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ORIENTADOR: PROF. DR. ARIOVALDO A. GIARETTA

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RESUMO

O gênero Phyllomedusa Wagler, 1830 (Anura, Hylidae) abriga 31 espécies e possui uma distribuição neotropical. Phyllomedusa é um agrupamento monofilético suportado por sinapomorfias morfológicas: presença de glândulas parotóides e o primeiro dedo mais longo do que o segundo dedo; e genéticas: 49 transformações em proteínas nucleares e mitocondriais e em genes ribossômicos. Por se tratar de um gênero com muitas espécies crípticas, estudos taxonômicos integrativos que utilizam caracteres morfológicos, bioacústicos, citogenéticos e moleculares são cada vez mais frequentes. O presente estudo obteve em campo espécimes vivos, dados acústicos e tecidos de uma população mato-grossense de Phyllomedusa do grupo de P. hypochondrialis do subclado das espécies de baixa altitude e com coloração não reticulada, visando atribuí-la uma identificação taxonômica apropriada. Com base em uma abordagem integrativa, propomos a ocorrência de uma espécie inédita do grupo de P. hypochondrialis município de Pontal do Araguaia, Mato Grosso. Nossa hipótese é sustentada por evidências morfométricas, acústicas e moleculares. Além disso, o presente estudo também descreve os cantos de anúncio e territorial de P. azurea e realiza comparações intraespecíficas com base em dados da literatura de populações da Bolívia e Argentina, bem como comparações interespecíficas com espécies atualmente atribuídas ao grupo de P. hypochondrialis.

ABSTRACT

The genus Phyllomedusa Wagler, 1830 (Anura, Hylidae) comprises 31 species with a neotropical distribution. Phyllomedusa is a monofiletic group supported by morphological synapomorphies: the presence of parotoid glands, toe I longer than toe II; and genetics: 49 transformations in nuclear and mitochondrial protein and ribosomal genes. Since this is a genus with many cryptic species, integrative taxonomic studies which use morphological, bioacoustics, cytogenetic and molecular characters are becoming more frequent. Through field works, the present study obtained live specimens, acoustic data and tissues of Phyllomedusa of subclade of lowland species within P. hypochondrialis group and with a non-reticulated coloration from Mato Grosso, Brazil, aiming to assign them an appropriate taxonomic identification. From an integrative approach, we propose the occurrence of a new species from P. hypochondrialis group from the municipality of Pontal do Araguaia, state of Mato Grosso. Our hypothesis is supported by morphological, acoustic and molecular evidence. Furthermore, the present study also describes the advertisement and territorial call of P. azurea and performs intraspecific comparisons based on literature data of populations from Bolivia and Argentina, as well as interspecific comparisons with species currently assigned to P. hypochondrialis group.

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FIG. 1—Phyllomedusa sp. nov., adult male, primary reference specimen (AAG-UFU 3444). Dorsal (A) and ventral (B) views. SVL = 30.6 mm

FIG. 2—Phyllomedusa sp. nov., adult male, primary reference specimen (AAG-UFU 3444). (A) Lateral and (B) Dorsal views of head; ventral views of (C) hand and (D) foot.

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1 INTRODUÇÃO

1.1 Introdução geral

Os anuros (p. ex. sapos, rãs e pererecas) possuem uma riqueza mundial que compreende ca. 6560 espécies (Frost, 2015) e a maior diversidade do grupo se encontra nos trópicos, principalmente na região Neotropical (Duellman & Trueb, 1986). Para o Brasil, se reconhece hoje ca. de 1000 de espécies de Anura, representando aproximadamente 15% da diversidade mundial do grupo (Frost, 2015). Grande parte dessa riqueza se deve a dimensão continental do país, o qual integra amplas zonas climáticas (Norte úmido, Nordeste árido e Sul subtemperado) (MMA, 2013) que formam zonas biogeográficas distintas (biomas) como a Amazônia, o Cerrado e a Mata Atlântica (MMA, 2013). No geral, o desmatamento, o avanço das fronteiras agrícolas, a mineração, e os projetos de desenvolvimento (p. ex., barragens, estradas, indústrias e empreendimentos imobiliários) são os fatores que mais provocam a destruição de habitats e perda de diversidade de espécies de anfíbios brasileiros (Silvano & Segalla, 2005). Dentre esses ambientes, do ponto de vista conservacionista, o bioma Cerrado é particularmente importante por ser considerado um dos vinte e cinco centros de alta diversidade biológica e que está sujeito a severas ameaças devido ao intenso avanço das atividades humanas (hot spots) (Myers et al., 2000). Klink e Machado (2005) constataram que cerca de 55% da extensão geográfica do Cerrado já foi desmatada ou modificada pela ação humana, e a taxa de desmatamento anual desse bioma é o dobro em relação a taxa de desmatamento da Amazônia.

Em um cenário mundial a situação da conservação de anuros também é muito preocupante. Stuart et al. (2004) ressaltam que os anfíbios possuem um maior risco de extinção do que qualquer outro táxon, uma vez que relatos de declínios populacionais e extinções de espécies desse grupo têm aumentado a partir de 1980, a uma velocidade maior do que a de mamíferos e aves. Os mesmos autores também apontam que desde 1970, 34 espécies de anfíbios devem ter se extinguido, 1800 espécies são consideradas como ameaçadas e 2468 espécies têm sofrido algum declínio populacional (Stuart et al., 2004).

1.2 Taxonomia e o conceito de espécie

Taxonomia é a disciplina que busca identificar, descrever e classificar táxons viventes e extintos (Padial et al., 2010). É uma área de pesquisa estimulante, pois além de trazer contribuições indispensáveis a ciência, também satisfaz a necessidade humana da descoberta (Kaiser et al., 2013).

Atualmente, um grande desafio da taxonomia moderna é de incorporar novas teorias, métodos e dados a partir de pesquisas que estudam a origem, os limites e a evolução das espécies (Padial et al., 2010). A falta de interação entre as diferentes linhas de pesquisa, envolvidas na delimitação das espécies, é um dos maiores problemas da chamada “crise da taxonomia” (Dayrat, 2005). Com o intuito de obter possíveis soluções para este problema, a chamada “Taxonomia Integrativa” tem ganhado espaço, inclusive na taxonomia de Anura (Dayrat, 2005; Padial & De la Riva, 2009; Padial et al., 2010) e têm impulsionado a descoberta e a descrição de anfíbios em todo o mundo (Dayrat, 2005). A Taxonomia Integrativa é definida como a “ciência que visa delimitar as unidades da diversidade da vida a partir de múltiplas e complementares perspectivas como a filogeografia, morfologia comparativa, genética de populações, ecologia, desenvolvimento, comportamento, etc.” (Dayrat, 2005). Uma consequência positiva dessa nova abordagem é que após 250 anos de predomínio da morfologia como única linha evidência na diagnose das espécies, novos métodos e novos dados (principalmente moleculares) têm conquistado espaço na área (Padial et al., 2010).

A unidade central da taxonomia é a espécie (Padial et al., 2010) a qual representa uma das unidades mais fundamentais na biologia, equiparando-a a genes, células e organismos (Mayr, 1982; de Queiroz, 2005; de Queiroz, 2007). Portanto, delimitá-las é crucial para o entendimento da diversidade da vida, uma vez que se procura estabelecer se dois organismos diferentes pertencem ou não a uma mesma entidade biológica (Dayrat, 2005). Essa diferenciação complica-se quando a delimitação de espécies é confundida com o próprio conceito teórico de espécie, gerando assim controvérsias a cerca da definição teórica dessa entidade e os métodos práticos utilizados pelos taxonomistas para inferir os limites e o número de espécies existentes (de Queiroz, 2007). Mayden (1997) listou ao menos 24 conceitos de espécie, dentre eles destacam-se o ecológico, o genético, o evolutivo, o filogenético e o mais tradicional de todos, o conceito biológico.

De Queiroz (2007) propõe um conceito unificado de espécie, baseando-se no que ele considera como elemento comum entre os diversos conceitos já existentes, denominando-o de “unidade conceitual subjacente”. Para este autor, a única propriedade necessária para definir espécies seria que elas representam linhagens metapopulacionais (populações que incluem subpopulações conectadas) com evolução independente. E os critérios operacionais que tradicionalmente são confundidos com a conceituação de espécie são secundários e relevantes na delimitação, separação e classificação dessas linhagens (de Queiroz, 2007).

De Queiroz (2007) também ressalta que tentativas prévias de unificar os conceitos de espécies fracassaram, pois se focaram em enfatizar as diferenças entre conceitos rivais em vez de suas semelhanças.

Em termos práticos, a delimitação de espécies depende do reconhecimento dessas linhagens que geralmente divergiram a ponto de serem feneticamente distinguíveis uma das outras e passíveis de serem diagnosticadas em termos de estados de caracteres fixos (e.g. genitália, gametas, sistemas de desenvolvimento e comunicação sonora). E, consequentemente, os sistemas de reconhecimento de parceiros divergiram a ponto dos organismos já não se reconhecerem mais como potenciais parceiros (de Queiroz, 2007).

1.3 Linhas de evidências no reconhecimento de espécies de Anura

Além da tradicional morfologia/morfometria, também utilizaremos a acústica e a genética como linhas de evidências na discriminação entre as espécies.

3 Acústica

A comunicação através da emissão de sinais acústicos (vocalizações) é amplamente difundida entre os vertebrados, como por exemplo, os anuros, aves, morcegos, primatas, cetáceos (Duellman & Trueb, 1986). Os anuros são vocalmente ativos e a vocalização é um componente importante de seu comportamento reprodutivo (Lukanov et al., 2015). A diversidade do repertório vocal de Anura é muita extensa, e, baseando-se em seu contexto social, foi organizado em três categorias: reprodutivo, agressivo e defesa (Toledo et al., 2015). O principal sinal acústico emitido pelos machos anuros durante a estação reprodutiva é o canto de anúncio, que tem como principal função a atração de fêmeas coespecíficas sexualmente receptivas (Blair, 1958; Duellman & Trueb, 1986), além de sinalizar a localização do emissor para outros machos vizinhos (Narins et al., 2007). Variações do canto de anúncio podem indicar importantes características do macho emissor (e.g. tamanho corporal), influenciando diretamente a escolha da fêmea e a territorialidade (Davies & Halliday, 1978; Asquith & Altig 1990; Gerhardt, 1991;

Wagner, 1992; Gerhardt & Ruber, 2002). Essa atração diferencial por parte das fêmeas (seleção sexual) determina o sucesso reprodutivo dos machos (Ryan & Rand, 1993).

Em geral, os caracteres fenotípicos selecionados por seleção sexual apresentam uma maior probabilidade de representar diferenças espécie-específicas do que os caracteres selecionados por seleção natural, uma vez que eles contribuem com uma coesão reprodutiva e isolamento das espécies. Por esse motivo, os caracteres mais importantes para estudos taxonômicos são aqueles que indicam um isolamento reprodutivo ou fluxo gênico limitado (Padial et al., 2010).

O canto de anúncio em Anura geralmente é espécie-específico (Salthe & Mecham, 1974) e possui um papel fundamental na manutenção das barreiras reprodutivas entre as espécies (Schneider & Sinsch, 2007). Além disso, o canto de anúncio também é amplamente aceito como mecanismo primário de isolamento reprodutivo (e.g. Blair, 1958; Littlejonh, 1965), tendo, portanto, um importante papel na taxonomia das espécies do grupo, uma vez que contribui para a diagnose de espécies e no reconhecimento de complexos de espécies crípticas com morfologia altamente conservada (Cocroft, 1995, Cardoso & Viellard, 1985).

[illegible] Sequências gênicas

A aplicação de métodos moleculares na taxonomia e sistemática tem revolucionado a catalogação da biodiversidade mundial (Funk et al., 2011). Trabalhos de taxonomia mais recentes têm-se utilizado cada vez mais de sequências gênicas (mitocondriais e nucleares) e princípios de "código de barras de ADN" (=barcoding) para a elaboração de diagnoses mais robustas para suas populações candidatas a espécie nova (Vieites et al., 2009; Padial et al. 2010). Entretanto, uma validação taxonômica baseada apenas em caracteres genéticos ainda não é muito clara e amplamente aceita entre os taxonomistas (Vieites et al., 2009). Geralmente, sequências de ADN são examinadas utilizando-se métodos baseados em árvores filogenéticas com o intuito de procurar grupos monofiléticos que possam representar espécies válidas (Padial et al., 2010). Dados de sequência de ADN também têm revelado uma diversidade críptica, ou seja, quando duas ou mais espécies feneticamente semelhantes são erroneamente identificadas como coespecificas (Faivovich et al., 2010; Bruschi et al., 2013).

Vieites et al. (2009) acessaram a variação morfológica, bioacústica e genética de espécies de anuros da ilha de Madagascar. Inventários taxonômicos em grande escala dos anuros de Madasgascar, realizados desde 1991, detectaram um aumento de 80% do número de espécies descritas (de 133 para 244 espécies). Baseando-se em sequências de ADN de 2850 espécimes amostrados em mais de 170 localidades, Vieites et al. (2009) projetam um aumento para no mínimo 373 espécies e de no máximo 465 espécies. Com isso, eles conseguiram recuperar em suas análises moleculares um maior número de espécies do que os nomes disponíveis até o momento.

1.4 Phyllomedusa como táxon modelo

A primeira hipótese (pré-cladística) mais abrangente das relações de parentesco dos Phyllomedusinae foi proposta por Funkhouser (1957) com base principalmente no grau de especialização dos pés, e segundo Faivovich et al. (2010), este estudo representa apenas um “arranjo filogenético” de Phyllomedusinae. Trabalhos mais recentes baseados em uma abordagem cladística têm sugerido diferentes hipóteses das relações de parentesco entre os Phyllomedusinae (Duellman, 2001; Faivovich et al., 2005; Wiens et al., 2005, Wiens et al., 2006; Moen & Wiens, 2009; Gomez-Mestre et al., 2008).

Até o presente, o gênero Phyllomedusa Wagler 1830 (Phyllomedusinae: Hylidae) abriga 32 espécies e possui uma distribuição neotropical desde o Panamá, incluindo a Colômbia, leste dos Andes até o sul da Argentina e Uruguai (Giaretta et al 2007; Frost, 2015). Phyllomedusa é um agrupamento monofilético (Faivovich et al., 2005; Faivovich et al., 2010) suportado por caracteres morfológicos como presença de glândulas parotóides e o primeiro dedo mais longo do que o segundo, e por 49 transformações em proteínas nucleares e mitocondriais e em genes ribossômicos.

Pela incerta posição filogenética do grupo de Phyllomedusa buckley, estudos pré-cladisticos indicavam Phyllomedusa como sendo parafilético (Duellman, 1968, 1969, Cannatella, 1980; Jungfer & Weygoldt, 1994). Jungler e Weyolat (1994) apontaram que o grupo “P. buckley” (sensu Duellman, 2001) seria mais proximamente relacionado à Phyllomedusa do que com qualquer outro Phyllomedusinae, baseando-se nas diferenças encontradas em morfologia e biologia reprodutiva. Entretanto, a partir da topologia recuperada em análises moleculares, Faivovich et al. (2005) sugeriram (com baixo suporte) que o grupo de P. buckley seja mais aparentado ao gênero Hylomantis Peters 1873 (atualmente sinonimizado a Agalychnis Cope 1864) do que com Phyllomedusa. Por esse motivo, os autores sugeriram a realocação de todas as espécies do grupo P. buckley para Hylomantis, com o intuito de resolver o parafiletismo de Phyllomedusa. Trabalhos posteriores a Faivovich et al. (2005) corroboraram a monofilia do gênero e dos grupos de Phyllomedusa (Faivovich et al., 2010; Bruschi et al., 2013).

A primeira tentativa (pré-cladistica) de organização das espécies de Phyllomedusa foi feita por B. Lutz (1950) que, com base em características morfológicas, considerou valido Pithecopus Cope 1866 como um subgênero. Esse subgênero incluiria dois sub-grupos, um de espécies grandes, como Phyllomedusa (Pithecopus) burmeisteri e outro de pequenas espécies como Phyllomedusa (Pithecopus) hypochondrialis. Duellman (1974) comparou populações amazônicas, de P. palliata Peters 1872 com P. hypochondrialis (Daudin 1800) e P. rohdei Mertens 1926, porém não as agrupou formalmente.

O grupo formado por espécies de Phyllomedusa de pequeno porte (< 42 mm CRC) só foi proposto por Bokermann (1965), como grupo “rohdei-hypochondrialis” que incluía P. hypochondrialis, P. rohdei e P. centralis Bokermann 1965. Posteriormente, B. Lutz (1966) propôs a validação de Pithecopus como gênero pleno que incluía P. burmeisteri burmeisteri Boulenger 1882, P. b. bahiana Lutz 1925, P. b. distincta Lutz 1950, P. b. iheringii Boulenger 1885, P. sauvagii Boulenger 1882, P. boliviana Boulenger 1902, P. coelestis (Cope 1874), P. nicefori Lutz 1966, P. tarsius, P. tomopterna, P. vaillantii Boulenger 1882, P. trinitatis Mertens 1926, P. ayeaye (Lutz 1966), P. hypochondrialis hypochondrialis, P. h. azurea Cope 1862 e P. rohdei.

Duellman (1968) contesta a validação do gênero Pithecopus feita por B. Lutz (1966) chamando-o de “não natural”. No mesmo trabalho, esse autor também cita que investigações subsequentes ao trabalho de B. Lutz (1966) indicavam que as Phyllomedusa de pequeno porte pareciam compor uma unidade filética natural.

Cruz (1982) baseando-se em caracteres larvais e dentro de um contexto fenetico agrupou P. hypochondrialis, P. burmeisteri, P. rohdei, P. distincta, P. centralis e P. ayeaye. Pombal e Haddad (1992) retiraram P. burmeisteri (incluindo P. b. burmeisteri e P. b. bahiana Lutz, 1925) e P. distincta desse grupo proposto por Cruz (1982), agrupando-as com P. iheringii e P. tetraploidea Pombal e Haddad

1992 formando um novo grupo denominado P. burmeisteri. Brandão (2002) revalida informalmente P. megacephala (Miranda-Ribeiro 1926), associando-a com P. ayeaye e P. centralis e descreve uma espécie nova. O mesmo autor estabelece o grupo de espécies de P. hypochondrialis: P. hypochondrialis, P. rohdei, P. centralis, P. ayeaye, P. megacephala e P. oreades Brandão 2002.

Caramaschi (2006) baseando-se em caracteres morfológicos redefine o grupo de P. hypochondrialis, revalida P. azurea (originalmente considerada subespécie de P. hypochondrialis), redescreve P. megacephala, descreve P. nordestina Caramaschi 2006 e inclui P. palliata no grupo. Embora Faivovich et al. (2005) tenham demonstrado uma relação de espécies irmãs entre P. palliata e P. hypochondrialis, por falta de uma amostra adequada não considerou esse resultado como sendo uma evidência conclusiva que suportasse P. palliata no grupo de P. hypochondrialis.

Após esta ultima redefinição, três novas espécies foram descritas para esse grupo, P. itacolomi Caramaschi, Cruz, e Feio 2006, P. araguari Giaretta, Oliveira, e Kokubum 2007 e P. rustica Bruschi, Lucas, Garcia e Recco-Pimentel (2014). Porém, a primeira foi sinonimizada a P. ayeaye por Baêta et al. (2009) e a segunda foi sugerida como sinônimo de P. oreades por Brandão & Álvares (2009). Entretanto, Valdujo et al. (2012) aceitaram P. araguari como uma espécie plena e uma sinonimização formal nunca foi feita.

Como freqüentemente aceito na literatura, o grupo de P. hypochondrialis é formando por 11 espécies (Caramaschi, 2006; Faivovich et al., 2010): P. araguari, P. ayeaye, P. azurea, P. centralis, P. hypochondrialis, P. megacephala, P. nordestina, P. palliata, P. oreades, P. rohdei e P. rustica. Esse grupo é caracterizado pelas espécies terem focinho reto, discos adesivos pouco desenvolvidos, polegar igual ou mais curto do que o segundo dedo e o primeiro artelho maior que o segundo (Bokermann, 1965; Brandão, 2002; Caramaschi, 2006; Caramaschi et al., 2006). Essas espécies possuem uma distribuição ao longo da América do Sul e a Leste do Andes, incluindo a Venezuela, Guianas, Peru, Equador, Brasil, Bolívia, Paraguai e norte da Argentina (Faivovich et al., 2010; Frost, 2015).

É possível identificar no grupo de P. hypochondrialis dois subclados bem suportados por dados moleculares. Um deles é composto pelas espécies de ocorrência em áreas de mais baixa altitude, sendo elas: P. hypochondrialis, P.

azurea, P. nordestina e P. palliata; e o outro subclado é composto por P. rohdei (Mata Atlântica) e espécies caracterizadas por um padrão reticulado nos flancos, e por, geralmente, habitarem altitudes acima de 800m (espécies serranas), sendo elas: P. araguari, P. ayeaye, P. centralis, P. megacephala, P. oreades e P. rustica (Bokermann, 1965; Cruz, 1982; Brandão, 2002; Carasmaschi, 2006; Giaretta et al., 2007; Brandão et al., 2009; Faivovich et al., 2010; Bruschi et al., 2013).

Bruschi et al. (2013) recentemente acessaram a posição taxonômica de diversas populações atribuídas a P. azurea e P. hypochondrialis através de caracteres morfológicos, citogenéticos e moleculares. Este trabalho corroborou Faivovich et al. (2010) em relação ao monofiletismo dos grupos taxonômicos (P. burmeisteri, P. hypochondrialis, P. perinesos e P. tarsius), bem como Caramaschi (2006) na revalidação de P. azurea. Bruschi et al. (2013) também apontaram que os caracteres fenotípicos diagnósticos propostos por Caramaschi (2006) não permitem a diagnose diferencial entre P. azurea e P. hypochondrialis, devido a considerável variação morfológica entre as populações dessas espécies. Além disso, Bruschi et al. (2013) indicam que mais estudos fenotípicos e sistemáticos são necessários para se identificar caracteres morfológicos e acústicos que permitam a distinção entre essas duas espécies. Esses mesmos autores também ampliaram a distribuição de P. hypochondrialis e apontaram que o entendimento dos limites da distribuição geográfica de P. azurea foram negativamente afetados por erros de identificação, e que esta última espécie deve ter distribuição restrita ao sul do Brasil, Paraguai, Norte da Argentina e sul do Uruguai.

As espécies do grupo de P. hypochondrialis com canto descrito atualmente são: P. araguari (Giaretta et al., 2007), P. azurea (Haga et al., submetido), P. centralis, (Brandão et al., 2009), P. hypochondrialis (Pyburn & Glidewell, 1971; Duellman & Pyles, 1983; Duellman, 1997; Guimarães et al., 2001), P. megacephala (Giaretta et al., 2007), P. nordestina (Vilaça et al., 2011), P. oreades (Brandão & Álvares, 2009), P. palliata (Duellman, 1978; Köhler & Lötters, 1999) e P. rohdei (Wogel et al., 2004).

Um audiospectrograma do canto de anúncio de P. hypochondrialis foi fornecido por Pyburn & Glidewell (1971) de Villavencio na Colômbia, mas sem descrições mais detalhadas de suas variáveis temporais e espectrais. Posteriormente, descrições do canto dessa espécie foram fornecidas por Barrio (1976) (população da Província do Chaco, Argentina), De la Riva et al. (1995)

(população de Puerto Almacén, Bolívia), e por Guimarães et al. (2001) (população de Palmeira e Silvânia de Goiás, estado de Goiás, Brasil). Entretanto, Brandão et al. (2009) assumiram que o canto de anúncio descrito por Guimarães et al. (2001) na verdade corresponde ao de P. azurea e o seu canto territorial descrito no mesmo trabalho se semelha ao canto de P. hypochondrialis descrito por De La Riva et al. (1995). Contudo, a distribuição geográfica de P. azurea fornecida por Bruschi et al. (2013) não abrange o estado de Goiás. Por esse motivo, o canto descrito por Guimarães et al. (2001) na verdade pertence a P. hypochondrialis (assim designado pela descrição original) e não a P. azurea como sugerido por Brandão et al. (2009) e o canto descrito por De la Riva et al. (1995) corresponde ao canto territorial de P. azurea, o que faz com que o canto de P. azurea ainda permaneça desconhecido para a ciência. Considerando-se que temos um manuscrito sobre o canto de P. azurea (capítulo 2) em fase avançada de revisão, consideramos esses dados para fins de comparação.

Em trabalhos de campo em área de Cerrado de Pontal do Araguaia, estado de Mato Grosso, Brasil, coletamos exemplares e gravamos cantos de uma população de Phyllomedusa do grupo de P. hypochondrialis do subclado das espécies de baixa altitude (não reticuladas) (sensu Faivovich et al., 2010) a qual parece ser distinta das demais ate agora reconhecidas.

2 OBJETIVOS

(1) Avaliar a posição taxonômica dessa população de Phyllomedusa, visando: (2) para fins comparativos, caracterizar e revisar morfometrica e acústicamente outras populações de espécies proximamente relacionadas; (3) integrar esses dados com os moleculares e citogenéticos do grupo; e (4) descrever cantos e espécies potencialmente inéditas para a ciência.

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4 RESULTADOS

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

A New Species of Phyllomedusa (Anura, Hylidae, Phyllomedusinae) From the Brazilian Cerrado

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RRH: NEW SPECIES OF PHYLLOMEDUSA

ABSTRACT

THE MONKEY frog, genus Phyllomedusa Wagler (Anura, Hylidae, Phyllomedusinae), currently comprises 32 valid species. The P. hypochondrialis species group contains 11 species and a recent molecular phylogeny suggested the existence of two well-supported subclades within it. One of them includes the lowland species: P. palliata, P. azurea, P. hypochondrialis, and P. nordestina. Herein we describe a new species of Phyllomedusa this lowland group, with a non-reticulated pattern from municipality of Pontal do Araguaia, state of Mato Grosso, Brazilian Cerrado. The new species recognition was based on adult morphology, advertisement call and molecular data. Phyllomedusa sp. nov differs from species of this group by: by (1) lacking the reticulate color pattern on flanks (2) smaller SVL (30.3–33.8 mm); (3) core portion of call with 5 to 7 pulses; and (4) higher dominant frequency (2239–3316 Hz).

KEY WORDS: Advertisement call, Morphometry, state of Mato Grosso, Phyllomedusa hypochondrialis, Species delimitation, Taxonomy.

1 INTRODUCTION

As Presently Defined, the monkey frog, genus Phyllomedusa Wagler, 1830 comprises 32 species with a Neotropical distribution from Panama, including Colombia, east of the Andes to the south of Argentina and Uruguay (Giaretta et al2007; Frost 2015). Based on molecular data (Faivovich et al. 2010), most Phyllomedusa species were assigned to four groups: that of Phyllomedusa burmeisteri Boulenger, 1882 (Pombal and Haddad 1992), P. hypochondrialis (Daudin, 1800) (Caramaschi 2006), P. perinesos Duellman, 1973 (Cannatella 1982), and P. tarsius (Cope, 1868) (Barrio-Amorós 2006).

The P. hypochondrialis group contains 11 species: P. ayeaye (Lutz, 1966); P. azurea Cope, 1862; P. araguari Giaretta, Oliveira, and Kokubum, 2007; P. centralis Bokermann, 1965; P. hypochondrialis; P. megacephala (Miranda-Ribeiro, 1926); P. nordestina Caramaschi, 2006; P. palliata Peters, 1873; P. oreades Brandão, 2002; P. rohdei Mertens, 1926; P. rustica Bruschi, Lucas, Garcia and Recco-Pimentel, 2014 (Frost 2015). This group is characterized by having truncate snout tip, finger pads poorly developed, thumb equal or shorter than toe II and the finger I greater than the toe II (Bokermann 1965; Caramaschi 2006).

The most comprehensive phylogenetic hypothesis for phyllomedusines was proposed by Faivovich et al. (2010). They points to two well-supported subclades within the P. hypochondrialis group. One including those essentially lowland species: (i) P. palliata, P. azurea, P. hypochondrialis, and P. nordestina; while the other: (ii) P. rohdei and the other the mid to highland species P. ayeaye, P. araguari, P. centralis, P. megacephala, P. oreades and P. rustica. A recent phylogeographic study detected a four well-supported clades from populations designed to P. hypochondrilais (Bruschi et al. submitted).

Herein we describe a new species of Phyllomedusa belonging to the P. hypochondrialis group and to the lowland group from the middle Araguaia River in Brazilian Cerrado, based on adult morphology, advertisement call and molecular data.

MATERIAL AND METHODS

Reference Specimens

We deposited type specimens in the Collection of Amphibians of the Museu de Biodiversidade do Cerrado (AAG-UFU) at Universidade Federal de Uberlândia (UFU), municipality of Uberlândia, state of Minas Gerais, Brazil; and in the Museu de Zoologia at Universidade Estadual de Campinas (UNICAMP), municipality of Campinas, state of São Paulo, Brazil. For morphometric and acoustic comparisons, we used specimens and calls of those species more closely related to new species as P. azurea, P. hypochondrialis and P. nordestina, as indicated by previous phylogenetic analysis (Bruschi, submitted). Details for examined specimens in Appendix I

Morphometric Analyses:

Morphometric features of adult males were measured using a Mitutoyo Absolute digital ccaliper CD-6" CSX to the nearest 0.1 mm, except for finger and toe discs that we measured under a stereomicroscope (Zeiss Stemi 2000) coupled to an ocular micrometer.

Seven morphometric traits follow Heyer et al. (1990): snout-vent length (SVL), hand length (HAL), forearm length (FAL), upper arm length (UAL), thigh length (THL), tarsus length (TAL), and foot length (FL). Six morphometric traits follow Cei (1980): head length (HL) (from corner of the mouth to snout tip), head width (HW), eye diameter (ED), internasal distance (IND), tibia length (TL) (=shank length), and tympanum diameter (TD). Five morphometric traits follow Napoli (2005): eye-nostril distance (END), disc diameter of third finger (3FD), fourth finger (4FD), fourth toe (4TD), and fifth toe (5TD). The axilla-groin lengths (AGL) were measured according to Clemente-Carvalho et al. (2012).

We also measured three specimens of the new species (called P. cf. hypochondrialis in Bruschi et al. 2013) from Chapada dos Guimarães (MT); six specimens of P. azurea from Bela Vista, state of Mato Grosso do Sul (MS) (our nearest sample from its type locality); 31 specimens of P. nordestina from Alagoinhas, state of Bahia (BA), Areia Branca and Laranjeiras (both state of Sergipe - SE); six specimens of P. rohdei from state of Rio de Janeiro (Itaguaí and Seropédica); 37 specimens of P. hypochondrialis from states of Pará and Amapá (our nearest sample from its type-locality), and 50 specimens from the states of: Minas Gerais (Uberlândia, Araguari and Ituiutaba), Goias (Guarani de Goiás, Uruaçu, Pirenópolis, Padre Bernardo, Chapada dos Veadeiros, and Mineiros), Mato Grosso (Barra do Garças), and Tocantins (Palmas and Paranã) (Appendix I). We treated separately P. hypochondrialis from Pará and Amapá as “P. hypochondrialis North” because the proximity to Suriname (type locality of P. hypochondrialis) and the specimens from Minas Gerais, Goiás, Mato Grosso, and Tocantins as “P. hypochondrialis South” (according to clades recovered in a forth coming phylogeny; Bruschi et al., submitted.).

Acoustic Analysis:

Vocalizations from Pontal do Araguaia (MT) 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 a sampling rate of 44.1□48.0 kHz and a resolution of 16 bits.

Fifty four advertisement calls from seven males of the new species were recorded between 20:40 to 03:00h on three field expeditions (12□16 February 2010; 06 □ 10 January 2014, and 01 □ 05 December 2014) with an air temperature ranging from 24 to 26°C and water from 26 to 31 °C. Voucher specimens for call recordings are: AAG-UFU 3444–45, 3449 and 4877–4878.

Sixty three calls from four males of P. azurea were recorded by T.R. Carvalho between 20:40–23:14h on 18 December 2010 in the municipality of Bela Vista (state of Mato Grosso do Sul) (around type locality) with air temperature from 26 to 31°C and water from 27 to 32 °C; vouchers: AAG-UFU 0148–0151.

Forty four calls from two males of P. nordestina were recorded between 19:35–22:07h on 05 – 06 May 2011 from Areia Branca and Muruim (SE), by D.P. Bruschi and M.A. Passos (air temperature = 27°C). Sound files are deposited in Fonoteca Neotropical Jacques Vielliard (FNJV) at UNICAMP.

One hundred and eighty calls of 33 males of P. hypochondrialis in our data basis (AAG record file) also were analyzed. Samples are from Mato Grosso (Barra do Garças); DF (Federal District) Brasília; Minas Gerais (Araguari, and Uberlândia); Goiás (Padre Bernardo, Pirenopolis and Uruaçu). The temperature of these records ranged from 18 to 26°C to air and 20 to 29 °C to water. Call vouchers of P. hypochondrialis are from Uruaçu: AAG-UFU 0991; Barra do Garças: AAG-UFU 1082–83; and Araguari: AAG-UFU 4832.

Call descriptions and most of acoustic terminology follow Duellman and Trueb (1994). The calls of some species of the P. hypochondrialis group sometimes have some few isolated weak and relatively isolated pulses at the end (more common) or at the beginning (unusual) (see results section). To compare call traits equivalently among specimens/species, we just used the main (stronger "core" portion) group of pulses of each call. Other traits, such as pulse duration and pulses per second, we also measured taking into account only the "core" portion (further details in Table 1). Means and standard deviations were calculated considering mean values of individual males. 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 = 270 Hz, brightness = 50%, contrast = 50%, overlap = 85% (locked), color map = “Cool”, DFT size = 1024 samples (locked), grid spacing (spectral resolution) = 46.9 Hz. We analyzed temporal traits in oscillograms and spectral traits in spectrograms; we measured the peak of dominant frequency through the ‘Peak Frequency’ function. We generate sound figures through package Seewave v. 1.6 package (Sueur et al. 2008), R (version 3.0.3) platform (R Core Team 2014). Seewave settings for the spectrograms were: Hanning window, 85% overlap, and 256 points resolution (FFT). Analyzed sound files are listed in Appendix II.

TABLE 1 – Acoustic terminology employed for P. hypochondrialis species group.

Acoustic traits	Definition
Call duration (ms)	Time from beginning to end of one call, including isolated pulses;
Intercall interval (ms)	Time from the end of one call to beginning of the next
Calls rate per minute	Numbers of calls recorded multiplied by 60s divided by time total (from begin to first call recorded into the end the last call recorded)
Pulses/call	Number of pulses into the entire call.
Pulse duration (ms)	Mean duration of all pulses in core. Ranges given from original data and not by individual average.
Interpulse interval within core (ms)	Mean interval between core pulses. Ranges given from original data and not by individual average).
Core duration (ms)	Time from beginning to end of one core. = Isolated pulses, if present, disregarded.
Pulses/core	Number of pulses into the core.
Duration of isolated pulses (ms)	Mean of duration of the isolated pulses. Range, which was given from original data and not by individual average).
Number of isolated pulses	Number of isolated pulses into the calls (summed when in more than a group).
Interval between core and isolated	Time from end to core to the beginning of first isolated pulse.

pulse (ms)	
Pulse rate per second	Total number of pulses per core/core duration
Minimum of dom. freq. (Hz)	Minimum frequency within the most energetic call band.
Maximum of dom. freq. (Hz)	Maximum frequency within the most energetic call band.
Peak of dominant frequency	Frequency with greatest energy within the most energetic call band (function “Peak Frequency” of Raven)
Fundamental frequency	Coincident to dominant frequency.

Statistical Analysis:

Considering the morphological and acoustic (multivariate) datasets, we seek for discrimination between populations/species by applying two functions: (1) "randomForest" (RF) (radomForest package; Liaw and Wiener 2002) and (2) "dapc" (package -Jombart 2008; Jombart et al. 2010). RandomForest (RF) algorithm constructs many (e.g. 500) classification trees using bootstrap samples of the data (each split using the best predictors randomly chosen at each node) than 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 Chupetlovska-Anastasova 2009) and displayed graphically with the "proximity.plot" function of the rfPermute package (Archer 2014). The classic Discriminant Analysis (DA) depends on multivariate normality (Pohar et al. 2004) and on a larger number of objects than variables. The multivariated normality of the original data was evaluated through the function "mardiaTest" (MVN package; Korkmaz et al 2014 - results details not shown). The “dapc” performs analyses on the Principal Component scores and not on the data directly (Jombart 2008; Jombart et al. 2010). The application of a DA on a few axes (preserving about 95% of the variance) of a Principal Component Analysis, as performed by "dapc", improves the imbalance between objects and traits (Jombart et al. 2010). Despite of the lack of normality in our both datasets, the results of "DAPC" are also presented within an exploratory context to assess the congruence between it and "randomForest" discriminations. The directly or indirectly packages related the application of both discriminant functions run in R (R Development Core Team 2014).

For the multivariate analysis and statistical tests we used all the morphometric features detailed above. To acoustic analysis we used call traits common to all species: call duration, number of pulses, pulse duration, interpulse interval, core duration, pulses/core, pulses/second, minimum of dominant frequency, maximum of dominant frequency and peak of dominant frequency. Considering that all multivariate analysis were highly concordant in species discrimination (see Results section) we present the RF classification results in tables and “dapc” in scatter plots.

The acoustic and morphometric traits were tested for statistical significance of the differences among population/species through the Exact Wilcoxon Mann Whitney Rank Sum Test using the package coin (Resampling Statistics models) (Hothorn et al. 2008; function wilcox_test) in R. As these tests were done between species/populations pairs, the significance levels (“p”) were adjusted considering the number of pairings through the method of "Holm" (p.adust function in R); significance was assumed when $P \leq 0.05$.

Molecular Divergences

Genomic DNA was extracted from liver or muscle tissue using the TNES method, as applied by Bruschi et al. (2012). The tissues are stored at -70°C in the tissue bank of the Departamento de Biologia Estrutural e Funcional of the Universidade Estadual de Campinas (UNICAMP), in São Paulo state, Brazil. The mitochondrial 12S rDNA, tRNA-Val, and 16S ribosomal genes were amplified using the primers MVZ 59(L), MVZ 50(H), 12L13, Titus I (H), Hedges16L2a, Hedges16H10, 16Sar-L, and 16Sbr-H (for primer sequences, see Goebel et al., 1999). The amplified PCR products were purified using Exonuclease I (10 units) and SAP (1 unit), with a 45-min incubation at 37°C and a 10-min denaturation at 85ºC, then used directly as templates for sequencing in an automatic ABI/Prism DNA sequencer (Applied Biosystems, Foster City, CA, USA) with the BigDye Terminator kit (Applied Biosystems, Foster City, CA, USA), as recommended by the manufacturer. The DNA samples were sequenced bidirectionally and edited in the software CodonCode Aligner 3.7.1 (Codon Code Corporation, Dedham, MA, USA).

Three paratopotypes specimens of the Phyllomedusa sp. n. from Pontal do Araguaia (ZUEC 21657, 21659 and 21660) was sequenced, and the data matrix was completed with sequences at least one individual of the Phyllomedusa hypochondrialis group available in GenBank database. We aligned sequences using CLUSTALW (Thompson et al. 1994) implemented in CodonCode Aligner 4.0. We used three different gap penalties (5, 10, and 15) for each gene to identify potential sites of ambiguous homology (Gatesy et al. 1993). Gap length was kept constant (0.20) and all other parameters were set at default settings.

Uncorrected genetic distances (p-distances) among the sequences of the species of the P. hypochondrialis group were calculated using the maximum composite likelihood model (Tamura et al. 2004) implemented in MEGA5 (Tamura et al. 2011). Gaps and missing data were eliminated in this analysis and all parameters were left as in the default settings.

4 SPECIES DESCRIPTION

Phyllomedusa sp. nov.

(Figs. 1□2, Tables 2–3)

Phyllomedusa cf. hypochondrialis: (Bruschi et al. 2013)

Primary reference specimen (holotype candidate).—AAG-UFU 3444, adult male (Figs. 1–2), collected in the municipality of Pontal do Araguaia (15°57'31.41"S, 52°20'W, 418 m above sea), state of Mato Grosso, Brazil, on 8 January 2014 by A. A. Giaretta, I. A. Haga, F. S. Andrade.

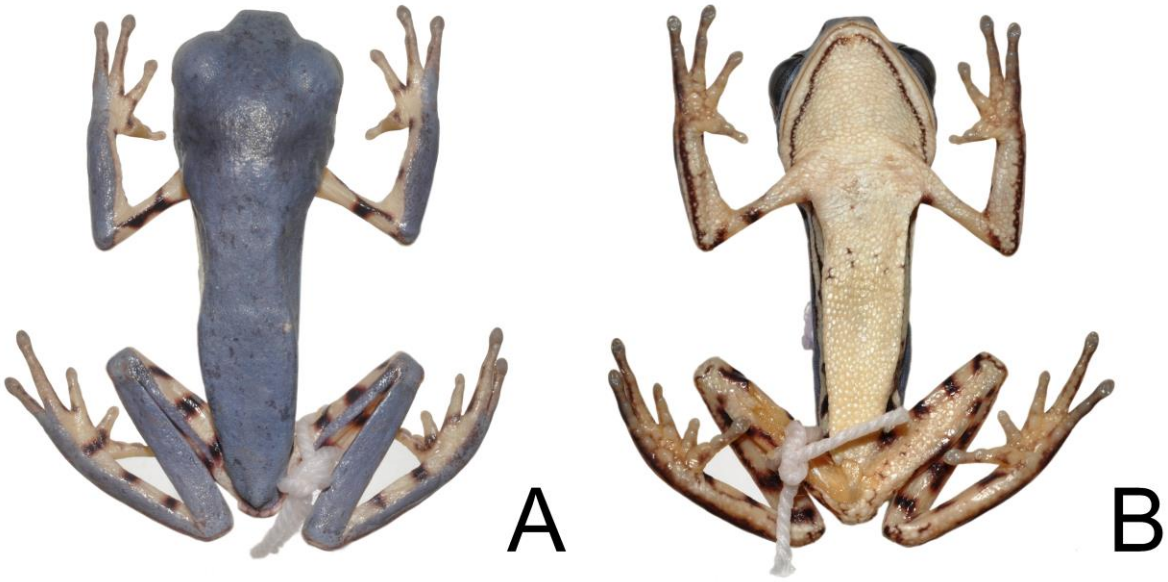

FIG. 1—`Phyllomedusa` sp. nov., adult male, primary reference specimen (AAG-UFU 3444). Dorsal (A) and ventral (B) views. SVL = 30.6 mm

Secondary reference specimens (paratopotypes candidates).—Seventeen adult males (Fig. 3), all collected at the type locality: AAG-UFU 4877–4882 collected in February 2010 by A. A. Giaretta; AAG-UFU 3442–3443, 3445–3449, collected in January 2014 by A. A. Giaretta, I. A. Haga, F. S. de Andrade, and ZUEC 21657–60 collected in December 2014 by A. A. Giaretta and C. S. Bernardes.

Diagnosis.—Phyllomedusa sp. nov. is assigned to the genus Phyllomedusa (sensu Faivovich 2005) by possessing toe I longer than toe II. The new species can be accommodated in the P. hypochondrialis species group (sensu Caramaschi 2006) by the following set of characters: (1) small body size (30.3–33.8 mm); (2) parotoid glands indistinct; (3) smooth skin on dorsum and granulose on ventral region; 4) fingers and toes long and slender with terminal discs poorly developed; (5) grasping finger I and toe I (opposable to the others). The new species differs from other species of the P. hypochondrialis species group by: (1) lacking the reticulate color pattern on flanks (2) smaller SVL (30–34 mm in adult males); (3) core portion of call with 5 to 7 pulses and (4) higher dominant frequency (2200 – 3400 Hz).

Comparison with highland species of the P. hypochondrialis group.—Phyllomedusa sp. nov. is promptly distinguished from P. ayeaye, P. araguari, P. centralis, P. oreades, P. megacephala, and P. rustica by lacking the reticulate color pattern on flanks and by having smaller head width (9.2–10.1 vs. 11.0–14.5, combined values) (Bokermann 1965; Lutz 1966; Brandão 2002; Caramaschi 2006; Giaretta et al. 2007; Bruschi et al. 2014).

Phyllomedusa sp. nov. can be diagnosed from P. ayeaye, P. araguari, P. centralis, P. megacephala, and P. rustica (33.9–42 mm, combined adult male SVL) by its smaller SVL (30.3–33.8) (Bokermann 1965; Lutz 1966; Brandão 2002; Caramaschi 2006; Giaretta et al.

2007; Bruschi et al. 2014). From P. rohdei, the new species can be distinguished by its smaller SVL (30.3–33.8 vs. 35.2–40.9) ($P<0.05$). Phyllomedusa sp. nov. differs from P. oreades, P. ayeaye, P. araguari, P. megacephala, and P. rustica (8.5–14.0 mm, combined adult male HL) by smaller head length (6.1–8.0) . The new species also promptly distinguished from P. rustica by lacking of slightly reticulated pattern on the palpebral membrane and throat region (pattern unique of P. rustica) (Bruschi et al. 2014).

The new species can be diagnosed from P. centralis, P. oreades, P. araguari and P. megacephala (1019–1808 Hz, combined values of dominant frequency) by having higher dominant frequency (2239–3316 Hz) (Giaretta et al. 2007; Brandão et al. 2009; Brandão and Álvarez 2009). From P. araguari and P. rohdei (1–5, combined values) by having a larger number of pulses per call (5–8) (Wogel et al. 2004; Giaretta et al. 2007). From P. centralis and P. oreades. (3–5 pulses, combined values), the new species differs by having more pulses in core portion (5–7) (Brandão et al. 2009; Brandão and Álvarez 2009). For comparisons with P. centralis, we only used its call "A" (Brandão et al. 2009) as we interpreted call "B" as composed by a doble “A” (Haga et al. under review). Phyllomedusa sp. nov. is promptly distinguished from P. megachephala by its shorter note duration (28–63 vs 90–140 ms) (Giaretta et al. 2007). Phyllomedusa araguari also emitted calls as call groups (5–9) while Phyllomedusa sp. nov. release it an isolated calls (Giaretta et al. 2007).

Comparison with the lowland species of the P. hypochondrialis group.— Morphometric comparisons.— Phyllomedusa sp. nov. can be diagnosed from P. palliata (37.7–43.8 mm) by its smaller SVL (30.3–33.8). Also, the new species is smaller than P. nordestina and P. azurea and P. hypochondrialis North and South in SVL ($P <0.05$) (Table 2). Both, randomForest and DAPC substantially discriminate the new species from P. azurea, P. nordestina and both population of P. hypochondrialis (North and South) (Table 4 and Fig.

4). The classification matrix of randomForest indicates an complete discrimination between the new species and the remainder compared species with and noteworthy lower error rate (Table 4).

Acoustic comparisons.—From P. palliata, the new species differs by having a higher dominant frequency (2239–3316 vs. 1580 Hz) and by not having the call emitted as double notes (Köhler and Lötters 1999). The new species can be distinguished from P. nordestina, P. azurea and P. hypochondrialis South by having more pulses per call (5–8 vs. 3–6, combined values, P = 0.05, 0.01, < 0.001, respectively), more pulses per core (5–8 vs. 3–6, combined values, P = 0.05, 0.01, < 0.001, respectively) and peak of dominant frequency (2240–3316 Hz vs. 1781–2250 Hz, combined values, P = 0.05, 0.01, < 0.001, respectively). From P. azurea and P. hypochondrialis South the new species differs by having longer core duration (28–48 ms vs. 18–70 ms, combined values, P = 0.01 and P = 0.012, respectively). From P. hypochondrialis South, the new species can be diagnosed by having: shorter pulse duration (2–18 ms vs. 4–11ms, P = 0.03) and faster call rate (2.2–59.8 vs. 1.3–95.8, P = < 0.001) (Table 3).

RandomForest model (Table 5) resulted in a total discrimination among the Phyllomedusa sp. nov., P. azurea and P. nordestina, and with all specimens classified correctly. The DAPC scatter plot reveled substantial differentiation between P. hypochondrialis South, P. azurea, P. nordestina and new species indicating the complete discrimination between these species (Fig. 4B).

Description of the reference specimen (holotype candidate).—General aspect slender (Fig. 1 A□B); snout truncate in lateral (Fig. 2A) and dorsal view (Fig. 2B) (sensu Cei 1980). Head wider than long; loreal region slightly concave; canthus rostralis rounded;

nostrils small, subcanthal, placed laterofrontally, closer to snout tip than to eyes; internasal distance larger than eye-nostril distance and tympanum diameter but smaller than eye diameter; eyes positioned dorsolaterally; tympanum nearly circular, with annuli undefined at the superior border; tympanum diameter smaller than half eye diameter; supratympanic dermal fold present, beginning on top of tympanum and ending nearly to corner of month; parotoids undistinguished; no vocal slits or external vocal sac; tongue nearly ovoid (sensu Cei 1980) free posteriorly, longer than wide; rudimentary vomerine teeth present, bordering choanae internally; choanae small, positioned laterally to mouth, slight rounded; upper arm thin and forearm robust; no finger webbing; comparative finger length when adpressed I<II<IV<III; finger disc poorly development, smaller than the tympanum diameter; finger I enlarged at the base; nuptial asperity covering most of the dorsal face of finger I, except the tip; palmar callosities poorly developmed (Fig. 2C), subarticular tubercle developed, rounded and distinct from supernumerary tubercles; inner and outer metacarpal tubercles undifferentiated; comparative toe length when adpressed II<III<I<V<IV; plantar callosities poorly developed (Fig. 2D), inner and outer metatarsal tubercles undifferentiated; no toe webbing; subarticular tubercle development, single and rounded; supernumerary tubercles rounded and poorly development; legs slender, thigh length slightly longer than tibia; heel reach the posterior border of tympanum when the leg is adpressed to body; dorsal skin smooth; ventral skin granulated on belly and throat and smooth of the thigh and foot. Thigh damaged ventrally due to tissue sampling.

Measurements of reference specimen (mm and % of SVL in parenthesis).—SVL 30.6, HL 6.6 (21.7), HW 9.6 (31.3), AGL 14.6 (47.8), ED 3.9 (12.6), TD 1.6 (5.4), END 2.1 (7.0), IND 2.8 (9.2), UAL 6.1 (19.9), FAL 6.8 (22.4), HAL 7.2 (23.5), THL 12.9 (42.2), TL

12.6 (41.2), TAL 8.4 (27.4), FL 10.1 (33.1), 3FD 0.8 (2.6), 4FD 0.8 (2.6), 4TD 0.8 (2.6), 5TD 0.9 (2.9).

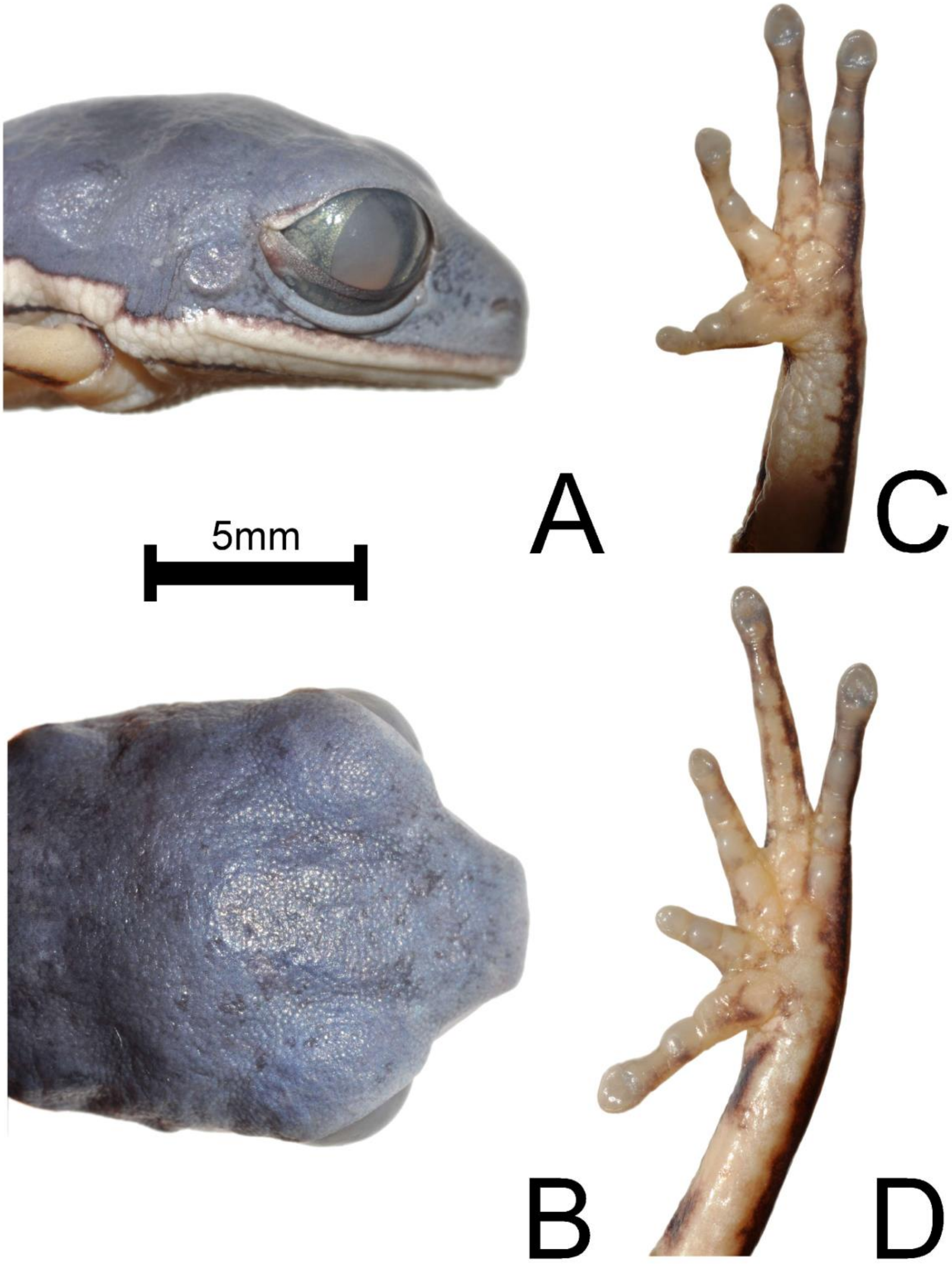

FIG. 2—Phyllomedusa sp. nov., adult male, primary reference specimen (AAG-UFU 3444). (A) Lateral and (B) Dorsal views of head; ventral views of (C) hand and (D) foot.

TABLE 2.—Morphometry of adult males of Phyllomedusa sp. nov. (candidate type series), two populations of P. hypochondrialis (north and south) and of P. nordestina. Values presented in millimeters as mean ± standard deviation (minimum–maximum); n = number of specimens analyzed. See text for the statistical significance of the difference in SVL.

Morphometric traits	Phyllomedusa sp. nov.	P. hypochondrialis North	P. hypochondrialis South	P. nordestina
	n = 17	n = 37	n = 50	n = 31
Snout-vent length	31.7±1.2 (30.3–33.8)	37.1±2.0 (33.6–42.0)	35.4±1.5 (32.5–39.5)	36.2±1.7 (32.9–39.5)
Head length	6.8±0.5 (6.1–8.0)	8.2±0.7 (7.1–9.4)	7.8±0.9 (5.9–9.9)	7.4±0.6 (6.3–8.7)
Head width	9.6±0.3 (9.2–10.1)	11.6±0.7 (10.5–13.0)	11.0±0.5 (10.1–12.3)	11.2±0.6 (9.9–12.4)
Axilla-groin length	14.4±1.3 (11.4–16.9)	18.1±1.7 (15.0–22.2)	16.1±1.5 (13.5–20.1)	17.3±1.6 (14.5–19.9)
Eye diameter	4.0±0.2 (3.7–4.4)	4.5±0.4 (3.6–5.3)	4.4±0.3 (3.8–5.1)	4.2±0.4 (3.4–4.9)
Tympanum diameter	1.6±0.2 (1.3–1.8)	1.8±0.3 (1.4–2.3)	1.7±0.3 (1.1–2.3)	1.8±0.3 (1.2–2.3)
Eye-nostril distance	2.0±0.2 (1.7–2.3)	2.6±0.2 (2.1–3.0)	2.4±0.2 (1.9–2.9)	2.6±0.2 (2.2–3.1)
Internasal distance	2.8±0.2 (2.5–3.2)	3.4±0.2 (3.1–3.9)	3.3±0.3 (2.8–3.9)	3.4±0.3 (2.9–3.9)
Upper arm length	6.9±0.4 (6.1–7.8)	7.6±0.5 (6.6–8.4)	7.3±0.4 (6.6–8.3)	7.3±0.4 (6.5–8.1)
Forearm length	7.4±0.4 (6.5–8.0)	8.7±0.7 (7.5–9.8)	8.1±0.5 (6.7–9.1)	8.3±0.6 (6.9–9.3)

Hand length	7.8±0.4 (7.2–8.6)	9.7±0.6 (8.3–10.7)	9.3±0.4 (8.4–10.3)	9.6±0.7 (8.6–11.2)
Thigh length	13.4±0.6 (12.3–14.4)	16.7±1.0 (14.9–18.7)	14.9±0.7 (13.2–16.3)	15.4±0.7 (13.9–16.6)
Tibia length	13.4±0.5 (12.3–14.1)	16.6±1.1 (14.8–19.3)	15.1±0.7 (13.6–16.5)	15.0±0.6 (13.5–16.0)
Tarsus length	9.0±0.6 (8.1–10.3)	11.5±1.0 (9.9–13.7)	10.5±0.5 (9.5–11.5)	9.9±0.6 (9.1–11.1)
Foot length	10.3±0.4 (9.6–11.1)	12.4±0.8 (10.7–14.1)	12.2±0.5 (11.4–13.3)	11.8±0.7 (10.5–13.1)
Disc diameter of third finger	0.8±0.1 (0.7–1.0)	1.2±0.2 (0.8–1.6)	1.0±0.1 (0.7–1.3)	0.9±0.2 (0.5–1.2)
Disc diameter of fourth finger	0.9±0.1 (0.7–1.0)	1.2±0.2 (0.8–1.6)	1.1±0.1 (0.9–1.4)	0.9±0.2 (0.6–1.3)
Disc diameter of fourth toe	0.9±0.1 (0.7–1.2)	1.2±0.2 (0.9–1.6)	1.1±0.1 (0.8–1.4)	0.9±0.2 (0.6–1.3)
Disc diameter of fifth toe	1.0±0.1 (0.9–1.1)	1.3±0.2 (0.9–1.7)	1.2±0.1 (1.0–1.6)	1.0±0.2 (0.7–1.4)

Color in life.—Dorsal surfaces of forearm, tibia, tarsus and portions of foot and hand leaf or swart green (Fig. 3). A green stripe on dorsal surface of the thigh, which never reaches the final proximal thigh portion (Fig. 3). Flanks, dorsal surface of arms, medial surface of forearm and hidden parts of the legs orange with vertical dark stripes (Fig. 3). The region below axilla light orange with the presence of dark line parallel to body length (Fig. 3B□D). Border of upper lip and upper eyelid with a white line, that on lip line never touching the border of lower eyelid. Dark lines rounding the dorsal surface of the fingers and toes (Fig. 3C–D). In preservative the green areas become bluish gray and the orange parts become light cream or whitish and the dark stripes, upper lip and upper eyelid lines remain unchanged (Fig. 1–2). A fine white line in edge of the mouth which reaches the border of lower eyelid. A fine white line in edge of the mouth that touch the border of lower eyelid (Fig. 2A), a small triangle in the snout tip (Fig. 1B), and the incomplete green strip in the dorsal face of the thigh (Fig. 1A)

Variation.—Size variation in Table 2. The specimens AAG-UFU 3443–3447 and ZUEC 21659 have small white points on dorsal surface on the back. The specimens AAG-UFU 3443–44, 3446–49, 4880 and ZUEC 21657–60 have a well-defined dark stripe bordering the jaw (Fig. 1B). The specimens AAG-UFU 3446, 3448, 4878 and 4882 have a symmetrical small dark spot in lower eyelid, next to the posterior corner of the eyes. The specimen ZUEC 21659 have dark spots in the vocal sac. The specimens 3442–46, 3448–49, 4877, 4879, 4881–82 and ZUEC 21657–59 have a fine white line in the border of upper eyelid surrounded by a fine dark line (Fig.2A).

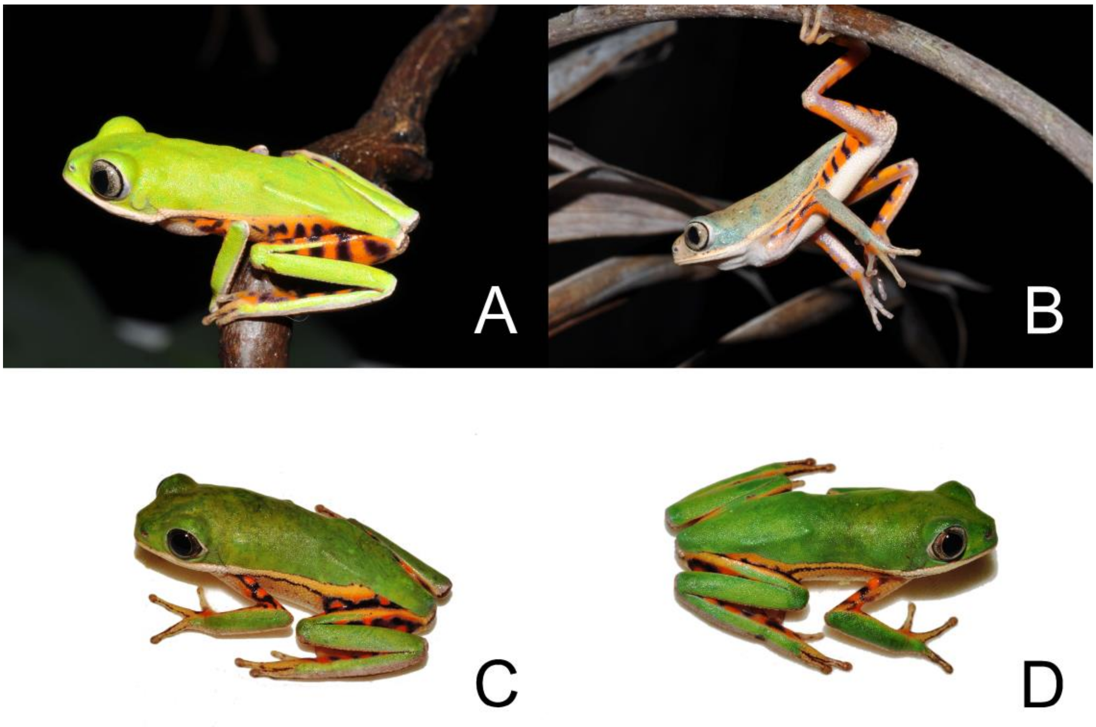

FIG. 3—Secondary reference specimens of Phyllomedusa sp. nov. in life. Specimens from Pontal do Araguaia, state of Mato Grosso, Brazil. Adult males (A) AAG-UFU 4877, SVL = 31.4 mm; (B) AAG-UFU 4878, SVL = 33.6 mm; (C) AAG-UFU 3442, SVL = 32.8 mm and (D) AAG-UFU 3443, SVL = 31.0 mm.

TABLE 3.—Advertisement call traits of three species of Phyllomedusa hypochondrialis group: candidate topotypes Phyllomedusa sp. nov. from the Pontal do Araguaia, state of Mato Grosso, Brazil; P. hypochondrialis South and P. nordestina. Mean+SD (minimum–maximum). n = number of specimens recorded (number of analyzed advertisement calls).

Call traits	Phyllomedusa sp. nov. n = 7 (54)	P. hypochondrialis South n = 33 (180)	P. nordestina n = 2 (44)
Call duration (ms)	41.2±4.9 (28–63)	39.1±8.7 (22–72)	42.3±5.6 (19–67)
Calls per minute	12.2±9.3 (2.2–59.8)	10.1±10.8 (1.3–95.8)	35.0±15.6 (11–46)
Pulses/call	6.0±0.56 (5–8)	4.3±0.4 (3–6)	3.9±0.24 (3–6)
Pulse duration (ms)	5.1±1.3 (2–18)	7.0±1.1 (4–11)	7.4±0.2 (3–16)
Interpulse interval within core (ms)	1.9±0.8 (1–4)	2.1±0.9 (1–5)	1.9±0.1 (1–3)
Core duration (ms)	39.3±5.4 (28–48)	33.2±3.4 (19–61)	23.4±1.3 (17–31)
Pulses/core	6.0±0.5 (5–8)	4.0±0.3 (3–6)	3.1±0.1 (3–4)
Duration of isolated pulses (ms)	3.5±1.1 (2–5)	2.5±3.1 (4–15)	8.6±1.2 (4–14)
Number of isolated pulses	1.0±0.0 (1–1)	1.0±0.1 (1–2)	1.2±0.1 (1–2)
Interval between core and isolated pulse (ms)	6.0±1.4 (5–8)	12.5±3. 6 (5–21)	14.4±3.3 (4–29)

Pulses per second	155±20 (114–206)	121±12.7 (66–160)	132.4±4.6 (96.8–176.5)
Minimum of dominant frequency (Hz)	1445±367 (1044–3029)	1082±209.8 (428–1592)	1388±7 (1133–1618)
Maximum of dominant frequency (Hz)	3489±409 (3088–4907)	3398±713 (2481–5373)	2624±182 (2451–3124)
Peak of dominant frequency (Hz)	2540±308 (2240–3316)	2170±146 (1781–2625)	2052±5(1969–2156)
Air temperature range	25±0.9 (24–26)	22.4±2.1 (18–26)	28.7±0.5(28–29)
Time	20h43min–2h53min	20h05min–03h41min	19h35min–22h07min

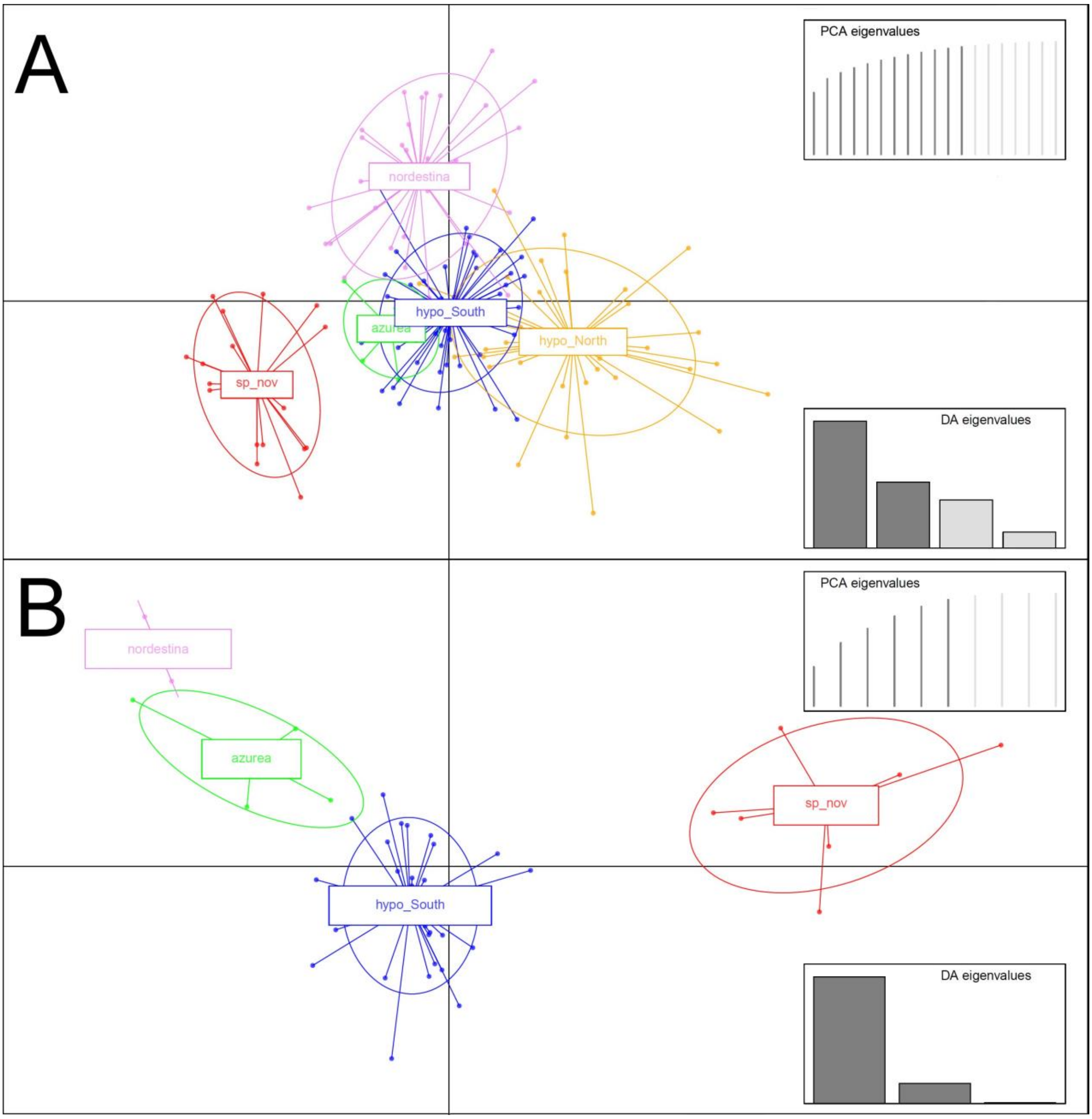

FIG. 4—(A) Scatterplot with the two first axes of the Discriminant Analysis (DAPC) on the first twelve Principal Components on the morphometric dataset of P. azurea; P. nordestina; P. hypochondrialis North; P. hypochondrialis South and Phyllomedusa sp. nov. (B) Scatterplot with the two first axes of the Discriminant Analysis (DAPC) on the first six Principal Components on the acoustic dataset of P. azurea; P. nordestina; P. hypochondrialis South and Phyllomedusa sp. nov. Abbreviations: azurea = P. azurea; nordestina = P. nordestina; sp_nov = Phyllomedusa sp. nov.; hypo_North = P. hypochondrialis North; and hypo_South = P. hypochondrialis South.

Advertisement call.—Seven males record, 54 calls analyzed. Quantitative call traits summarized in Table 3. The advertisement call of Phyllomedusa sp. nov. consists of a pulsed note emitted sporadically. Generally, the calls are composed by: (1) a long group of pulses, the "core" portion (see Material and Methods and Table 1); and (2) rarely, by isolated weak final pulses. The core pulses had a duration of 28 to 48 ms (mean = 39.3; SD = 5.4; n = 54) and 5 to 8 pulses/core (mean = 6.0; SD = 0.5; n = 54). The pulses arrangement in the calls can have the following patterns: core with six pulses without isolated pulse (40.7%; n = 22 calls; n = 7 males); core with five pulses without isolated pulse (33.3%; n = 18 calls; n = 4 males); core with seven pulses without isolated pulse (12.9%; n = 7 calls; n = 4 males); core with six pulses with one isolated pulse (5.6%; n = 3 calls; n = 1 male). Core with seven pulses followed by one isolated pulse and core with eight pulses without isolated pulse had the same frequency (3.9%; n = 2 calls; n = 1 male). Just one isolated pulse was recorded (mean = 1.0; SD = 0; n = 5) which can last from 2 to 5 ms (mean = 3.5; SD = 1.1; n = 5). The interval between the core and isolated pulse (Table 3) varies from 5 to 8 ms (mean = 6.0; SD = 1.4; n = 5) and is only provided in 6 x1 and 7 x 1 arrangements (n = 5 calls). The call duration (see Table 1) lasted from 28 to 63 ms (mean = 41.2, SD = 4.9; n = 54) a call rate of 2.2 to 59.8 calls/minute (mean = 12.2, SD = 9.3, n = 14). The call has 5 to 8 pulses (mean = 6.0, SD = 0.6, n = 398), whose duration varied from 2 to 18 ms (mean = 5.1, SD = 1.3, n = 398), emitted at rates of 114 to 206 pulses/second (mean = 155, SD = 20, n = 54). The peak of dominant frequency varied from 2240–3316 Hz (mean = 2540 Hz, SD = 308; n = 54). The amplitude was generally ascendant in the begging and descendent at the end (n = 33 calls, 89.19%; n = 7 males) or without noticeable modulation in the begging and descendent at the end (n = 4 calls, 10.8%; n = 4 males). The frequency modulation can be ascendant in beginning and descendent at the end (n = 11 calls, 29.7%; n = 5 males); no modulation in beginning and descendent at the end (n = 9 calls, 24.3%; n = 5 males); descendent along the call (n = 8 calls, 21.6%; n = 2 males); without modulation along the call (n = 6 calls, 16.2%; n = 4 males); descendent in beginning and at the end (n = 3 calls, 8.1%; n = 2 males) (Table 3; Fig. 5 A–B).

The advertisement call of Phyllomedusa hypochondrialis [quantitative call traits are summarized in Table 3 (N = 33 males, 180 calls)] consists of a short signal, with sporadically emission. The core duration varied from 19 to 61 ms (mean = 33.2, SD = 3.4; n = 180) and the intervals between core and isolated pulses varies from 5 to 21 ms (mean = 12.5, SD = 3.6; n = 64). The number of pulses per core varied from 3 to 6 pulses (mean= 4.0, SD = 0.3; n = 64) (Fig. 5); with intervals within core pulse from 1 to 5 ms (mean = 2.1, SD = 0.9; n = 64). Pulses were more often arranged in the following patterns: a four pulse group with no isolated pulse (53.3%; n = 96 calls); a four pulse group followed by a single isolated pulse (28.3%; n = 51 calls); a five pulse group with no isolated pulse (7.2%; n= 13 calls). Two other types of pulse arrangements were observed with same frequency: a three pulse group with no isolated pulse (3.88%; n = 7 calls); a three pulse group followed by a single isolated pulse (3.88%; n = 7 calls) and two other types of pulse arrangements were observed with less frequency: a five pulse group with one isolated pulse (2.2%; n= 4 calls) and a four pulses followed by two isolated pulses (1.1%; n = 2 calls). The call duration lasted from 22 to 72 ms (mean = 39.1, SD = 8.7; n = 180 calls), with 3–6 pulses (mean = 4.3, SD = 0.4, n = 721), whose duration varied from 4–11 ms (mean = 7.0 ms, SD = 1.1; n = 721), emitted at rates of 66–160 pulses/second (mean= 121, SD = 12.7; n = 721). The peak of dominant frequency varied from 1781 to 2625 (mean = 2170, SD = 146; n= 180) (Table 3; Fig. 6 A–B).

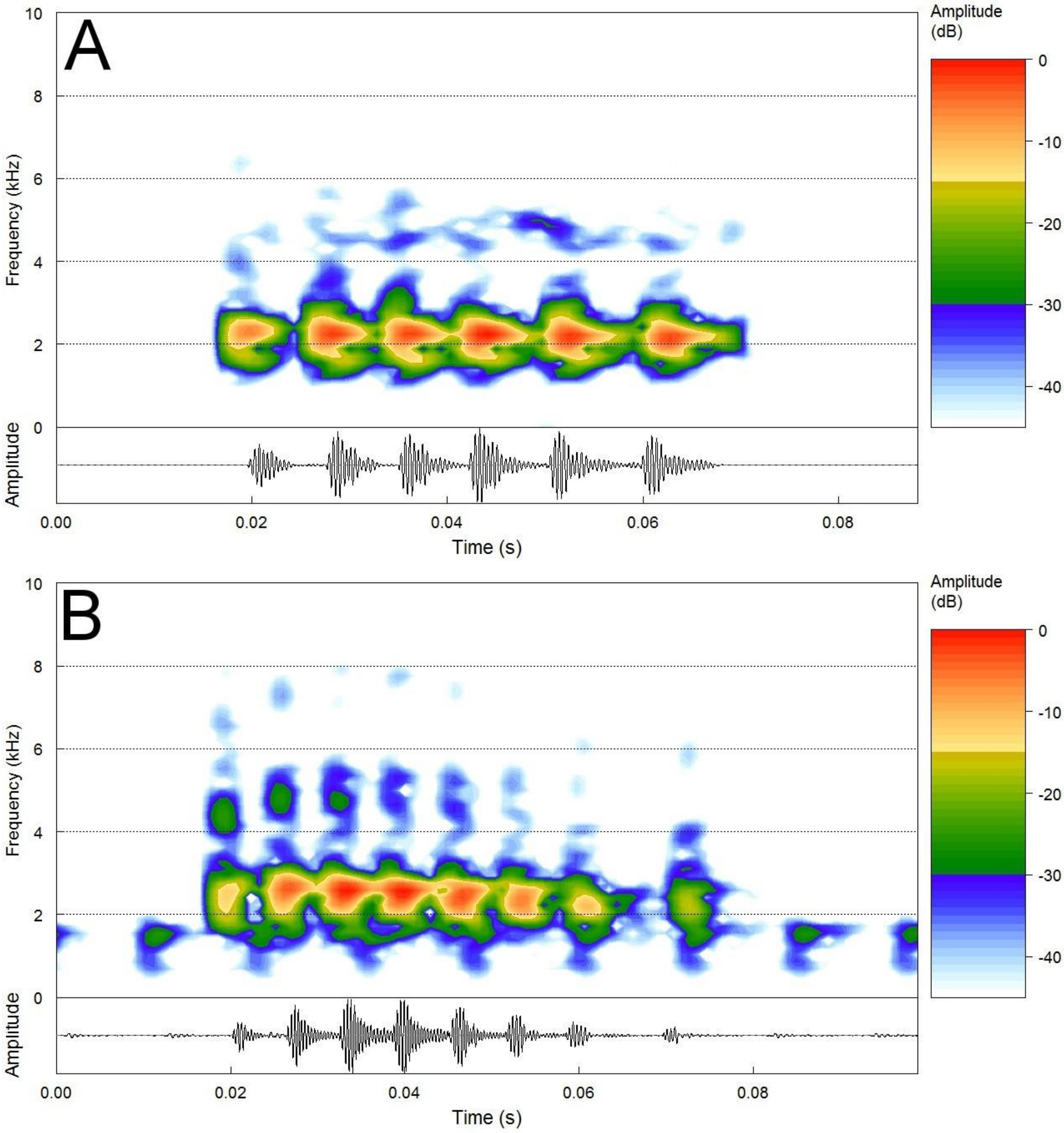

FIG. 5—Audiospectrograms (above) and corresponding oscillograms (below) of the advertisement call of `Phyllomedusa` sp. nov. from Pontal do Araguaia, state of Mato Grosso, Brazil. (A) Sound file: Phyllom_PontAraguMT1aFSA_AAGb; recorded on 07 Jan 2014: temperature of air 25 ºC and water 31 ºC. Voucher recording (AAG-UFU 3449); (B) Sound file: Phyllom_nordestPonAraguMT1cAAGmt; recorded on 15 Feb 2010 at 22:30h: temperature of air 24 ºC and water 26 ºC. Unvouchered recording. Evidencing the last pulse isolated of core (at 0.07 s approximately).

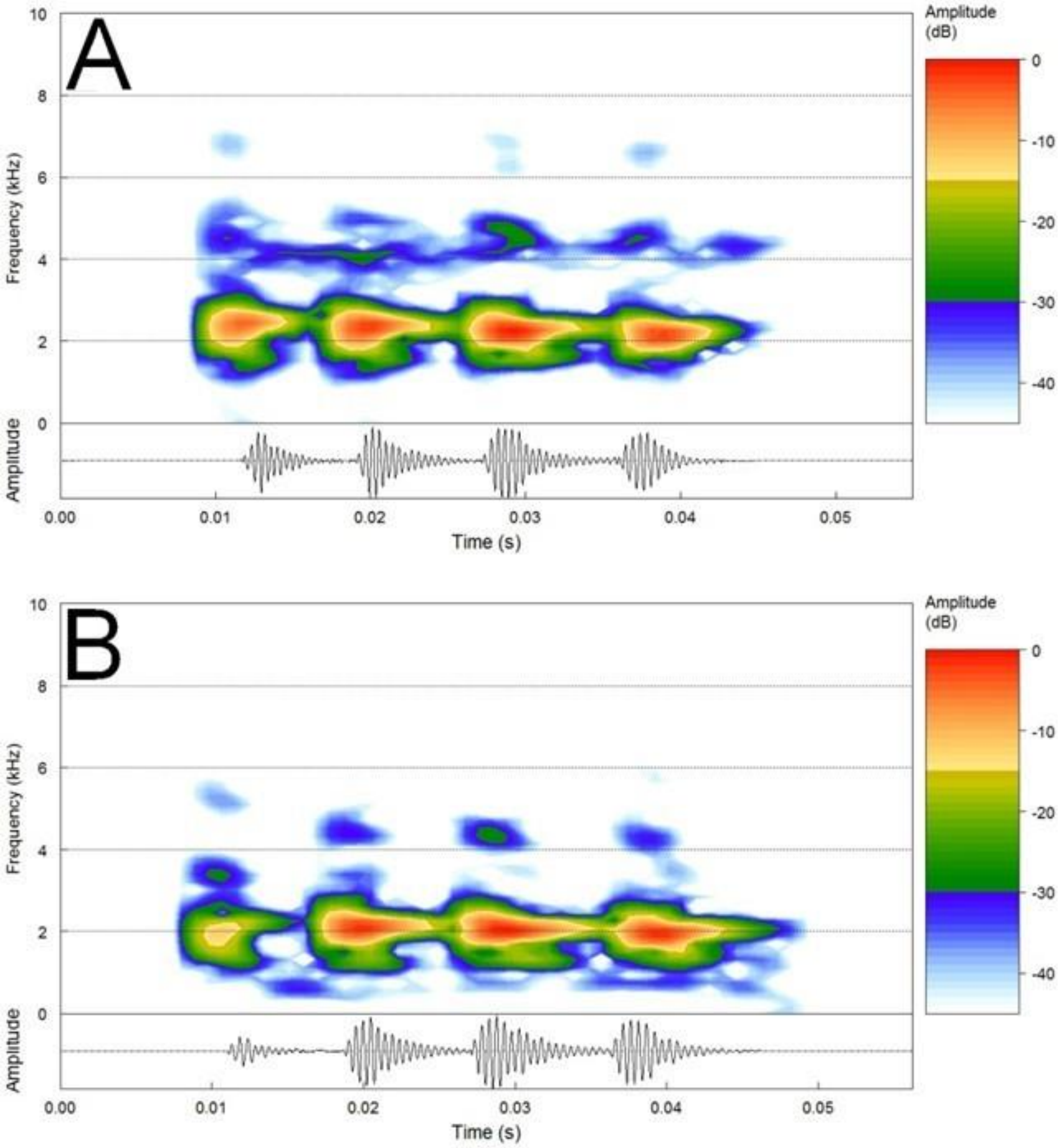

FIG. 6—Audiospectrograms (above) and corresponding oscillograms (below) of the advertisement call of Phyllomedusa hypochondrialis from (A) municipality of Barra do Garças, state of Mato Grosso, Brazil. Sound file: Phyllom_hypochBGarcasMT2bAAGmt; recorded on 19 Feb 2012, at 23:39h; temperature of air 22 ºC and water 25 ºC. Voucher recording (AAG-UFU 1083). (B) municipality of Uberlândia, state of Minas Gerais, Brazil. Sound file: Phyllom_hypochiUberlMG9aAAGm671; recorded on 14 Out 201; temperature of air 21 ºC and water 25 ºC.

TABLE 4.—RandomForest classification matrix for four species of Phyllomedusa hypochondrialis group based on morphometric data. Settings: number of tree permutations = 500; number of traits tried at each split = 4; error rate = 15.71%.

	Phyllomedusa sp. nov.	P. azurea	P. hypochondrialis North	P. hypochondrialis South	P. nordestina	class.error
Phyllomedusa sp. nov.	18	0	0	0	0	0.0
P. azurea	0	4	1	0	1	0.3
P. hypochondrialis North	0	0	28	8	1	0.2
P. hypochondrialis South	0	0	3	44	2	0.2
P. nordestina	0	0	1	5	24	0.2

TABLE 5.—RandomForest classification matrix for four species of Phyllomedusa hypochondrialis group based on acoustic data. Settings: number of tree permutations = 500; number of traits tried at each split = 3; error rate = 10.9%.

	Phyllomedusa sp. nov.	P. azurea	P. hypochondrialis South	P. nordestina	class.error
Phyllomedusa sp. nov.	7	0	0	0	0.0
P. azurea	0	2	2	0	0.5
P. hypochondrialis South	0	1	32	0	0.03
P. nordestina	0	1	1	0	1.0

Genetic divergence analyses.—The genetic distances between Phyllomedusa sp. n. and other species of the P. hypochondrialis group ranged from 6% to 13%; within the lowland species the genetic divergence levels varied from 6% to 10% (Table 6).

TABLE 6.—Uncorrected pairwise p-distances between the 16S mitochondrial sequences of some species of the *Phyllomedusa hypochondrialis* group to emphasise the differences between the new species and the other species with available comparative data.

Species	1	2	3	4	5	6	7	8	9	10
Phyllomedusa sp. n.	-									
P. paliatta	0.10									
P. nordestina	0.08	0.11								
P. azurea	0.10	0.12	0.09							
P. hypochondrialis	0.06	0.10	0.08	0.10						
P. centralis	0.11	0.13	0.11	0.11	0.11					
P. ayeaye	0.11	0.12	0.10	0.10	0.10	0.02				
P. oreades	0.11	0.13	0.11	0.10	0.10	0.01	0.02			
P. megacephala	0.10	0.11	0.10	0.09	0.09	0.07	0.06	0.07		
P. rustica	0.10	0.11	0.11	0.10	0.10	0.06	0.06	0.06	0.05	
P. rhodei	0.11	0.12	0.11	0.10	0.10	0.07	0.06	0.07	0.05	0.06

Distribution.— *Phyllomedusa* sp. nov. is known from the type locality (municipality of Pontal do Araguaia, Mato Grosso, Brazil).

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4.1.7 APPENDICES

APPENDIX I– Additional examined specimens for morphological analyses

Phyllomedusa hypochondrialis – BRASIL:PARÁ: Belterra (ZUEC 16605, 16504–09); Prainha (ZUEC 16511, 16561, 16520, 16515, 16529–30); Monte Alegre (ZUEC 19916, 19938, 19940, 19944); Alenquer (ZUEC 19917, 19920, 19923, 19927, 19930, 19932); Oriximiná (ZUEC 19918, 19922, 19926, 19928); Óbidos (ZUEC 19921,19933, 19943, 19945). AMAPÀ: Laranjal do Jari: (ZUEC 16550–51, 16553, 16555, 16559, 16562); MATO GROSSO: Barra do Garças (ZUEC 21650, AAG-UFU 3489–94,1078–84); GOIÁS: Guarani de Goiás (AAG-UFU 1963–64); Uruaçu (AAG-UFU 0991–93, 0996–99); Pirenópolis (AAG-UFU 0331, 0334); Padre Bernardo (AAG-UFU 0117–18); Chapada dos Veadeiros (AAG-UFU 1333); Mineiro (AAG-UFU 3410); TOCANTINS: Paranã (AAG-UFU 2827–30); Palmas (AAG-UFU 2779, 2817); MINAS GERAIS: Uberlândia (AAG-UFU 2311, 2313, 2315, 2299, 3256); Araguari (1276, 3101, 3116, 4573, 4689, 4936–37, 4832, 4834); Ituiutaba (AAG-UFU 0455);

Phyllomedusa azurea – BRASIL: MATO GROSSO DO SUL: Bela Vista (AAG-UFU 0148–53);

Phyllomedusa nordestina – BRASIL: BAHIA: Alagoinhas (ZUEC 18623–26); SERGIPE: Areia Branca (ZUEC 19882–85, 19887–94, 19898–99, 19901–02, 19906–07, 19909, 19911); Laranjeiras (ZUEC 19895, 19897, 19900, 19903, 19908,19912, 19913).

Phyllomedusa rohdei– BRASIL: RIO DE JANEIRO: Itaguaí (ZUEC 1223–24, 5229–30, 7716); Seropédica (ZUEC 16130)

APPENDIX II – Analyzed sound file calls (*.wav); all files from the AAG collection (UFU) or from Fonoteca (N=2 males/ 44calls) Neotropical Jacques Vielliard (FNJV) (UNICAMP).

Phyllom_azurAraguariMG1bAAGb.WAV
Phyllom_azurAraguariMG1cAAGb.WAV
Phyllom_azurAraguariMG2aTRC_LM_AAGmt
Phyllom_azurAraguariMG3aTRC_LMmt
Phyllom_azurAraguariMG4aTRC_LMmt
Phyllom_azurAraguariMG5aTRC_LMmt.wav
Phyllom_azurAraguariMG6aTRC_LMmt.wav
Phyllom_azurAraguariMG6bTRC_LMmt.wav
Phyllom_azurAraguariMG7aTRC_LMmt.wav
Phyllom_azurAraguariMG7bTRC_LMmt.wav
Phyllom_azurAraguariMG8aTRC_LMmt.wav
Phyllom_azurAraguariMG9aAAGm.wav
Phyllom_azurAraguariMG10aAAGm.wav
Phyllom_azurAraguariMG10bAAGm.wav
Phyllom_azurBarraGarcasMT1aAAGm.wav
Phyllom_azurBarraGarcasMT2aAAGmt.wav
Phyllom_azurBarraGarcasMT2bAAGmt.wav
Phyllom_azurBarraGarcasMT2cAAGmt.wav
Phyllom_azurBarraGarcasMT3aAAGm671.wav
Phyllom_azurBarraGarcasMT4aAAGm671.wav
Phyllom_azurBarraGarcasMT4bAAGm671.wav
Phyllom_azurBarraGarcasMT5aCSB_AAGm671.wav
Phyllom_azurBarraGarcasMT6aFSA_AAGb.wav
Phyllom_hypochBrasiliaDF1aAAGm671.wav
Phyllom_hypochBrasiliaDF1bAAGm671.wav
Phyllom_hypochBrasiliaDF1cAAGm671.wav
Phyllom_azurItuiutMG1aAAGmt.wav
Phyllom_azurPadBernarGO1aAAGm.wav
Phyllom_azurPadBernarGO1bAAGm.wav
Phyllom_azurPirenopGO1aAAGm.wav
Phyllom_azurPirenopGO2aAAGm.wav
Phyllom_azurPirenopGO3aAAGm.wav
Phyllom_azurUberlMG1aAAGm.wav
Phyllom_azurUberlMG1bAAGm.wav
Phyllom_azurUberlMG2aAAGm.wav
Phyllom_azurUberlMG2bAAGm.wav

Phyllom_azurUberlMG3aAAGm.wav
Phyllom_azurUberlMG4aAAGm.wav
Phyllom_azurUberlMG4bAAGm.wav
Phyllom_azurUberlMG5aAAGm.wav
Phyllom_azurUberlMG6aLM_AAGmt.wav
Phyllom_azurUberlMG7aAAGm671.wav
Phyllom_azurUberlMG8aAAGm671.wav
Phyllom_azurUberlMG8bAAGm671.wav
Phyllom_azurUberlMG9aAAGm671.wav
Phyllom_azurUberlMG10aAAGm671.wav
Phyllom_hypochUruacuGO1aAAGm.wav
Phyllom_hypochUruacuGO1bAAGm.wav
Phyllom_hypochUruacuGO2aAAGm.wav
Phyllom_hypochUruacuGO2bAAGm.wav
Phyllom_hypochUruacuGO3aAAGm.wav
Phyllom_hypochUruacuGO4aAAGm.wav
Phyllom_hypochUruacuGO5aAAGm.wav
Phyllom_hypochUruacuGO6aAAGm.wav
Phyllom_hypochUruacuGO6bAAGm.wav
Phyllom_azurBelVistMS1aTRC_AAGmt.wav
Phyllom_azurBelVistMS1bTRC_AAGmt.wav
Phyllom_azurBelVistMS1cTRC_AAGmt.wav
Phyllom_azurBelVistMS2aTRC_AAGmt.wav
Phyllom_azurBelVistMS2bTRC_AAGmt.wav
Phyllom_azurBelVistMS2cTRC_AAGmt.wav
Phyllom_azurBelVistMS2dTRC_AAGmt.wav
Phyllom_azurBelVistMS3aTRC_AAGmt.wav
Phyllom_azurBelVistMS4aTRC_AAGmt.wav
FNJV 12243
FNJV 12244
FNJV 12245
FNJV 12247
Phyllom_nordestPonAraguMT1aAAGmt.wav
Phyllom_nordestPonAraguMT1bAAGmt.wav
Phyllom_nordestPonAraguMT1cAAGmt.wav
Phyllom_nordestPonAraguMT2aAAGmt.wav
Phyllom_nordestPonAraguMT2bAAGmt.wav
Phyllom_nordestPonAraguMT2cAAGmt.wav
Phyllom_nordestPonAraguMT2dAAGmt.wav
Phyllom_nordestPontalAraguMT3aAAGm671.wav
Phyllom_nordestPontalAraguMT3bAAGm671.wav
Phyllom_nordestPontalAraguMT3cAAGm671.wav
Phyllom_nordestPontalAraguMT4aAAGm671.wav
Phyllom_nordestPontalAraguMT4bAAGm671.wav
Phyllom_nordestPontalAraguMT4cAAGm671.wav

Phyllom_nordestPontaldoAragMT5aIAH_AAGmt.wav

Phyllom_nordestPontaldoAragMT5bIAH_AAGmt.wav

Phyllom_nordestPontaldoAragMT5cIAH_AAGmt.wav

Phyllom_nordestPontaldoAragMT5dIAH_AAGmt.wav

Phyllom_nordestPontaldoAragMT5eIAH_AAGmt.wav

Phyllom_nordestPontalAraguMT7aAAGm671.wav

Phyllom_nordestPontalAraguMT8aAAGm671.wav

Phyllom_nordestPontalAraguMT9aAAGm671.wav

Phyllom_nordestPontalAraguMT10aAAGm671.wav

5 CONCLUSÕES GERAIS

Nossa hipótese de ocorrência de uma espécie nova do grupo de Phyllomedusa hypochondrialis no município de Pontal do Araguaia (MT) é sustentada por evidências morfométricas, acústicas e moleculares. Por fim, os resultados do presente estudo corroboraram a relevância da "taxonomia integrativa" como uma ferramenta de alto potencial para descrever e catalogar as espécies ainda desconhecidas pela ciência, além de contribuir para resolver os perdurantes problemas taxonômicos da literatura e de aprimorar os conhecimentos sobre a ainda subestimada biodiversidade da anurofauna brasileira.

6 APÊNDICE

6.1 Manuscrito II

Advertisement and territorial calls of Phyllomedusa azurea Cope, 1862 (Anura, Hylidae, Phyllomedusinae) from Brazil-Paraguay border, southwestern state of Mato Grosso do Sul

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Running title: Vocalizations of Phyllomedusa azurea

Cited references: 30

Figures: 2

Zootaxa section: Chordata; Vertebrata; Amphibia; Hylidae

Advertisement and territorial calls of Phyllomedusa azurea Cope, 1862 (Anura, Hylidae, Phyllomedusinae) from Brazil-Paraguay border, southwestern state of Mato Grosso do Sul

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Abstract

We describe the advertisement and territorial calls of Phyllomedusa azurea from a site nearer to P. azurea's type locality, making intraspecific comparisons with previously available calls from Bolivia and Argentina, as well as interspecific comparisons with species assigned to the P. hypochondrialis group. Considering the similarities among the calls of P. azurea, P. hypochondrialis, and P. nordestina, acoustic traits should no longer be employed, at least solely, as reliable diagnostic characters among these species.

Keywords

Amphibia, Bioacoustics, Monkey frog, Phyllomedusa hypochondrialis group, Taxonomy.

1 Introduction

The leaf frogs, genus Phyllomedusa (Phyllomedusinae), are represented by 31 species distributed from Panama to northern Argentina and Uruguay (Frost 2015). According to Faivovich et al. (2010), most species are assigned to four monophyletic groupings: the Phyllomedusa burmeisteri, Phyllomedusa hypochondrialis, Phyllomedusa perinesos, and Phyllomedusa tarsius groups. The P. hypochondrialis species group currently contains ten species associated to two well-supported clades recovered by Faivovich et al. (2010) and Bruschi et al. (2013). One group contains P. rohdei Mertens and the five other highland species: P. ayeaye (B. Lutz), P. centralis Bokermann, P. megacephala (Miranda-Ribeiro), P. oreades Brandão, and P. rustica Bruschi, Lucas, Garcia and Recco-Pimentel. The other group contains lowland species: P. azurea Cope, P. hypochondrialis Daudin, P. nordestina Caramaschi and P. palliata Peters. In spite of the unequivocal identification of these closely related species (P. azurea, P. hypochondrialis, and P. nordestina) being difficult due to morphological similarities, the specific identity of these nominal species were previously addressed by Faivovich et al. (2010) and Bruschi et al. (2013), the latter study even proposed distribution limits for these species.

Cope (1862) did not designate any type specimen in the original description of P. azurea. Cochran (1961) referred to a specimen from Paraguay as a syntype of P. azurea, and Caramaschi (2006) considered "Paraguay" as a valid restriction for its type locality, providing morphometric and morphological data for P. azurea from Bolivia, Brazil, Paraguay, and Argentina. Bruschi et al. (2013) stated that P. azurea's distribution limits might have been hampered by misidentifications, then restricting its distribution to populations from Argentina, Bolivia, and Paraguay.

Species of the P. hypochondrialis group with advertisement call described are: P. centralis (Brandão et al. 2009), P. hypochondrialis (Pyburn & Glideweel 1971;

Duellman & Pyles 1983; Duellman 1997; Guimarães et al. 2001), P. megacephala (Giaretta et al. 2007), P. nordestina (Vilaça et al. 2011), P. oreades (Brandão & Álvares 2009), P. palliata (Duellman 1978; Köhler & Lötters 1999), and P. rohdei (Wogel et al. 2004). The advertisement call of P. azurea (sensu Bruschi et al. 2013) was described from Resistencia, Chaco Province, Argentina (Barrio 1976), and Puerto Almacén, Santa Cruz Department, Bolivia (De la Riva et al. 1995), originally under the name P. hypochondrialis. However, Brandão et al. (2009) stated that the call described by De la Riva et al. (1995) probably corresponded to the territorial call.

Herein, we describe the advertisement and territorial calls of Phyllomedusa azurea from Brazil-Paraguay border in southwestern state of Mato Grosso do Sul, and make intraspecific and interspecific comparisons with the available acoustic data for congeners of the P. hypochondrialis group.

Materials and Methods

Field work was conducted on 18 December 2010 from 20:40 to 23:15 h, in the municipality of Bela Vista (22º06'32"S, 56º31'16"W, approximately 180 m a.s.l.), state of Mato Grosso do Sul, Brazil. Males were calling perched from 0.5 to 1.0 meters high on herbaceous vegetation surrounding an artificial temporary pool next to a road. Snout-vent length (SVL) from six adult males were measured using a Mitutoyo digimatic caliper CD-6" CSX (0.01 mm) according to Heyer et al. (1990). Specimens were deposited in the Collection of Amphibians of the Museu de Biodiversidade do Cerrado, Universidade Federal de Uberlândia, municipality of Uberlândia, state of Minas Gerais, Brazil: AAG-UFU 0148–0153 (SVL 34.1–38.5 mm); see Appendix 1 for call voucher males.

Acoustic definitions for call, note, and pulse followed McLister et al. (1995); overall acoustic terminology followed Duellman and Trueb (1994); call type definitions followed Toledo et al. (2015). Advertisement call description was based on 62 calls from four males; territorial call description was based on 20 calls from two males. Mean and standard deviation (SD) were obtained from individual mean values. Given that we could not get access to raw data from Phyllomedusa acoustic descriptions to test for normality and homocedasticity, we decided not to apply any statistical test. Therefore, we restricted our acoustic comparisons to the ranges of values.

Calls were recorded with a Sennheiser K6/ME66 directional microphone and a M-audio Microtrack II digital recorder set at a sampling rate of 44.1 kHz and a resolution of 16 bits, and 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 = 270 Hz, brightness = 50%, contrast = 50%, overlap = 85% (locked), color map = "Cool", DFT size = 1024 samples (locked), grid spacing (spectral resolution) = 46.9 Hz. Temporal traits were analyzed in oscillograms; dominant frequency was measured in spectrograms through 'Peak Frequency' measurement function. Figures were generated using Seewave v. 1.6 package (Sueur et al. 2008), R (version 3.0.3) platform (R Core Team 2014). Seewave settings for the spectrograms: Hanning window, 85% overlap, and 256 points resolution (FFT). Analyzed sound files are listed in Appendix 1.

Toledo et al. (2015) proposed terminologies for basic bioacoustic analyses, mostly applied in call descriptions. In the same paper, the authors stated that "territorial calls" (within the aggressive call category) were emitted during male dispute by resources and interspacing in a reproductive chorus without a close-range distance or physic combat between them. Based on field annotations, the aggressive calls recorded by us fitted the behavioral definition of territorial calls described by Toledo *et al.* (2015), so the “territorial call” nomenclature was applied to our aggressive calls (Fig. 2; Table 2).

Brandão *et al.* (2009) described the vocal repertoire of *P. centralis* with three types of call but we only included call type “A” and both notes of call type “B” for our acoustic comparisons, considering the traits “note duration” and “pulses per note” as “call duration” and “pulses per calls”, respectively, since call type “B” probably represents two calls “A” emitted in a row (see discussion below). Wogel *et al.* (2004) described the advertisement call of *P. rohdei* as a sequence from 1 to 7 multipulsed notes. This sequence might actually correspond to a sequence of calls instead of one call composed of multipulsed notes. So, we regarded *P. rohdei*’s multipulsed notes as advertisement calls. Advertisement calls of *P. megacephala* was described by Giaretta *et al.* (2007) from Serra do Cipó, state of Minas Gerais, as a long call composed of 6 or 7 notes. We regard herein each note as one advertisement call, emitted in long series of 6 or 7 calls per series.

Results

During field observations, it was possible to observe two different types of acoustic signals. In the cases that there were interactions between nearby conspecific males, the longer acoustic signal (hereinafter referred to as “territorial call”) were more often recorded, while in the cases of isolated males that were recorded with no male-male interactions, the shorter acoustic signal (hereinafter referred to as “advertisement call”) were almost always the only type of call recorded.

Advertisement call consisted of a single type of short, pulsed note emitted in series or isolated at irregular intervals. Calls (Fig. 1A□B, Table 1) had regular and deep amplitude modulations throughout their duration, whereas frequency modulation was barely detected, being either ascendant or descendent, when present. Calls lasted from 18–70 ms (mean = 45.7, SD = 2.9; N = 62), with 3–6 pulses per call (mean = 4.3, SD = 0.3; N = 62). Pulse duration varied from 2–16 ms (mean = 7.5 ms, SD = 0.6; N = 262), emitted at rates of 57–167 pulses per second (mean = 98.4, SD = 7.5; N = 62). Pulses were more often arranged in the following patterns: a three-pulse group followed by a single isolated pulse (40.3%; N = 25 calls); a four-pulse group followed by a single isolated pulse (29.0%; N = 18 calls); a three-pulse group with no isolated pulse (12.9%; N = 8 calls); a four-pulse group with no isolated pulse (9.7%; N = 6 calls); a five-pulse group with no isolated pulse (4.8%; N = 3 calls). Two other types of pulse arrangements were observed once (1.6%; N = 1 call): a five-pulse group followed by a single isolated pulse and one isolated pulse followed by a three-pulse group. Pulse number within pulse groups varied from 3–5 pulses per group (mean= 3.5, SD = 0.4; N = 62) (Fig. 1B). Pulse group duration varied from 18–41 ms (mean = 25.4, SD = 2.8; N = 62), with intervals within pulse group varying 1–6 ms (mean = 2.7, SD = 2.3; N = 16) or with no interval, and intervals between pulse groups and isolated pulses varying 2–40 ms (mean = 16.4, SD = 2.5; N = 47). Dominant frequency varied from 1781–2250 Hz (mean = 2075.8 Hz, SD = 61.9; N = 62).

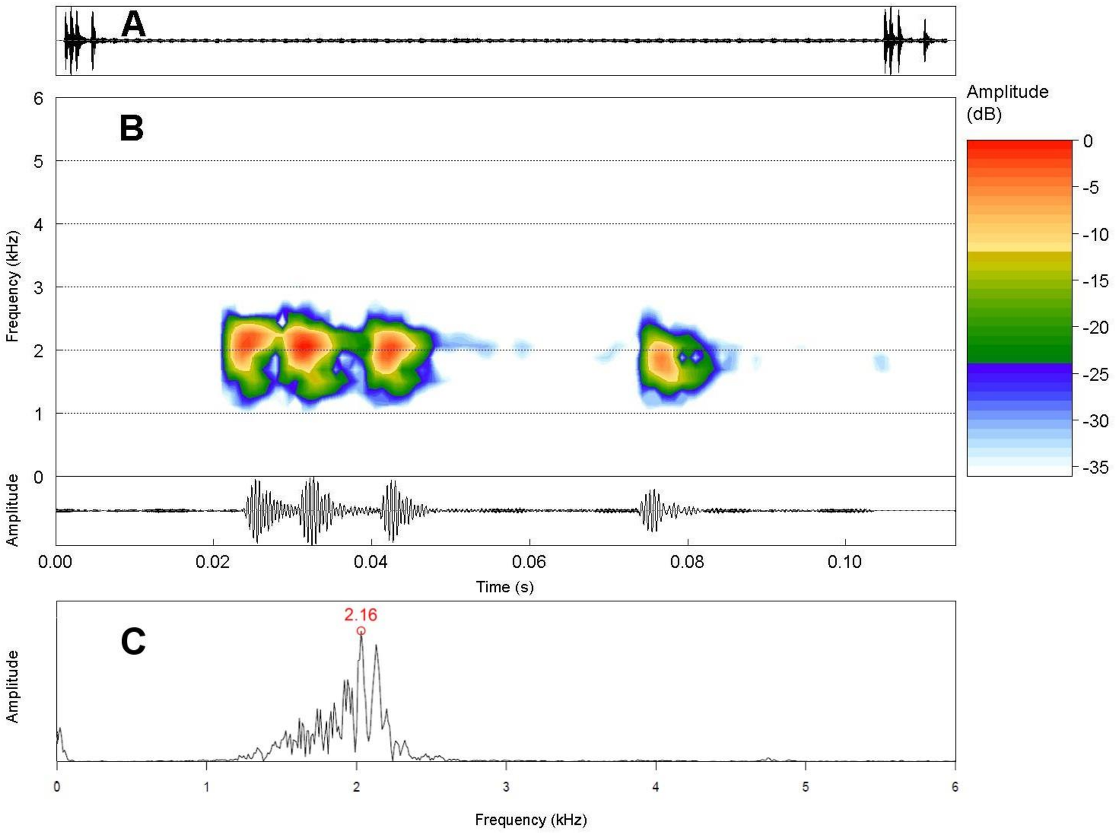

FIGURE 1. A - Waveform section (ca. 1.0 s) of two advertisement calls of Phyllomedusa azurea. B - Spectrogram and corresponding oscillogram detailing the second call marked from A. C – Power spectrum of the second call marked from A. Sound file: Phyllom_azurBelVistMS1bTRC_AAGmt. Recorded on 18 Dec 2010, at 20:40h; air temperature 26 ºC. Recorded in Bela Vista, state of Mato Grosso do Sul, Brazil. Voucher recording (AAG-UFU 0148)

Territorial call of P. azurea (Fig. 2A–B, Table 2) was a longer, single type of pulsed note compared to the advertisement call, also emitted at irregular intervals. Calls could have a slight frequency modulation (ascendant or descendent). Call duration lasted from 222–498 ms (mean = 334.8, SD = 73.1; N = 20). Pulse number varied from 14–43 (mean = 26.7, SD = 8.0; N = 20), which could be arranged either in 4–7 groups (mean = 5.4, SD = 1.0; N = 17) with duration ranging from 13–244 ms (mean = 41.4, SD = 35.3; N = 82), separated by intervals of 5–41 ms (mean = 21.5, SD = 10.4; N = 65), with 2–26 pulses per group (mean = 4.5, SD = 3.6; N = 82), or without well-defined pulse groups, either with irregular or regular spacing throughout call duration. Pulse duration lasted from 5–8 ms (mean = 6.5, SD = 1.1; N = 414), separated by intervals of 4–14 ms (mean = 8.4, SD = 2.4; N = 351), and emitted at rates of 63–96 pulses per second (mean = 78.5, SD = 9.7; N = 414). Dominant frequency varied from 1781–2109 Hz (mean = 2005.8, SD = 79.0; N = 20).

Territorial call differs from advertisement call by a longer duration (territorial: 222–498 ms; advertisement: 18–70 ms) and a more pulses per call (territorial: 14–43 pulses/call; advertisement: 3–6 pulses/call).

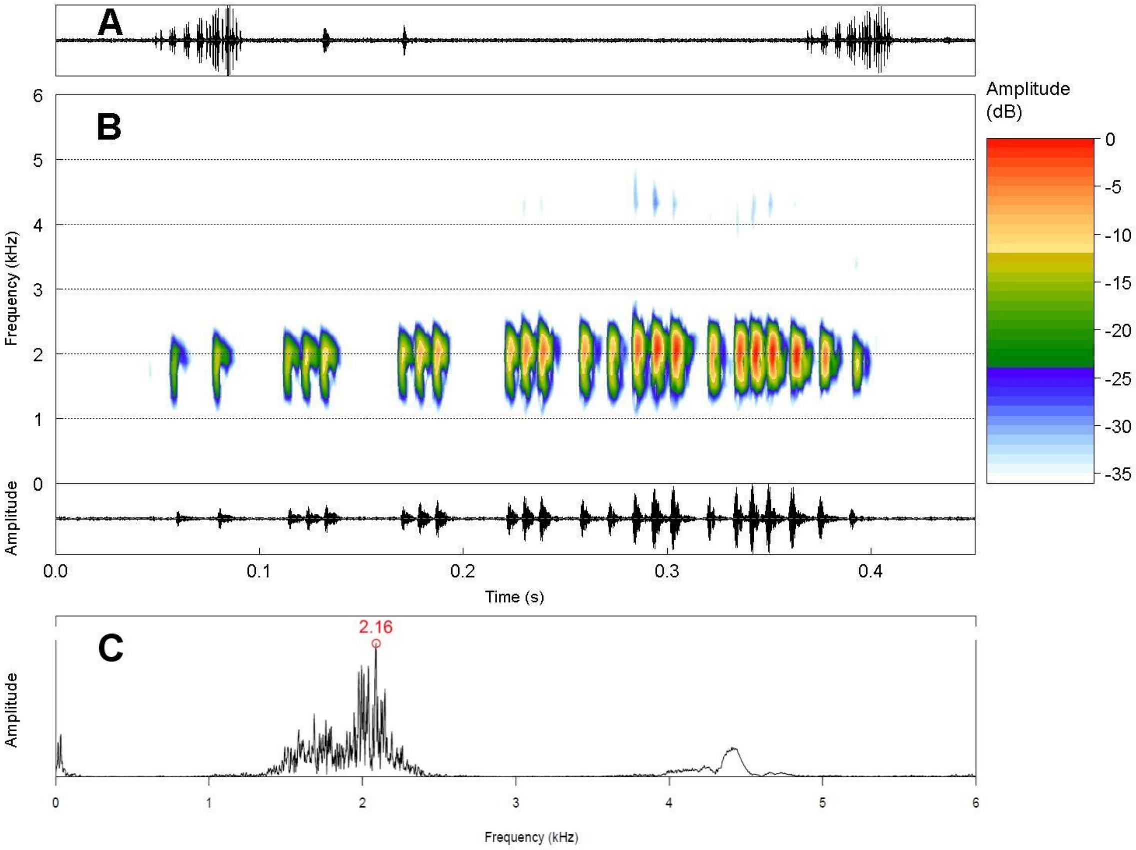

FIGURE 2. A - Waveform section (ca. 3.5 s) of two territorial calls of *Phyllomedusa azurea*. B – Spectrogram and corresponding oscillogram detailing the first call from A. C – Power spectrum of the first call from A. Sound file: Phyllom_azurBelVistMS4aTRC_AAGmt. Recorded on 18 Dec 2010, at 23:14h; air temperature 27 ºC. Recorded in Bela Vista, state of Mato Grosso do Sul, Brazil.

6 Discussion

The territorial calls of P. azurea (see Table 2) were similar in the three available temporal traits to the “first and second phases” of the calls presented by Barrio (1976): call duration (Barrio 1976: 450–650 ms; present work: 222–498 ms), pulses per call (Barrio 1976: 25–60; present work: 14–43), and pulses per second (Barrio 1976: 55–90; present work: 63–96), whereas the “third phase” described as “series composed of note groups” was similar to our advertisement calls (see Table 1), particularly in pulses per call (described as pulses/note) (Barrio 1976: 2–4; present work: 3–6 pulses) and pulse rate (Barrio 1976: 55 pulses/second; present work: 57–167 pulses/second). Our territorial calls were also quite similar in all acoustic traits to calls described as the advertisement call in De la Riva et al. (1995): call duration (as note duration) (De la Riva et al. 1995: 372–642 ms; present work: 222–498 ms), pulses per call (De la Riva et al. 1995: 26–54; present work: 14–43), pulses per second (De la Riva et al. 1995: 61–99; present work: 63–96), calls per minute (De la Riva et al. 1995: 10–43; present work: 14–35), and dominant frequency (De la Riva et al. 1995: 1757–2181 Hz; present work: 1781–2109 Hz) (see Table 2). Therefore, the acoustic similarities between our calls and those ones described by Barrio (1976) and De la Riva et al. (1995) indicate that the described calls from Argentina and Bolivia actually represent the same call type that we classified as territorial call, corroborating the statement of Brandão et al. (2009).

Territorial calls of P. hypochondrialis described as aggressive calls by Guimarães et al. (2001) from the state of Goiás differed from ours, being remarkably longer than those of P. azurea (P. hypochondrialis: 528–607 ms; P. azurea: 222–498 ms), with a higher pulse number per call (P. hypochondrialis: 68–79; P. azurea: 14–43), and a higher dominant frequency (P. hypochondrialis: 2140–2244 Hz; P. azurea: 1781–2109 Hz).

TABLE 1. Advertisement call data for Phyllomedusa azurea from the municipality of Bela Vista, state of Mato Grosso do Sul, Brazil (present study), and comparative data for the other species of the P. hypochondrialis group. Mean±SD (minimum–maximum). N = number of recorded males [analyzed calls].

	Call duration (ms)	Pulses/call	Pulse rate/sec.	Dominant frequency (Hz)
P. azurea (present study) N = 4 [62]	45.7±2.9 (18–70)	4.3±0.3 (3–6)	98.4±7.5 (57–167)	2075.8±61.9 (1781–2250)
P. azurea “Third phase” (Barrio 1976) N = not available [?]	–	(2–4)	55.0	–
P. centralis “Call type A” (Brandão et al. 2009) N = 1 [75]	33.8±8.5 (26–63)	3.9±0.5 (3–5)	–	1450.8±175.9 (1019–1743)
P. centralis “Call type B”(First note) (Brandão et al. 2009) N = not available	43.0±11.5 (26.4–68)	4.4±0.8 (3–6)	–	1520.2±147.9 (1207–1743)
P. centralis “Call type B” (Second note) (Brandão et al. 2009) N = not available	53.57±9.07 (28.1–77.2)	5.1±0.6 (4–7)	–	1520.2±147.9 (1207–1743)
P. hypochondrialis (Guimarães et al. 2001) N = 3 [15]	56.7±43.3 (29–107)	4.0±1.0 (3–6)	–	2327.8±153.9 (2180–2487)
P. hypochondrialis (Duellman & Pyles 1983) N = 4 [12]	100.0 (80–130)	–	233.0 (213–240)	2534.0 (2325–2685)
P. megacephala (Giaretta et al. 2007) N = 1 [10]	110.0±20.0 (90–140)	(6–10)	–	1722.0
P. nordestina (Vilaça et al. 2011) N = 5 [100]	33.8±15.2 (13–73)	4.2±1.9 (3–9)	–	2076.5±67.7 (1972–2227)
P. oreades (Brandão & Álvares 2009) N = 5 [114]	29.7±4.0 (25–56)	3.9±0.2 (3–5)	–	1681.0±65.0 (1550–1808)
P. paliatta (Duellman 1978) N = not avaiable [2]	135.0 (130–140)	–	190.0 (180–200)	3000.0

P. paliatta (Köhler & Lötters 1999) N = 1 [6]	26.2±3.1 (23–32)	–	–	1580.0
P. rohdei (Wogel *et al.* 2004) N = not available [270]	18.0±5.0 (7–34)	2.3±0.5 (1–4)	–	1300±0.100 to 2600±0.200 (1100–3200)

Duellman and Pyles (1983) described the advertisement call of P. hypochondrialis from near the mouth of the Amazonas River near Belém, state of Pará, northern Brazil. Its call (= note) differed from ours in the following acoustic traits: by longer call duration (P. hypochondrialis: 80–130 ms; P. azurea: 18–70 ms), higher pulse rate (P. hypochondrialis: 213–240 pulses/s; P. azurea: 57–167 pulses/s), and higher dominant frequency (P. hypochondrialis: 2325–2685 Hz; P. azurea: 1781–2250 Hz). In opposite, the advertisement call of P. hypochondrialis described from the state of Goiás, central Brazil (Guimarães et al. 2001), could not be distinguished from our data for P. azurea in any call trait.

Calls of P. palliata from Bolivia (N = 1 male; Köhler & Lötters 1999) could be distinguished from those of P. azurea (see Table 1) by a lower dominant frequency [P. palliata: 1580 Hz; P. azurea: mean = 2075.8±61.0 (1781–2250 Hz)] (see Table 1); calls of P. palliata from Ecuador (Duellman 1978) differed from calls of P. azurea by having a longer duration (P. palliata: 130–140 ms; P. azurea: 18–70 ms), a higher pulse rate (P. palliata: 180–200 ms; P. azurea: 57–167 pulses per second), and a higher dominant frequency [P. palliata: 3000 Hz; P. azurea: mean = 2075.8±61.0 (1781–2250 Hz)].

The acoustic repertoire of P. centralis included: call type “A”, which was more often heard and recorded, call type “B”, which consisted of two notes emitted repeatedly and generally followed by calls emitted by nearby males, and call type “C”, which was probably a territorial call (sensu Toledo et al. 2015) due to agonist interactions among males (as described by Brandão et al. 2009). Also, the comparison of the call types “A” and “B” with call type “C” was similar to our comparison between advertisement and territorial calls of P. azurea. Besides, the similarities in call type “A” duration (25.7–63.4 ms) and that of both notes of call type “B” (first note: 26.4–68.0; second note: 28.1–77.2 ms), as well as the number of pulses of call type “A” (3–5 pulses) and that of both notes of call type “B” (first note: 3–6; second note: 4–7 pulses), led us to assume that the classification of the advertisement call of P. centralis composed of two different call types (calls A and B) is not necessary, instead, both call types might represent a single call type with different emission patterns. Calls of P. centralis differed from those of P. azurea (see Table 1) by its lower dominant frequency (P. centralis “call type A”: 1019–1743 Hz; P. centralis “call type B”: 1207–1743 Hz; P. azurea: 1781–2250 Hz).

Advertisement call of P. megacephala (N = 1 male; Giaretta et al. 2007) could be distinguished from that of P. azurea by having a longer call (= note) duration (P. megacephala: 90–140 ms; P. azurea: 18–70 ms), a lower dominant frequency (P. megacephala: 1722 Hz; P. azurea: 1781–2250 Hz), and by being released in long, regular sequences.

TABLE 2. Territorial call data for *Phyllomedusa azurea* from the municipality of Bela Vista, state of Mato Grosso do Sul, Brazil (present study), and comparative data for *P. azurea* populations from Bolivia (De la Riva *et al.* 1995) and Argentina (Barrio 1976), and for *P. hypochondrialis* from the state of Goiás, central Brazil (Guimarães *et al.* 2001). Mean±SD (minimum–maximum). N = number of recorded males [analyzed calls = notes].

	P. azurea			*P. hypochondrialis*
	Present study N=2 [20]	De la Riva *et al.* (1995) N = not available [15]	Barrio (1976) N = not available [?]	Guimarães *et al.* (2001) N = 3 [9]
Call duration (ms)	377.2±85.6 (222–498)	473.4 (372–642)	(450–650)	577.4±33.2 (528–607)
Calls/minute	24.1±14.9 (14–35)	25.9 (10–43)	–	7.0
Pulses/call	31.7±10.3 (14–43)	40.0 (26–54)	(25–60)	72.0±5.0 (68–79)
Pulse rate/second	82.8±8.8 (63–96)	84.0 (61–99)	(55–90)	–
Dominant frequency (Hz)	1952.6±109.8 (1781–2109)	2047.5 (1757–2181)	–	2197.7±39.4 (2140–2244)
Air temperature (°C)	26.0	26.0	28.0	20.5

With respect to P. nordestina (Vilaça et al. 2011), P. rohdei (Wogel et al. 2004) and P. oreades (Brandao et al. 2009), we did not observe great differences compared to P. azurea in any acoustic trait so they could not be differentiated based on their calls (see Table 1).

Molecular and cytogenetic datasets supported the independent specific identities of P. azurea, P. hypochondrialis, and P. nordestina (Bruschi et al. 2013), which are unaccompanied of acoustic evidence. Considering the similarities observed among the advertisement calls of P. azurea, P. hypochondrialis, P. nordestina, P. rohdei, and P. oreades (see Table 1), and the resulting difficulty in discriminating them with the currently available data, call traits should not be used solely as reliable diagnostic characters while additional data are assessed.

Furthermore, P. azurea and P. nordestina are allopatric species (see Bruschi et al. 2013). Lack of unambiguous acoustic diagnosis - even taking different degrees of statistical differentiation into consideration - among well-supported species hypothesis based on other phenotypic and/or genetic evidence has already been reported for other frog groups, such as Allobates (Tsuji-Nishikido et al. 2012), Dendropsophus (Teixeira et al. 2013), Proceratophrys (Martins & Giaretta 2013), and Leptodactylus (Carvalho et al. 2013). Still, differences in the territorial calls of P. azurea and P. hypochondrialis call attention to the importance of further studies with acoustic data from additional localities to better assess intra- and interspecific variation, so that such call type might have potential congeneric diagnosis applicability.

In conclusion, further sampling efforts are required as well to increase acoustic information at a population-level, as well as the correct assignment of call types for Phyllomedusa species in the behavioral framework so as to establish acoustic homology and facilitate direct acoustic comparisons in this treefrog group and thereafter clarify whether or not their call types and acoustic traits might supplement species differentiation.

6.1.6 Acknowledgements

Hellen H. Haga made an English review and Luis F. Toledo for providing laboratory facilities. Financial support by CNPq and FAPEMIG. Fellowships by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) to FSA and IAH, and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) to TRC.. Collection permit: ICMBio 30059□1.

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6.1.8 Appendice I

Analyzed sound files of `Phyllomedusa azurea`, deposited in AAG acoustic database, with their respective voucher specimen accession numbers in parentheses.

Phyllom_azurBelVistMS1aTRC_AAGmt.wav (AAG-UFU 0148)

Phyllom_azurBelVistMS1bTRC_AAGmt.wav (AAG-UFU 0148)

Phyllom_azurBelVistMS1cTRC_AAGmt.wav (AAG-UFU 0148)

Phyllom_azurBelVistMS2aTRC_AAGmt.wav (AAG-UFU 0149)

Phyllom_azurBelVistMS2bTRC_AAGmt.wav (AAG-UFU 0149)

Phyllom_azurBelVistMS2cTRC_AAGmt.wav (AAG-UFU 0149)

Phyllom_azurBelVistMS2dTRC_AAGmt.wav (AAG-UFU 0149)

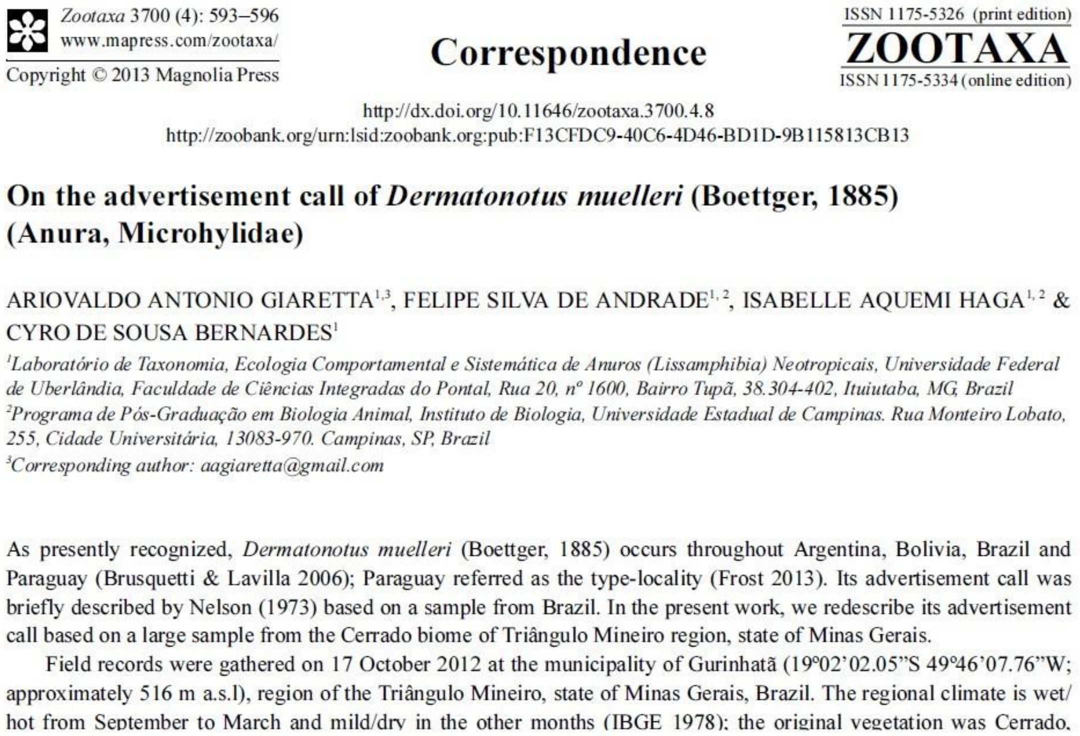
Phyllom_azurBelVistMS3aTRC_AAGmt.wav (AAG-UFU 0150)

Phyllom_azurBelVistMS4aTRC_AAGmt.wav (AAG-UFU 0151)

7 ANEXOS

7.1 Produção bibliográfica com enfoque relacionado a dissertação do candidato durante a vigência do mestrado

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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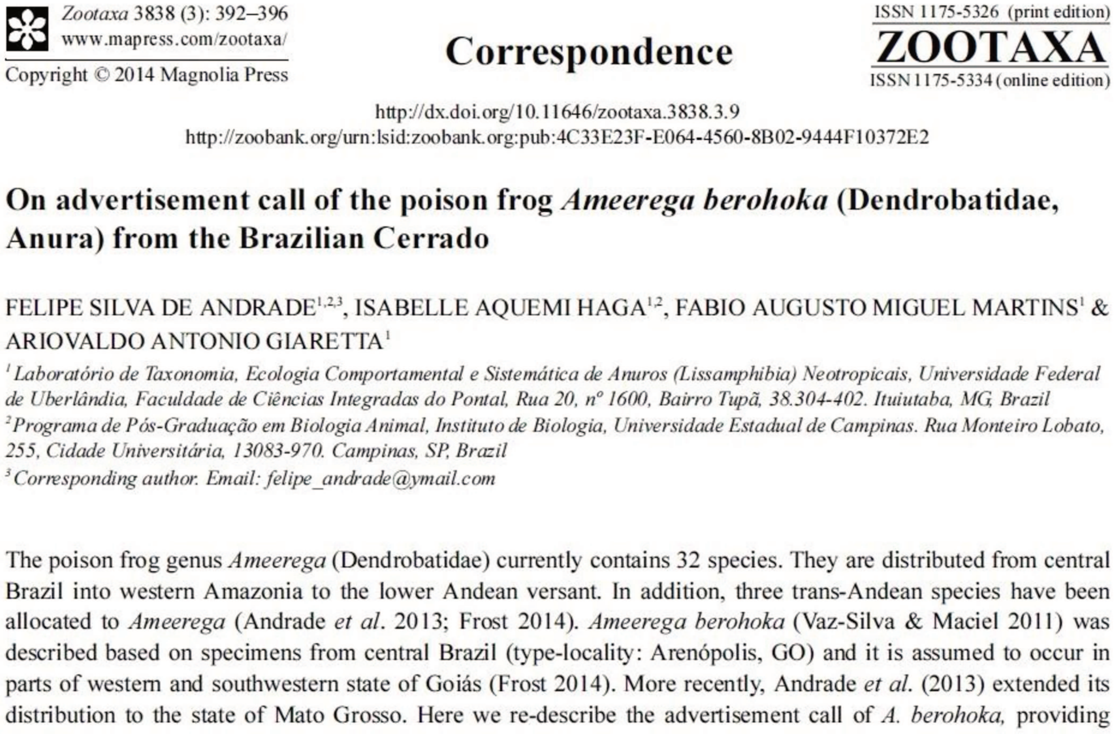
As presently recognized, *Dermatonotus muelleri* (Boettger, 1885) occurs throughout Argentina, Bolivia, Brazil and Paraguay (Brusquetti & Lavilla 2006); Paraguay referred as the type-locality (Frost 2013). Its advertisement call was briefly described by Nelson (1973) based on a sample from Brazil. In 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,

1 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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http://zoobank.org/urn:lsid:zoobank.org:pub:4C33E23F-E064-4560-8B02-9444F10372E2

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

1 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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Correspondence

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

Declaração referente a Bioética e/ou Biossegurança

DECLARAÇÃO

Declaro para os devidos fins que o conteúdo de minha dissertação de Mestrado intitulada Posição taxonômica de populações de *Phyllomedusa* do grupo de *Phyllomedusa hypochondrialis* Daudin (Anura, Hylidae, Phylomedusinae) com o reconhecimento de uma espécie nova, não se enquadra no § 4º do Artigo 1º da Informação CCPG 002/13, referente a bioética e biossegurança.

Isabelle Haga

Aluno: Isabelle Aquemi Haga

Orientador: Dr. Ariovaldo Antonio Giaretta

Para uso da Comissão ou Comitê pertinente:

( ) Deferido ( ) Indeferido

Carimbo e assinatura

Para uso da Comissão ou Comitê pertinente:

( ) Deferido ( ) Indeferido

Carimbo e assinatura

Declaração sobre direito autoral

Profa. Dra. Rachel Meneguello
Presidente
Comissão Central de Pós-Graduação
Declaração

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Campinas, 23/10/2015

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