW
SED
HAL
FL
SL
SVL
FAL
ED
IND
0
5
10
MeanDecreaseAccuracy
HL
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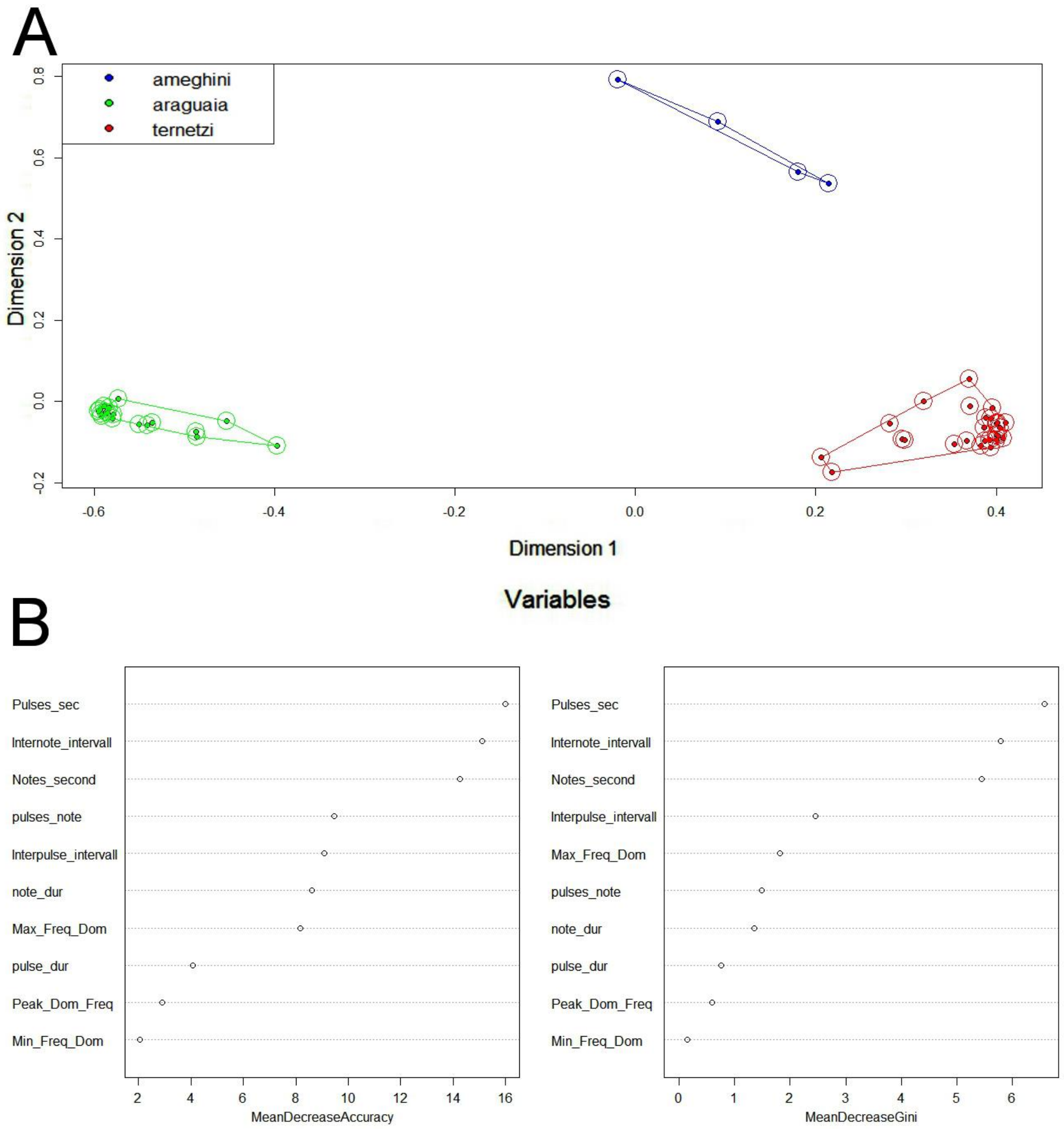
MeanDecreaseGini

FIGURE 4


A
ameghini
araguaia
ternetzi
Dimension 2
0.8
0.6
0.4
0.2
0.0
-0.2
-0.6
-0.4
-0.2
0.0
0.2
0.4
Dimension 1
B
Variables
Pulses_sec
Internote_intervall
Notes_second
pulses_note
Interpulse_intervall
note_dur
Max_Freq_Dom
pulse_dur
Peak_Dom_Freq
Min_Freq_Dom
2
4
6
8
10
12
14
16
MeanDecreaseAccuracy
Pulses_sec
Internote_intervall
Notes_second
Interpulse_intervall
Max_Freq_Dom
pulses_note
note_dur
pulse_dur
Peak_Dom_Freq
Min_Freq_Dom
0
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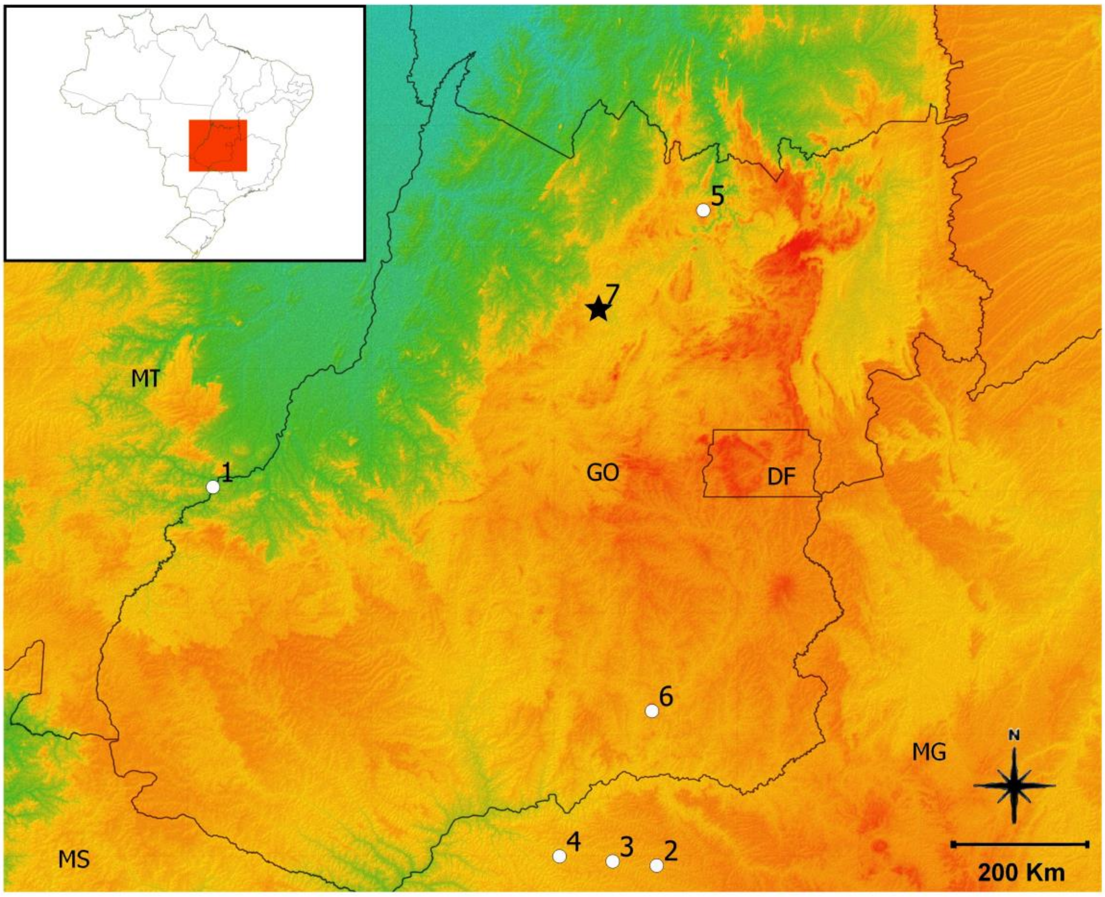
MeanDecreaseGini

FIGURE 5



5 CONCLUSÕES GERAIS

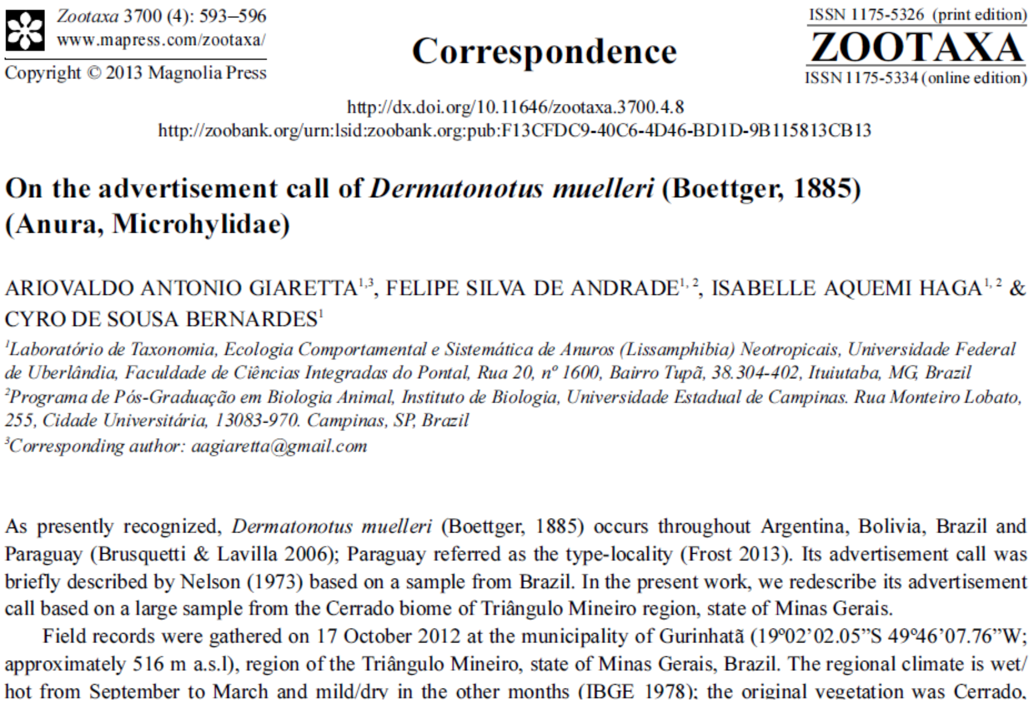
No presente trabalho defendemos a hipótese de uma espécie nova do grupo de *Pseudopaludicola saltica* no nordeste brasileiro. Tal hipótese é sustentada por evidências acústicas, moleculares, citogenéticas e filogenéticas. Além disso, avaliamos a posição taxonômica de populações supostamente relacionadas à *P. ternetzi*. Com base em dados acústicos, morfológicos e moleculares atribuímos a elas o nome *P. ternetzi*, e propomos uma diagnose acústica desta espécie em relação às outras espécies do gênero. Adicionalmente, detectamos uma variação intraespecífica morfológica e acústica da população do Pontal do Araguaia. As variações na temperatura do ar e da água aferidas durantes as gravações dos machos dessa população justificam as altas taxas de emissão de pulsos e notas dos machos quando comparados a outras populações estudadas de *P. ternetzi*. Além disso, a baixa divergência genética (0,2%) entre as sequências de DNA dos espécimes da população do Pontal do Araguaia e de espécimes da localidade-tipo de *P. ternetzi* (Uruaçu-GO) corroborou a hipótese de variação intraespecífica dos indivíduos dessa população de *P. ternetzi*.

Uma abordagem integrativa nos permitiu avaliar objetivamente várias linhas de evidências para a definição de um bom nome para as populações amostradas no presente trabalho. Portanto, nós acreditamos que dentro de uma estrutura formalizada esta é a abordagem mais eficiente e teoricamente fundamentada para uma melhor delimitação da distribuição de uma espécie válida ou para a consolidação de uma hipótese robusta de existência de uma espécie nova.

Os resultados obtidos com esse estudo contribuem para o melhor conhecimento da biodiversidade de Anura no Brasil. Estudos taxonômicos detalhados que levem em consideração uma abordagem integrativa ainda são necessários e se mostram relevantes para a ciência brasileira, uma vez que a nossa biodiversidade esta enfrentando uma grave ameaça devido o avanço desproporcional das atividades humanas sob os habitats em todo território nacional. Além disso, ainda há muito que se fazer no que diz respeito a estudos taxonômicos de populações e espécies de *Pseudopaludicola*, uma vez que a riqueza de espécies desse grupo ainda esta subestimada.

6 ANEXOS

6.1 GIARETTA, A. A.; **ANDRADE, F. S.**; HAGA, I. A.; BERNARDES, C. S. On the advertisement call of *Dermatonotus muelleri* (Boettger, 1885) (Anura, Microhylidae). Zootaxa (Online), v. 3700, p. 593–596, 2013.



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On the advertisement call of *Dermatonotus muelleri* (Boettger, 1885) (Anura, Microhylidae)

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As presently recognized, *Dermatonotus muelleri* (Boettger, 1885) occurs throughout Argentina, Bolivia, Brazil and Paraguay (Brusquetti & Lavilla 2006); Paraguay referred as the type-locality (Frost 2013). Its advertisement call was briefly described by Nelson (1973) based on a sample from Brazil. In the present work, we redescribe its advertisement call based on a large sample from the Cerrado biome of Triângulo Mineiro region, state of Minas Gerais.

Field records were gathered on 17 October 2012 at the municipality of Gurinhatã (19º02’02.05”S 49º46’07.76”W; approximately 516 m a.s.l), region of the Triângulo Mineiro, state of Minas Gerais, Brazil. The regional climate is wet/hot from September to March and mild/dry in the other months (IBGE 1978); the original vegetation was Cerrado,

6.2 **ANDRADE, F. S.**; HAGA, I. A.; MARTINS, F. A. M.; GIARETTA, A. A. On advertisement call of the poison frog *Ameerega berohoka* (Dendrobatidae, Anura) from the Brazilian Cerrado. Zootaxa (Online), v. 3838, p. 392–396, 2014.

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On advertisement call of the poison frog *Ameerega berohoka* (Dendrobatidae, Anura) from the Brazilian Cerrado

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The poison frog genus *Ameerega* (Dendrobatidae) currently contains 32 species. They are distributed from central Brazil into western Amazonia to the lower Andean versant. In addition, three trans-Andean species have been allocated to *Ameerega* (Andrade *et al*. 2013; Frost 2014). *Ameerega berohoka* (Vaz-Silva & Maciel 2011) was described based on specimens from central Brazil (type-locality: Arenópolis, GO) and it is assumed to occur in parts of western and southwestern state of Goiás (Frost 2014). More recently, Andrade *et al.* (2013) extended its distribution to the state of Mato Grosso. Here we re-describe the advertisement call of *A. berohoka,* providing

6.3 HAGA, I. A.; **ANDRADE, F. S.**; TOSCANO, N. P.; KWET, A.; GIARETTA, A. A. Advertisement call and habitat of *Vitreorana uranoscopa* (Müller, 1924) (Anura, Centrolenidae) in Brazil. Salamandra (Frankfurt), v. 50(4), p. 236–240, 2014.

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Advertisement call and habitat of *Vitreorana uranoscopa* (Anura: Centrolenidae) in Brazil

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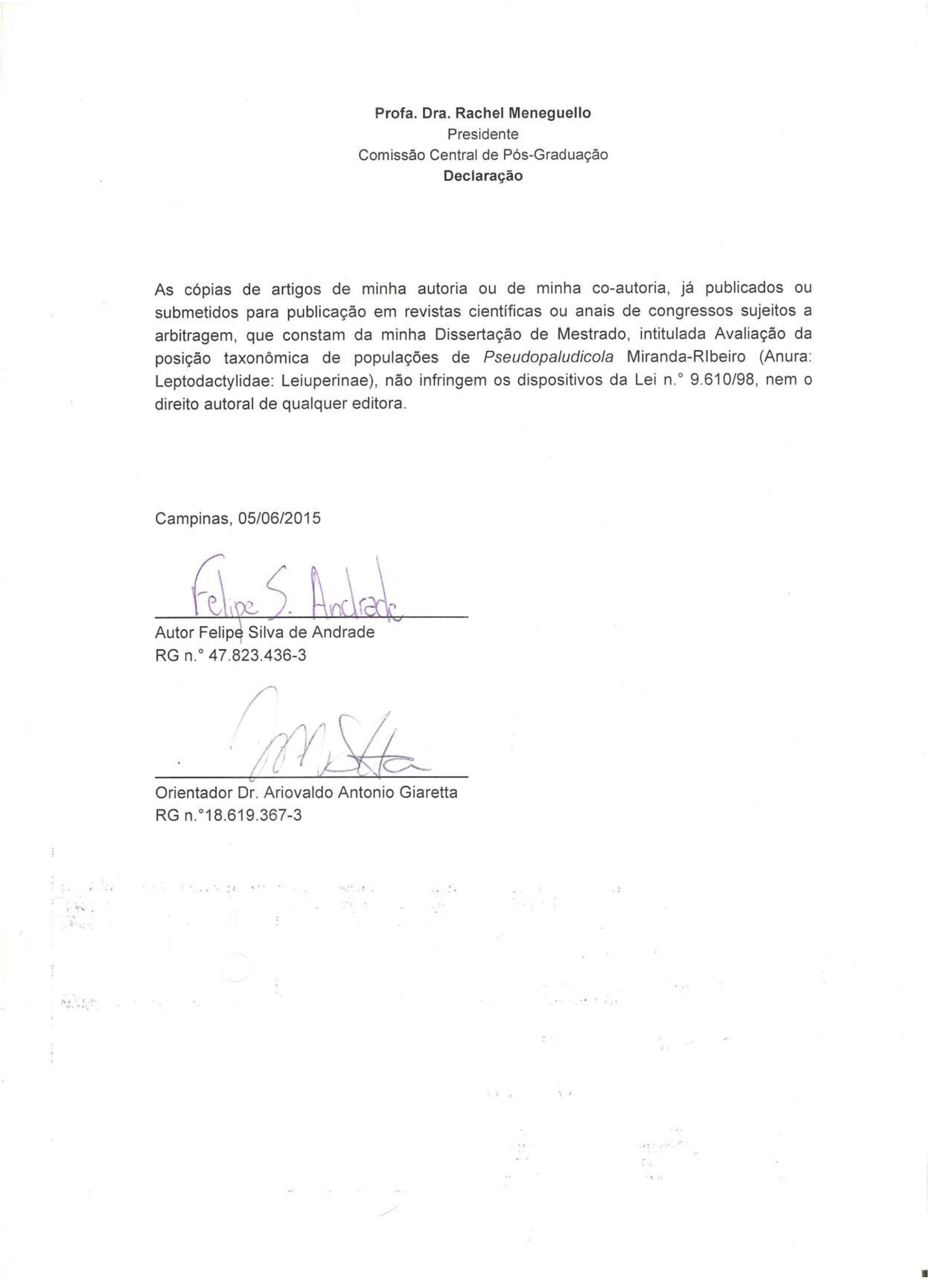
As presently defined, the glass frogs of the genus *Vitreorana* are comprised of nine disjunctively distributed species, five of which occur in the Cordillera de la Costa of Venezuela and the Guianas to French Guiana; Amazonia of Colombia and Ecuador, and four in the Atlantic Forest of Brazil and Argentina (Guayasamin et al. 2009, Frost 2014, Pontes et al. 2014). As presently recognized, *Vitreorana uranoscopa* (Müller, 1924) is distributed through the eastern and

published a detailed call description of the species from the Argentinean Atlantic Forest, allowing for proper comparison with our data from Brazil.

Data were gathered at Córrego Grande, municipality of Florianópolis, Island of Santa Catarina, state of Santa Catarina, southern Brazil (27°36'21.98" S, 48°30'34.47" W, approximately 140 m a.s.l) on 9 October 2002 from 20:45–22:00 h. This site has a warm humid subtropical climate

6.4 Declaração sobre direito autoral



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