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Desvendando a diversidade de espécies do grupo *Scinax catharinae* (Anura: Hylidae) no Cerrado: um estudo de caso com *Scinax skaios* Pombal, Carvalho, Canelas, and Bastos, 2010

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Brasília, 20 de agosto de 2020

Este trabalho é dedicado ao meu filho, Caio Carvalho Cieslak.

" O correr da vida embrulha tudo, a vida é assim: esquenta e esfria, aperta e daí

afrouxa, sossega e depois desinquieta. O que ela quer da gente é coragem."

Grande Sertão: Veredas – Guimarães Rosa

AGRADECIMENTOS

SUMÁRIO

LISTA DE FIGURAS

Capítulo 1

Capítulo II

LISTA DE TABELAS

Capítulo I

Capítulo II

RESUMO

O reconhecimento da diversidade críptica está gradualmente se tornando uma tendência nos biomas Neotropicais que são ricos em espécies. A morfologia sempre teve um papel fundamental na taxonomia, contudo outras ferramentas como a molecular e a bioacústica se tornaram tão importantes quanto a análise morfológica dos organismos para a delimitação de um táxon. O gênero *Scinax* é um dos mais especiosos gêneros de pererecas da família Hylidae com muitos representantes no Cerrado brasileiro. Apesar do grande número de potenciais novas espécies reveladas em trabalhos recentes, populações de várias espécies ainda carecem de mais pesquisas para avaliar seus status taxonômico. *Scinax skaios* é uma espécie pertencente ao grupo *Scinax catharinae*, encontrada apenas em poucas localidades em matas de galerias dentro do Cerrado. A fim de investigar de modo mais profundo as populações conhecidas de *S. skaios*, uma avaliação da variação intraespecífica na morfologia, padrões de coloração, canto de anúncio e genética foi conduzida para esclarecer a real identidade desta espécie e desvendar espécies potencialmente novas. Além disso, uma modelagem de nicho foi feita para prever áreas adequadas de ocorrência e melhor avaliarmos seu status de conservação. A descrição de uma nova espécie e a delimitação da sua área geográfica tem impactos não somente na taxonomia de um grupo de organismos, mas também pode beneficiar outros campos da biologia.

Palavras-chave: Anuros, Cerrado, Conservação, Diversidade críptica, Taxonomia

ABSTRACT

The recognition of cryptic diversity is gradually becoming a trend in Neotropical biomes that are rich in species. Morphology has always played a fundamental role in taxonomy, however other tools such as molecular, and bioacoustic have become as important as the morphological analysis of organisms for the delimitation of a taxon. The *Scinax* genus is one of the most specious genera of treefrogs in the Hylidae family with many representatives in the Brazilian Cerrado. Despite the large number of potential new species revealed in recent works, populations of several species still lack further research to assess their taxonomic status. *Scinax skaios* is a species belonging to the *Scinax catharinae* group, found only in a few locations in gallery forests within the Cerrado. In order to further investigate the known populations of *S. skaios*, an assessment of intraspecific variation in morphology, coloration patterns, advertisement call and genetics was conducted to clarify the real identity of this species and to unravel potentially new species. In addition, a niche modeling was done to predict suitable areas of occurrence and better assess their conservation status. The description of a new species and the delimitation of its geographic area have impacts not only on the taxonomy of a group of organisms, but can also benefit other fields of biology.

Keywords: Anurans, Cerrado, Conservation, Cryptic diversity, Taxonomy.

INTRODUÇÃO GERAL

Níveis de biodiversidade nos Neotrópicos são amplamente subestimados, apesar de séculos de pesquisa nessas regiões (Rull, 2011; Scheffers et al., 2012; Fouquet et al., 2014) e grande quantidade de espécies esperam para ser descritas (Mora et al. 2011; Costello et al., 2013).

Diante de um cenário com enorme insuficiência de conhecimento sobre a biodiversidade, o déficit Lineano (Brown & Lomolino, 1998) foi imaginado para descrever a falta de conhecimento taxonômico e os obstáculos que isso traz para estudos biológicos, e ainda reconhecer o problema em termos de práticas de conservação (Possingham et al. 2007). O déficit Wallaceano, de modo similar, apresenta uma falta de conhecimento acerca da distribuição das espécies e dificuldades associadas (Whittaker et al., 2005). Ambos déficits estão correlacionados e podem restringir fortemente ações em medidas de conservação, especialmente em hotspots de biodiversidade (Bini et al., 2006). Além disso, mesmo resolvendo estes problemas, informação sobre as relações filogenéticas ainda estaria faltando para muitas espécies em todo mundo, déficit Darwiniano (Diniz-Filho et al., 2013), e isso prejudicaria ainda mais os esforços de conservação. Estes três déficits são altamente acentuados nos Neotrópicos, em que a alta diversidade e o distanciamento de várias áreas causa certa dificuldade para os pesquisadores superarem essas lacunas de conhecimento (Silva et al., 2014).

Apesar da posição central e incontestável da taxonomia baseada na morfologia em pesquisas sobre biodiversidade, a percepção visual dos humanos provavelmente nunca será suficiente para capturar toda complexidade natural (Beheregaray & Caccone, 2007). Exemplo claro disso, é o crescente número de estudos utilizando ferramenta molecular para reportar espécies crípticas (Bickford et al., 2007).

75   Espécies crípticas, ou 'sibling species', são espécies discretas que são difíceis, ou até mesmo impossíveis, de serem distinguíveis morfologicamente e assim tem sido incorretamente atribuídas a um único táxon (Beheregaray & Caccone, 2007). Podem ser encontradas em todos os principais grupos taxonômicos aquáticos e terrestres (Knowlton, 1993; Pfenninger & Schwenk, 2007) e tem sido reportada para vários grupos de anfíbios

80   anuros (Elmer et al. 2007; Fouquet et al., 2007; Elmer & Cannatela, 2008; Funk et al., 2012; Prado et al., 2012; Fouquet at al., 2014).

Os anfíbios representam uma significativa parcela da biodiversidade global do seu grupo taxonômico (14%) contando com 8.177 espécies ao todo, sendo 7.210 delas de anuros. O Brasil é o país com a maior diversidade de anfíbios do mundo (1.136 espécies),

85   a maioria delas de anuros (93%), incluindo 1.093 espécies, representadas em 20 famílias e 105 gêneros (Segalla et al., 2019).

Apesar do declínio populacional dos anfíbios em todo o mundo (Stuart et al., 2004), o número de espécies reconhecidas deste grupo de animais tem aumentado dramaticamente nos últimos anos (Glaw & Kolher, 1998; Hanken, 1999; Kolher et al.,

90   2005; Ron et al., 2006; Frost, 2020), tornando os anfíbios um dos grupos de vertebrados com a mais alta taxa proporcional de descrições de novas espécies (Hanken, 1999).

Mesmo com o grande número de descrições de novas espécies de anfíbios nas últimas três décadas, sugerindo que a diversidade de espécies é ainda altamente subestimada (Walker et al., 2018), os anfíbios são o grupo de vertebrados mais ameaçados

95   de extinção com mais de um terço das espécies avaliadas (41%) globalmente ameaçadas ou extintas (IUCN, 2020). Além disso, cerca de 30% das espécies avaliadas, carecem de mais informações sobre o status de ameaça (sendo classificadas como "Dados Deficientes" – DD, categoria da IUCN), e uma porção significativa dessas espécies estão globalmente ameaçadas (IUCN, 2020). Atualmente, o status de conservação de uma

100   espécie é apoiado por regras de decisão baseadas em alguns parâmetros, como a faixa de

distribuição, tamanho e histórico da população, e riscos de extinção (Stuart et al., 2004).

Portanto, o entendimento apropriado da taxonomia e da distribuição de ocorrência de uma

espécie é imprescindível para uma avaliação completa das ameaças (Stuart et al., 2004;

Gehara et al., 2013). Estudos que avaliam a relação entre a distribuição de anfíbios e seus

105   hábitats podem fornecer informação científica vital auxiliando na configuração dos

planos de conservação, pois para proteger uma espécie é necessário entender melhor o

que constitui um ambiente adequado e onde estão essas áreas (Blank & Blaustein, 2012).

O mapeamento de hábitats adequados pode identificar áreas que necessitam de

restauração ou preservação (Gibson et al., 2004), e identificar áreas candidatas para

110   reintrodução (Olsson & Rogers, 2009).

Anfíbios em geral, tendem a exibir evolução morfológica conservativa (Cherry et

al., 1978), e a utilização de múltiplas linhas de evidência, tais como a molecular e

bioacústica, tem se mostrado particularmente eficaz para revelar espécies

morfologicamente muito similares ou crípticas que até então eram atribuídas a um único

115   táxon (Hillis et al., 1983; Wynn & Heyer, 2001; Gower et al., 2005; Fouquet et al., 2007;

2016; Sheridan et al., 2010; Prado et al., 2012; Magalhães et al., 2018; Marinho et al.,

2018). Embora diferentes conjuntos de dados possam gerar respostas distintas quando

examinados individualmente, para saber se dois indivíduos são coespecíficos,

coletivamente devem dar uma indicação mais confiável de delimitações de espécies

120   (Sheridan et al., 2010).

As evidências relacionadas ao isolamento reprodutivo são particularmente

poderosas, pois elas atendem ao critério de espécies biológicas, um meio inequívoco e

indiscutível para delimitação de uma espécie (Padial et al., 2010). Em anuros, os

mecanismos de reconhecimento e escolha de parceiros envolvem vários comportamentos

125 e atributos, contudo, o mais reconhecido e estudado são os sinais acústicos (Wells, 2007). Estes sinais tem uma grande utilidade taxonômica, uma vez que, as vocalizações contêm informações temporais e espectrais que são importantes para o reconhecimento específico (Cocroft & Ryan, 1995). Os sinais acústicos são influenciados pelas características morfológicas e ambientais (Duellman & Trueb, 1994; Lingnau & Bastos, 2007). Alguns

130 estudos já relataram a existência de uma relação negativa entre a frequência dominante e o comprimento rostro-cloacal ou a massa dos machos (Ryan, 1988; Toledo & Haddad, 2005; Bastos et al., 2011), e uma relação positiva entre parâmetros temporais do canto (como a duração do canto, taxa de repetição, número de pulsos) e a temperatura (Ryan, 1988; Bastos et al., 2003; Lingnau & Bastos, 2007). Os machos também conseguem

135 alterar seus cantos quando outros machos coespecíficos estão muito próximos do seu território (Bastos et al., 2011). Apesar dessas variações nos atributos do canto, os componentes básicos da vocalização permitem o reconhecimento de coespecíficos (Wells, 2007).

Como o canto dos anuros é particularmente um poderoso mecanismo de

140 isolamento pré-zigótico, ele tem sido amplamente utilizado em estudos de taxonomia e sistemática como um meio indiscutível para delimitar espécies (Köhler et al., 2017) e ainda, a comparação das análises bioacústicas têm também resultado na descoberta de muitas espécies morfologicamente crípticas de anuros (Padial et al., 2008; Vences & Kolher, 2008; Glaw et al., 2010).

145

A família Hylidae Rafinesque, 1815

Hylidae é uma família de anuros amplamente distribuída nas Américas, Austrália/Pápua Nova Guiné e Eurásia, abrigando atualmente 730 espécies (Duellman,

150   2001; Faivovich et al., 2005; Frost, 2020). Duellman (1970) dividiu a família em quatro subfamílias: Amphignathodontinae, Hemiphractinae, Hylinae e Phyllomedusinae. Desde então, vários trabalhos foram realizados visando esclarecer as relações de parentesco dentro do táxon. Há algum tempo, a classificação dos representantes deste grupo tem mudado drasticamente daquela baseada apenas em evidência morfológica para uma

155   baseada em dados moleculares (Duellman et al., 2016). Por exemplo, Trueb (1974) sugeriu a inclusão de Hemiphractinae com Amphignathodontinae em um único grupo. Baseados no trabalho de Tyler (1971) e Savage (1973), Dowling & Duellman (1978) removeram os hilídeos australianos de Hylinae e os transferiram para a subfamília Pelodryadinae. Ruvinsky & Maxson (1996) sugeriram em seu trabalho que Hylidae

160   (sensu lato) seria polifilético e Duellman (2001) apresentou um cladograma das subfamílias incluindo Pseudidae como uma subfamília: (Pelodryadinae + (("Hylinae" + Pseudinae) + (Phyllomedusinae + Hemiphractinae))). Haas (2003) por meio de evidência da morfologia larval sugeriu que Hylidae é polifilético, com Hemiphractinae sem uma relação próxima com outros hilídeos, Pelodryadinae sendo parafilético à Hylinae e este

165   último não seria claramente monofilético e com Pseudinae e Phyllomedusinae possivelmente incorporados dentro dele. Utilizando uma abordagem molecular, Darst e Cannatela (2004) sugeriram que Hemiphractinae (sensu lato) seria polifilético e não proximamente relacionado aos hilídeos das outras três subfamílias. Faivovich et al. (2005) encontraram resultados similares e removeram formalmente Hemiphractinae de

170   Hylidae e os consideraram como uma subfamília de Leptodactylidae, Hylidae estaria então restrita a Hylinae, Pelodryadinae e Phyllomedusinae, e corroboram uma relação de grupos irmãos entre estas duas últimas subfamílias, que juntas correspondem ao táxon irmão de Hylinae. Frost et al. (2006) também sugeriram o polifiletismo de Hemiphractinae, e que seria composto por três grupos distantemente relacionados e

175   reconheceu essas como famílias: Amphignathodontinae (*Flectonotus* e *Gastrotheca*), Cryptobatrachidae (*Cryptobatrachus* e *Stefania*) e Hemiphractidae (*Hemiphractus*). Roelants et al. (2007) sugeriram que Hylidae seria polifilético, com Phyllomedusinae + Pelodryadinae como táxon irmão de Brachyicephalidae e Hylinae incorporado dentro de Ceratophryidae. Wiens et al. (2010) apresentaram uma análise expandida de Hylidae e

180   encontraram monofiletismo fortemente suportado para Hylinae, Phyllomedusinae + Pelodryadinae, Phyllomedusiane, Pelodryadinae, Cophomantini e Hylini com Dendropsophini também monofilético, mas fracamente suportado. Revisando a filogenia em larga escala para Amphibia, Pyron & Wiens (2011) forneceram uma grande árvore filogenética de hilídeos como parte da grande árvore de todos os anfíbios e propondo

185   novos arranjos.

O grupo todo foi revisado por Duellman et al. (2016), no qual apresentaram uma nova hipótese filogenética. A nova classificação baseada na árvore possui três famílias (Hylidae, Pelodryadidae e Phyllomedusidae) que compõem um táxon sem classificação (Arboranae), um total de nove subfamílias (Acridinae, Hylinae, Pseudinae,

190   Dendropsophinae, Lophyohylinae, Scinaxinae, Cophomantinae, Pelobiinae e Pelodryadinae, sendo cinco destas revalidadas - Acridinae, Dendropsophinae, Lophyohylinae, Cophomantinae e Pelobiinae, e uma nova - Scinaxinae), cinco nomes de gêneros revalidados (*Hyliola*, *Dryophytes*, *Ololygon*, *Pithecopus* e *Dryopsophus*) e cinco novos nomes de gêneros (*Sarcohyla*, *Rheohyla*, *Julianus*, *Colomascirtus* e *Callimedusa*).

195   Contudo, muitos pesquisadores não concordam com a maioria das mudanças propostas (Colaço & Da Silva, 2016; Conte et al., 2016; Lourenço et al. 2016; Faivovich et al., 2018; Ron et al., 2018; Lourenço et al., 2019) devido aos critérios inconsistentemente aplicados, alguns valores de confiança baixos e pela discussão superficial dos autores.

200   Faivovich et al. (2018) em um trabalho sobre a monofilia e as relações entre Hylini fazem comentários pertinentes às alterações taxonômicas implicadas por Duellman et al. (2016). Eles argumentam que as relações de parentesco para os hilídeos têm sido estável desde o estudo de Faivovich et al. (2005) e que, além disso, o crescimento do número de espécies foi restrito a poucos gêneros (*Boana*, *Dendropsophus*, *Litoria*, *Nyctimystes*,

205 *Ranoidea* e *Scinax*) sem causar impactos na estrutura taxonômica fora desses gêneros. Isso anularia o argumento de uma necessidade hipotética de mais subdivisões taxonômicas. Segundo Faivovich et al. (2018), a melhor medida a ser tomada seria continuar reconhecendo Hylinae, Phyllomedusinae e Pelodryadinae como subfamílias (devido a falta de clareza na real vantagem para modificar a classificação); as antigas

210 tribos de Hylinae reconhecidas por Faivovich et al. (2005) e elevadas à subfamília por Duellman et al. (2016) serem consideradas tribos novamente (Hylini, Scinaxini – restrito à *Scinax*, considerando *Julianus* e *Ololygon* sinônimos de *Scinax*, Sphaenorhynchini – restrito ao gênero *Sphaenorhynchus*.

215 **Histórico do gênero *Scinax* Wagler, 1830**

        O gênero *Scinax* é composto principalmente por espécies originalmente descritas ou anteriormente incluídas no grupo *Hyla rubra*. O grupo foi primeiro reconhecido por Dunn (1933) para abrigar quatro espécies da América Central e anos mais tarde foi

220 redefinido e aumentado mediante os trabalhos de Cochran (1952; 1955), Duellman (1970; 1972a e 1972b), Lutz (1951; 1952; 1973) e Savage & Heyer (1969).

        Vários agrupamentos dentro de *Hyla rubra* foram definidos. O grupo *Hyla catharinae*, brevemente definido por Lutz (1954), foi também mencionado por Bokermann (1964) que sugeriu uma relação com o grupo *H. rubra*. Duellman (1972a)

225   comentou sobre a existência de alguns grupos de espécies, grupo *H. rubra*, *Hyla boulengeri*, *H. catharinae* e *Hyla parkeri*, o qual definiu como "complexo *Hyla rubra*". Lutz (1973) fez referência aos "complexos" de espécies de *H. rubra* – *Hyla x-signata* e *H. catharinae* separadamente. Ao contrário dos autores anteriores que não reconheceram a existência desses complexos.

230   Fouquette & Delahoussaye (1977) descreveram que as espécies do grupo *H. rubra* apresentavam esperma com cauda dupla. Considerando essas observações morfológicas mais as características relatadas por León (1969) e por Duellman (1970), sugeriram transferir as espécies do grupo *H. rubra* para compor um gênero próprio, *Ololygon* Fitzinger, 1843. A partir da combinação de caracteres morfológicos externos (como 235   comprimento rostro-cloacal, formato do focinho e padrão de coloração) e tipo de esperma, Fouquette & Delahoussaye (1977) agruparam muitas das espécies em cinco grupos: os grupos *Ololygon rubra*, *Ololygon catharinae*, *Ololygon rostrata*, *Ololygon staufferi* e *Ololygon x-signata* (algumas espécies não foram atribuídas a nenhum desses grupos).

    Muitos autores não reconheceram *Ololygon* após esses estudos por diferentes 240   razões (Faivovich, 2002). *Ololygon* foi considerado *nomen dubium* por Cardoso & Sazima (1980) devido a problemas na determinação de topótipos de *Hyla strigilata* Spix, 1824 (espécie-tipo para o gênero *Ololygon*). Cardoso & Haddad (1982) endossaram o não reconhecimento de *Ololygon* e argumentaram ainda sobre a forma do esperma ser um critério questionável de difícil avaliação e por não refletir mecanismos de isolamento 245   reprodutivo. Almeida & Cardoso (1985) demonstraram variabilidade morfométrica nos espermatozóides de *Hyla fuscovaria,* indicando então que o caracter "forma do esperma" não era convincente. Os autores também sugeriram que o grupo *H. rubra* e o complexo *H. catharinae* não formavam um grupo natural, pois exibiam uma grande variação morfológica comportamental assim como outras espécies de *Hyla*, sendo assim, não havia

250   justificativa para colocar os dois grupos em um único gênero. A solução nesse caso seria

incluir as espécies do grupo *H. rubra* no gênero *Garbeana* Miranda-Ribeiro, 1926.

Alguns grupos de espécies foram definidos a partir de sinapomorfias (características derivadas compartilhadas), como a capacidade de dobrar o polegar e o primeiro artelho para trás (apontadas como sinapomorfia para o gênero, observados em

255   algumas espécies de *Ololygon* notada por Jungfer (1986); oviposição na água nas axilas de bromeliáceas terrestres e membranas reduzidas (ausentes entre os artelhos I e II e reduzidos ou ausentes entre os artelhos III e IV) observada nas espécies abrigadas no grupo *Ololygon perpusilla* proposta por Peixoto (1986); a presença de saco vocal lateralmente expandido observada nas espécies do grupo *Ololygon rizibilis* (Andrade &

260   Cardoso, 1987).

Pombal & Gordo (1991) também não reconheceram o gênero *Ololygon* e argumentaram que se as espécies do grupo *H. rubra* realmente deveriam se diferenciar genericamente, então *Scinax* Wagler, 1830 teria prioridade sobre *Ololygon*.

O gênero *Scinax*, foi então revalidado por Duellman & Wiens (1992) (espécie-

265   tipo *Hyla aurata* Wied-Neuwied, 1821) para todas as espécies antes incluídas em *Ololygon*, e sugeriram três sinapomorfias que suportavam a monofilia de *Scinax*: região loreal deprimida; membranas entre os artelhos I e II ausentes ou reduzidas a uma franja na margem do segundo artelho; discos adesivos nos dedos das mãos dilatados, truncados e mais amplos que longos. Assim eles agruparam a maioria das espécies em sete grupos

270   de espécies: os grupos *Scinax rubra*, *Scinax rostrata*, *Scinax catharinae*, *Scinax x-signata*, *Scinax perpusilla* e *Scinax staufferi*. Faivovich et al. (2005) forneceram uma lista completa de sinapomorfias moleculares e morfológicas para *Scinax*. As sinapomorfias morfológicas apontadas pelos autores incluem a presença de membranas entre os artelhos I e II que não extendam além do tubérculo subarticular do artelho I, a capacidade de

dobrar o artelho I e o dedo I, origem do músculo *peitoralis abdominalis* através de tendões bem definidos e do músculo *pectoralis abdominalis* sobrepondo o músculo *obliquus externus*.

Mais tarde, Pombal et al. (1995b) propuseram algumas alterações baseados na existência de similaridades morfológicas e bioacústicas, e transferiram todas as espécies do grupo *S. x-signata* para o grupo *Scinax rubra*.

Segundo Faivovich (2002), todos os grupos de espécies previamente reconhecidos para *Scinax* estão na verdade agrupados em dois clados (clado *Scinax catharinae* e o clado *Scinax ruber*). Ele reconheceu dois grupos de espécies no clado *S. catharinae* (os grupos *Scinax catharinae* e *Scinax perpusillus*) e dois grupos de espécies no clado *Scinax ruber* (os grupos *Scinax rostratus* e *Scinax uruguayus*).

O grupo *Scinax catharinae*

O grupo de espécies *Scinax catharinae* é bem suportado por estado de caracteres miológicos e osteológicos que incluem parte posterior do anel cricóide extensivamente alongado e curvado, mineralização parcial dos elementos intercalares entre a última e a penúltima falange e a origem laterodistal do músculo extensor *brevis distalis digiti III* (Faivovich, 2002).

Como discutido acima, Duellman et al. (2016) em seu trabalho de reanálise de toda a família Hylidae, propôs muitas mudanças taxonômicas e uma delas foi a revalidação do gênero *Ololygon*. Neste trabalho, ele transfere todas as espécies do clado *S. catharinae* para *Ololygon* e todas as espécies que estavam abrigadas no clado *S. ruber* permanecem no gênero *Scinax*. Contudo, muitos pesquisadores não concordam com todas as propostas sugeridas por Duellmann et al. (2016) (Colaço & Silva, 2016; Conte et al., 2016; Lourenço et al., 2016; Faivovich et al., 2018; Ron et al., 2018; Lourenço et al.,

300 2019) principalmente devido aos critérios adotados pelos autores, pouco discutidos, e por

não levarem em consideração as sinapomorfias dos grupos em questão.

Apesar das alterações serem estritamente opcionais, pois neste caso específico, elas não são necessárias para preservar a monofilia do arranjo taxonômico e elas resultaram de pouca discussão dos dados, muitos autores continuam mantendo o arranjo

305 proposto por Faivovich et al. (2005), ao menos até surgirem novas evidências que justifiquem mudanças.

Atualmente, o grupo *S. catharinae* compreende 37 espécies distribuídas na Argentina, Paraguai, Uruguai e no Brasil (sendo a maioria das espécies encontradas na Mata Atlântica) (Lourenço et al., 2014; Duellman et al., 2016; Lourenço et al., 2016;

310 Lourenço et al. 2019; Frost, 2020), que são: *Scinax agilis* (Cruz & Peixoto, 1983); *Scinax albicans* (Bokermann, 1967a); *Scinax angrensis* (Lutz, 1973); *Scinax argyreornatus* (Miranda- Ribeiro, 1926); *Scinax ariadne* (Bokermann, 1967a); *Scinax aromothyella* Faivovich, 2005; *Scinax berthae* (Barrio, 1962); *Scinax brieni* (De Witte, 1930); *Scinax caissara* Lourenço, Zina, Catroli, Kasahara, Faivovich & Haddad, 2016; *Scinax*

315 *canastrensis* (Cardoso & Haddad, 1982); *Scinax cardosoi* (Carvalho-e-Silva & Peixoto, 1991); *Scinax carnevallii* (Caramaschi & Kisteumacher, 1989); *Scinax catharinae* (Boulenger, 1888); *Scinax centralis* Pombal & Bastos, 1996; *Scinax flavoguttatus* (Lutz & Lutz, 1939); *Scinax garibaldae* Lourenço, Lingnau, Haddad & Faivovich, 2019; *Scinax goya* (Andrade, Santos, Rocha, Pombal e Vaz-Silva, 2018); *Scinax heyeri* (Peixoto &

320 Weygoldt, 1986); *Scinax hiemalis* (Haddad & Pombal, 1987); *Scinax humilis* (Lutz & Lutz, 1954); *Scinax jureia* (Pombal & Gordo, 1991); *Scinax kautskyi* (Carvalho-e-Silva & Peixoto, 1991); *Scinax littoralis* (Pombal & Gordo, 1991); *Scinax longilineus* (Lutz, 1968); *Scinax luizotavioi* (Caramaschi & Kisteumacher, 1989); *Scinax machadoi* (Bokermann & Sazima, 1973); *Scinax melanodactylus* Lourenço, Luna & Pombal, 2014;

Scinax muriciensis Cruz, Nunes & Lima, 2011; *Scinax obtriangulatus* (Lutz, 1973); *Scinax pombali* Lourenço, Carvalho, Baêta, Pezzuti & Leite, 2013; *Scinax ranki* (Andrade & Cardoso, 1987); *Scinax rizibilis* (Bokermann, 1964); *Scinax skaios* Pombal, Carvalho, Canelas & Bastos, 2010; *Scinax skuki* Lima, Cruz & Azevedo, 2011; *Scinax strigilatus* (Spix, 1824); *Scinax trapicheiroi* (Lutz & Lutz, 1954); *Scinax tripui* Lourenço, Nascimento & Pires, 2010.

A taxonomia do gênero *Scinax* é complexa e possivelmente, sua real diversidade é subestimada no Cerrado. Neste contexto, o objetivo geral deste trabalho é testar o status taxonômico de *Scinax skaios* por meio de uma abordagem taxonômica integrativa. Os objetivos específicos englobam (i) investigar as populações conhecidas de *S. skaios*; (ii) avaliar a variação da morfologia externa dos adultos, morfométrica, acústica e molecular (iii) verificar a real identidade de *S. skaios* no Cerrado, checando se as populações apresentam divergência genética significativa; (iv) contribuir com o conhecimento sobre a taxonomia do grupo pela descrição de novas espécies e (v) prever novas áreas de ocorrência para *S. skaios*.

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CAPÍTULO I

Phenotypic and molecular variation in a poorly known treefrog from Brazilian Cerrado, *Scinax skaios* Pombal, Carvalho, Canelas, and Bastos, 2010 (Anura: Hylidae) along its geographical range

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Abstract

In recent years, several studies have revealed cryptic lineages among populations considered endemic to the Cerrado, showing that the diversity of frogs in this biome is still underestimated. We analyzed the morphological, acoustic and molecular variation of some populations recognized as *Scinax skaios*, an endemic little known species of the Cerrado belong to the *Scinax catharinae* group. Additionally, we performed ecological niche models, using the ensemble approach, to predict suitable areas for its occurrence. Our results bring novelties on intraspecific variation based on morphological, acoustic and molecular data, indicated candidate species of the *S. catharinae* group for the Cerrado, and highlighting the importance of take into consideration to multiple lines of evidence for better taxonomic accuracy.

Introduction

Taxonomic identification of anurans was traditionally based on morphological variation (Sheridan et al., 2010). From the mid-60's the frog's vocalizations started to gain more attention, because they were biologically meaningful in delimiting species, given that they may function as premating isolation mechanism (Cocroft & Ryan, 1995; Gerhardt & Huber, 2002) where the typically conservative morphological variation was not able to separate taxons (e.g., Ball & Jameson, 1966; Littlejohn & Oldham, 1968). Subsequently, molecular data became commonly used for delimiting species (Goldberg, et al., 2004; Lougheed, et al., 2006; Stuart, et al., 2006; Fouquet, et al., 2007a, b; 2016; Prado et al., 2012; Magalhães et al., 2018; Cassini et al., 2020).

The Cerrado is the most species rich savanna in the world (Cavalcante & Joly, 2002; Silva & Bates, 2002), has been the focus of an increasing number of anurans species descriptions in recent years (e.g., Pugliesi et al., 2009; Pombal et al. 2010; Martins &

745 Giaretta, 2011; Brandão et al., 2012; 2013; Lourenço et al., 2013; Araujo-Vieira et al., 2015; 2016; Berneck et al., 2017; Andrade et al., 2018). Several studies have recently recognized cryptic lineages among previously described Cerrado endemic herpetofauna (Gamble et al., 2012; Prado et al., 2012; Werneck et al., 2012; Domingos et al., 2014; 2017; Giugliano et al., 2013; Recoder et al., 2014; Magalhães et al., 2017).

750 Currently, 37 valid species of *Scinax catharinae* group occur in Brazil, mostly in Atlantic Forest, and only seven species of this group are found in gallery forests within the Cerrado domain (Frost, 2020). An integrative approach, combining different tools, such as morphological, bioacoustics, and molecular data, has revealed the existence of several confirmed candidate species in anurans (Fouquet et al., 2007a; b; Prado et al.,

755 2012; Ferrão et al., 2016; 2018; Orrico et al., 2017; Escalona et al., 2019). The species recognition in *S. catharinae* group is complex (Pombal & Bastos, 1996), due to the high morphological similarity between adult specimens (Carvalho-e-Silva, 1986) that result in a large number of unidentified specimens in collections (Lourenço et al., 2014; 2016). Taxonomic inaccuracy can affect other areas of biology, generating an error cascade that

760 can lead to a variety of negative consequences, compromising, for example, ecological and environmental management studies and programs (Bortolus, 2008).

Conservation of species requires detailed knowledge on its natural history and on its biology, including information about on its distribution and potential occurrence (Papes & Gaubert, 2007). Ecological niches are manifested in environmental spaces that

765 comprise sets of abiotic variables that shape the potential occurrence of a species (Escobar et al., 2018). They translate into geographic distribution according to the combined effects

of the distribution of abiotic conditions, biotic interactions and accessibility by dispersion (Soberón & Peterson, 2005). Even with records of a species that is abundant, they may be biased, characterizing only a portion of the species' niche, limited by biotic factors (eg. interspecific competition), dispersion limit, biased by the sampling effort, or simply the existence of sets conditions in relevant landscapes (Soberón & Peterson, 2005).

Most studies on modeling ecological niche has focused on species of wide geographical distribution (Ron, 2005; Phillips et al., 2006; Siqueira & Durigan, 2007) and there are still few published works on distribution potential geographical of rare species with restricted distribution (Engler et al., 2004; Guisan et al., 2006; Peterson & Papes, 2006; Young, 2007; Papes & Gaubert, 2007; Giovanelli et al. 2008). *Scinax skaios* is a endemic Cerrado species of treefrog belonging to the *S. catharinae* group that has few occurrence records: municipality of Santa Rita do Novo Destino (type locality) (Pombal et al., 2010), Chapada dos Veadeiros (Santoro & Brandão, 2014), Pirenópolis (Serra dos Pirineus), and Caiapônia (Serra das Gales) (Brandão et al., 2016) in the state of Goiás; and Brasília in the Federal District (Brandão et al., 2016). Considering that this species is related to streams restricted to gallery forests, sometimes isolated mountain ranges in the central portion of the Cerrado biome, it is possible that some degree of genetic differentiation and phenotypic variation should be recorded for these local populations, revealing a greater diversity of frogs than previously thought for these types of environments in the Cerrado.

Herein, we analyzed the morphological, morphometric, acoustic and molecular variation of *S. skaios* to evaluate the cryptic diversity of the taxa. We also performed a niche modeling to predict new areas of occurrence of the species.

Material and Methods

General procedures

We examined specimens in herpetological collections to be able to assess individuals from all localities where *S. skaios* already been registered. Type material also was examined. The Brazilian collections where the specimens examined are deposited are: Coleção Herpetológica da Universidade de Brasília (CHUNB), Brasília, Federal District; Coleção Zoológica da Universidade Federal de Goiás (ZUFG), Museu Nacional do Rio de Janeiro (MNRJ), Rio de Janeiro, state of Rio de Janeiro, and Centro de Estudos e Pesquisas Biológicas (CEPB), Pontifícia Universidade Católica de Goiás (PUC Goiás), State of Goiás. The adult specimens collected by us were euthanized with 5% xylocaine, fixed in 10% formaldehyde and stored in 70% ethanol.

Morphological observations

The qualitative phenotypic data were conducted by observation and comparison among adult specimens and on literature information (see below). Sex was determined by the presence of nupcial pads and vocal slits in males, and oocytes in females by direct visualization through transparence of the skin. Description of snout shape follows Heyer et al. (1990). Webbing formula follows Savage & Heyer (1967) as modified by Myers & Duellman (1982). Fingers nomenclature follows Fabrezi & Alberch (1996). Coloration always refers to living specimens.

We evaluate the morphology of 128 specimens of *S. skaio*s in total: nine from Alto Paraíso de Goiás, one from Barro Alto, 33 from Caiapônia, two from Formoso, eight from Luziânia, two from Niquelândia, 13 from Pirenópolis, five from the type locality, Santa Rita do Novo Destino, in State of Goiás; 18 from Brasília, Federal District, one

specimen from Unaí, Minas Gerais state, and 36 from Paracatu, Minas Gerais state. (see the list of specimens examined in Appendix 1).

Morphometric analysis

820   Measurements of adults following Duellman (1970): SVL (snout-vent length), HL (head length), HW (head width), IND (internarial distance), END (eye-nostril distance), NSD (nostril-snout distance), ED (eye diameter), UEW (upper eyelid width), IOD (interorbital distance), TD (tympanum diameter), HDL (hand length), FAL (forearm length), AL (arm length), THL (thigh length), TL (tibia length), TSL (tarsus length), FL

825 (foot length), TFDD (third finger disk diameter) and FTDD (fourth toe disk diameter). Measurements of TFDD and FTDD follows nomenclature of Fabrezi & Alberch (1996) (Table 1). All measurements were taken with a digital caliper (precision 0.01 mm) in millimeters. Specimens used for comparisons are listed in Appendix1.

To summarize and visualize the variation on morphometric dataset in populations

830 of *S. skaios*, we used a Principal Component Analysis (PCA) with the function PCA from R-package FactoMineR (Lê et al., 2008). This analysis was performed using SVL and 12 morphometric ratios (measure divided by SVL) from 76 males of 10 populations and five males of the *S. goya* (recent described species that formerly involving a population of *S. skaios*). We excluded from the analysis: the UEW, TFDD and FTDD, measures that can

835 easily involve high variation from specimen fixation issues; HL, HDL and THL do not follow normality assumptions; and females individuals are enough samples across populations. Component loadings, eigenvalues, and variation explained in each component are provided in Table 2.

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Acoustic analysis

We analysed 78 advertisement calls of 11 males in total across *S. skaios* distribution. It is from Santa Rita do Novo Destino, state of Goiás (type locality) (recording used by Pombal et al., 2010 for the original description of the species call and that we reanalysed), one call from one male; Caiapônia, state of Goiás on June 2010, seven calls from one male; Pirenópolis, state of Goiás on July 2019, around 1:00 a.m and 3:00 a.m, air temperature 18 Cº, 17 calls from two males; and Paracatu, state of Minas Gerais, on June 2018, 53 calls from seven different males. Vocalizations were recorded with Marantz PMD 660 digital recorder set at 44.100 Hz sample rate and 16 bits resolution coupled to directional microphone Sennheiser ME66. Calls were analysed using Raven Pro 1.5 (Cornell Lab of Ornithology, 2011) with the following settings: window type: Hanning, window size = 256 samples, 3dB filter bandwidth = 270 Hz, brightness = 75%, overlap = 85% and DFT = 1.024 samples. The sound figures were obtained using Seewave v. 2.1.0 package (Sueur et al. 2008) on the RStudio plataforma v. 1.1.463 (R Team, 2019). Seewave settings: window type = Hanning, sampling rate = 44100 Hz, overllap = 90%, window length = 256 points of resolution. The terminology used followed Köhler et al., 2017.

Molecular data procedures

Molecular analyses included the mitochondrial genes 16S (575bp) and Cytochrome c oxidase subunit I (COI, 646 bp). These genes have been important to delimiting species (Fouquet et al., 2007a). Whole cellular DNA was extracted from frozen and ethanol-preserved tissues (liver or muscle) using either phenol-chloroform extraction methods or the Qiagen Dneasy isolation kit (QIAGEN, Valencia, CA). Primers used to amplify the 16S were AR (forward - CGCCTGTTTATCAAAAACAT - Palumbi et al.,

1991; Wilkinson2 (Reverse - GACCTGGATTACTCCGGTCTGA - Wilkinson et al.,
1996), and primers used to amplify the COI were AnF1 (Forward -
ACHAAYCAYAAAGAYATYGG - Lyra et al., 2017; AnR1(Reverse -
CCRAARAATCARAADARRTGTTG - Lyra et al., 2017); AnCOIR (Reverse -
870 CCAAAGAATCARAADAAGTGTTG - Mariana Lyra, pers. comm.); COIf-F (Forward
- CCTGCAGGAGGAGGAGAYCC - Palumbi et al., 1991); COIa-R (Reverse-
AGTATAAGCGTCTGGGTAGTC - Palumbi et al., 1991). PCR amplifications was
carried out in 25 µL TAQ (Fermentas). The PCR protocol consisted of an initial
denaturation step of 3 minutes at 94ºC, 35 cycles of 30 seconds at 94ºC, 40 seconds at
875 48ºC, and 30-60 seconds at 72ºC, and final extension step of 10-15 minutes at 72ºC. The
PCR amplified products were cleaned with 0.5 µL of Exonuclease plus 1 µL of Alkaline
Phosphatase per 20 µL of reaction. Sequencing was done on an automatic sequencer ABI
3730XL (Applied Biosystems) in both directions to check for potential errors and
polimorphisms. The chromatograms obtained from the automatic sequencer were read
880 and contigs made using the molecular evolutionary analysis software MEGA version X
(Kumar et al., 2018) under default parameters for sequence alignment using CLUSTALW
(Thompson et al., 1994). Here we used the comparison of molecular distances, the
divergences corresponding to intraspecific distances over 3% can be considered as deep,
and this threshold is reasonable predictor of lineages describing potential candidate
885 species (Fouquet et al., 2007b).

Distribution modelling analysis

We conducted ecological niche models (ENMs) to predict the potential
distribution of *S. skaios* in order to support field sampling efforts and refine its
890 distributional model across Brazilian Cerrado (Syfert et al., 2014). First, we retrieved

occurrence records from museums and published literature. We only included in our analysis localities with precise georeferencing. Second, we selected six environmental (Mean Diurnal Range, Isothermality, Mean Temperature of Warmest Quarter, Precipitation of Wettest Quarter, Precipitation of Driest Quarter, Precipitation of Coldest Quarter) and one geospatial (terrain slope) data layers at 30 arc second (~1km at the Equator) spatial resolution. Environmental variables were choose from 19 bioclimatic variables of the worldclim database (http://www.worldclim.org; Fick & Hijmans, 2017) using a factor analysis with "varimax" rotation of R-package Psych (Revelle, 2019). The geospatial layer was constructed from the digital elevation model (http://srtm.csi.cgiar.org; Jarvis et al., 2008) using the terrain function of the R-package raster (Hijmans & van Etten, 2014). We modelled occurrence records with environmental-geospatial layers using 20 simulations by four presence-only ENM-methods (Bioclim, Gower distance, Mahalanobis distance and Maxent) with cross-validation (75% training and 25% test) using the R-package dismo (Hijmans et al., 2015). Next, a consensus map was built under ensemble approach (Araújo & New, 2007), averaging the suitabilities predicted from all ENM simulations considering only models with Area Under a Curve (AUC) higher than 70% (69 out of 80 models: mean AUC=0.91, sd=0.093). To know the sources of uncertainties in the ensemble, we used a two-way Analysis of Variance (ANOVA) using the standardized suitability values for each grid cell as dependent variable and methods as factors (Diniz-Filho et al., 2009). Next, we mapped the sum of squares (methods and residuals) allowing observed where each effect is more important in explaining the distributions. Finally to describe the potential area of distribution and discuss areas of importance to conservation of *S. skaios*, we overlaying the consensus map with shapefile of the municipalities (obtained from R-package

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915   brazilmaps; Siqueira, 2020) and conservation units (obtained from georeferenced data;

MMA, 2020) of the Brazilian Cerrado.

Results

Species identification

920   We found some unreported variation in diagnostic morphological/chromatic traits

defined in the original species description, especially in SVL, HW, END, ED, IOD, TL,

snout shape, and patterns of spots, blotches and stripes. Below, we comment on traits used

in species identification and describe trait variation previously unreported.

Diagnosis of the *Scinax skaios* by Pombal et al. (2010): A small species (SVL of

925   males 23.2–27.6 mm and females 29.7–36.1 mm, Fig. 1-A; B) belonging to the *Scinax*

catharinae species group, characterized by snout subovoid in dorsal view and protruding

in lateral view; no tubercle on the *canthus rostralis*; an inverted triangular interorbital

blotch; no vocal sac expanded externally; dorsal skin texture moderately rugose; absence

of a thick and well differentiated inguinal gland; hidden areas of thigh with vermiculate

930   pattern. Images of the living individuals of the populations of type locality (holotype),

Brasília, Alto Paraíso de Goiás, Caiapônia, Paracatu, and Pirenópolis, can be seen in

figure 1.

With regard to external morphology, the differences are subtle, more qualitative

than quantitative. Individuals from the populations of Brasília (DF), Caiapônia, and

935   Paracatu exhibited morphological differences in head shape, eye-nare distance, head

length, *canthus rostralis* and tibia length when compared to topotypes. The head shape is

acuminated in topotypical population of *S. skaios*, more rounded in dorsal view when

compared to the population of Brasília, and even more rounded on individuals from

Caiapônia than others populations (see Figure 2-3). In relation to topotypical population,

940   eye-nare distance and head length are bigger in the population of Caiapônia, which gives

the impression of more sparse eyes (see Figure 2-3). The *canthus rostralis* of the

specimens of all populations are well defined, however, the difference between the

individuals of Paracatu and the others is visible, being much deeper (see Figure 1I-J). The

tibia length also differs between populations, being higher in individuals of the type

945   locality, and smaller in specimens of Paracatu.

We noticed that inguinal region and hidden areas show differences in color and

blocthes pattern (Figs. 4-5) when compared to topotypes (vermiculate dark brown spots

on light green background: see also Figure 1). Some specimens from Brasília show

vermiculate dark brown to black spots on yellowish background in males or greenish

950   background in females (C-D from Figure 1). The population of Caiapônia exhibited

irregular dark brown spots, spaced and barely evident under a pale yellow background in

males, well-defined dark brown vermiculate pattern on a yellowish background in

females (E-F from Figure 4-5). Paracatu population show irregular dark brown blotches

on pale yellowish background (Figure 5-I).

955   There is variation in the pattern of color and size between males and females. The

females are always bigger than the males and have a more striking and marked color than

the males, which generally show a more uniform color throughout the body, and a paler

yellow with the pattern of little blotches (Fig. 1-D; G-H; K; N). We also can see

differences in adhesive disc sizes (broader in Brasília population and even wider in

960   Caiapônia population). The individuals from Brasília may have greater pigmentation on

the dorsum and belly (Fig. 1-E).
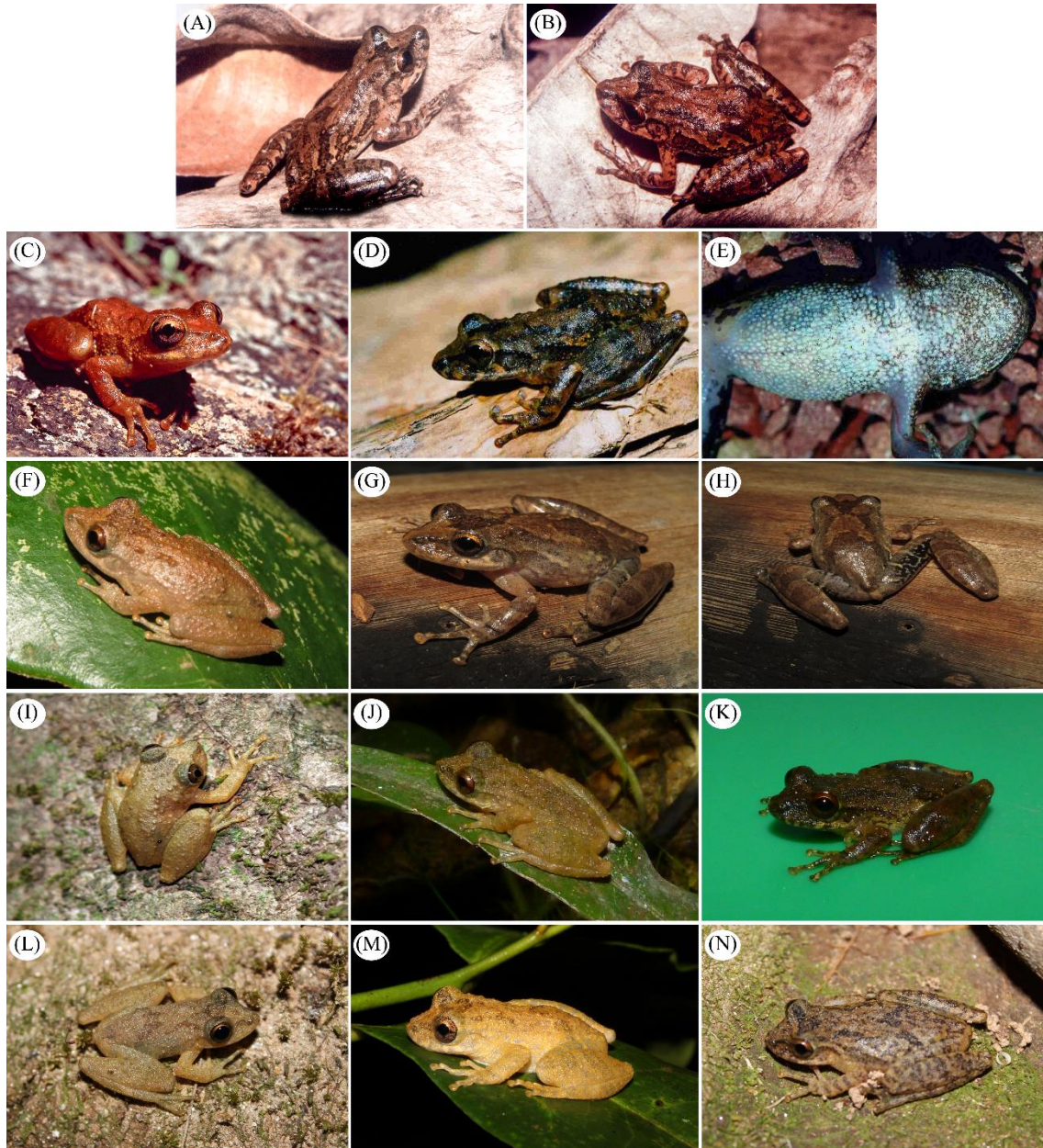
Figure 1. *Scinax skaios* from different localities: Santa Rita do Novo Destino, State of Goiás (type locality) - (A) lateral view and (B) dorsal view of a female; Brasília, Federal District - (C) male (D) view dorsolateral of a female, (E) ventral view of a female; Alto Paraíso de Goiás (Chapada dos Veadeiros), State of Goiás - (F) male (note the paler coloration in males) (G) view dorsolateral of a female (H) dorsal view of a female, evidence of the hidden parts of the thighs; Caiapônia, State of Goiás - (I) male; Paracatu, State of Minas Gerai - (J) male and (K) female; Pirenópolis, State of Goiás - (L-M) males and (N) female. Photos by Ronald Carvalho (A-B); Marcelo Kokobum (C-E); Reuber A. Brandão (F-H); Natan M. Maciel (I), and Alejandro Zuleta (J-N).
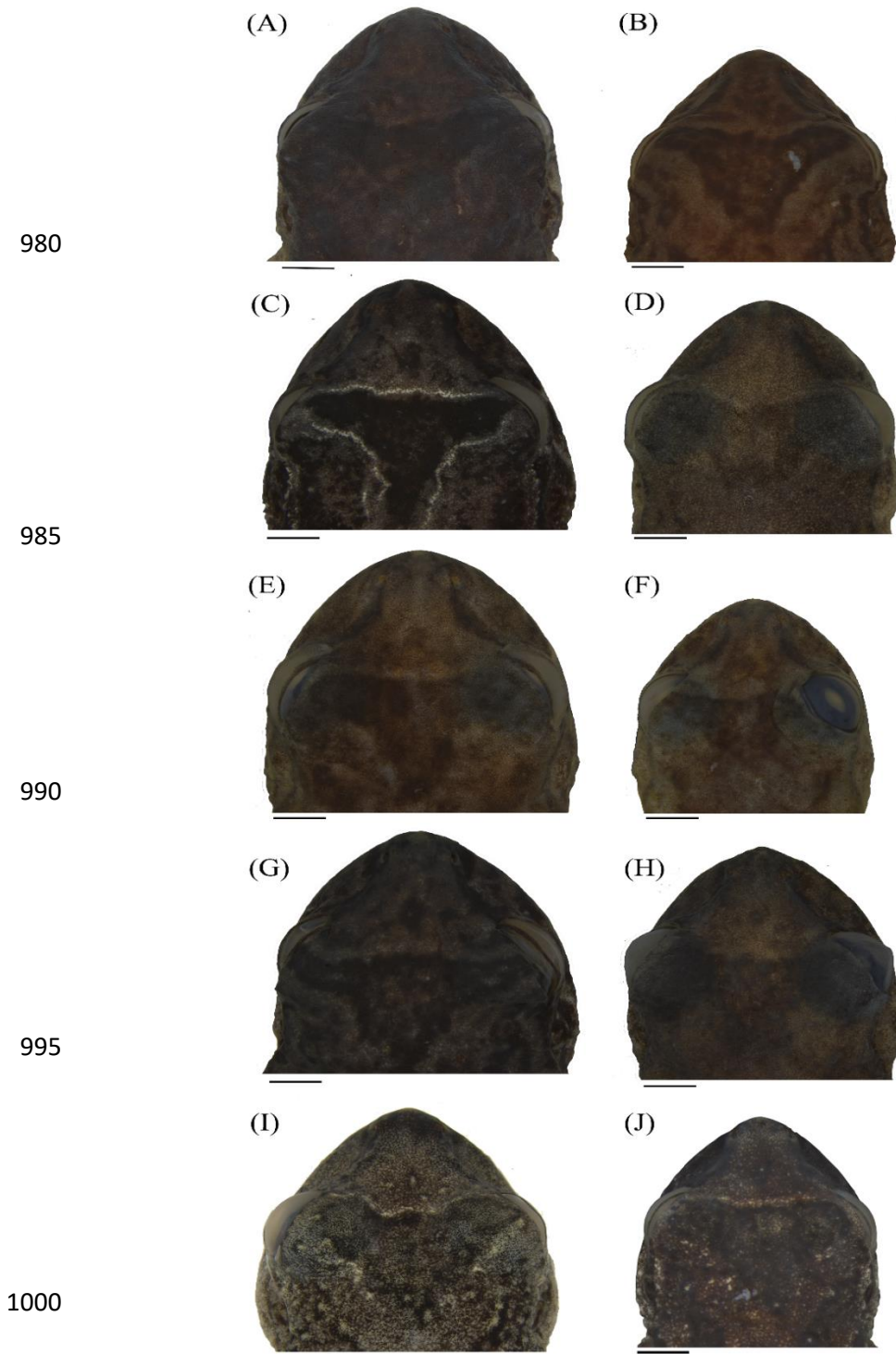
Figure 2. Dorsal head view of the different populations of *Scinax skaios*. Females on the left and males on the right. *Scinax skaios* from Santa Rita do Novo Destino (type locality) (A); Niquelândia (B); Pirenópolis (C-D); Caiapônia (E-F); Brasília (G-H); Paracatu (I-J). Scale bar = 2mm.
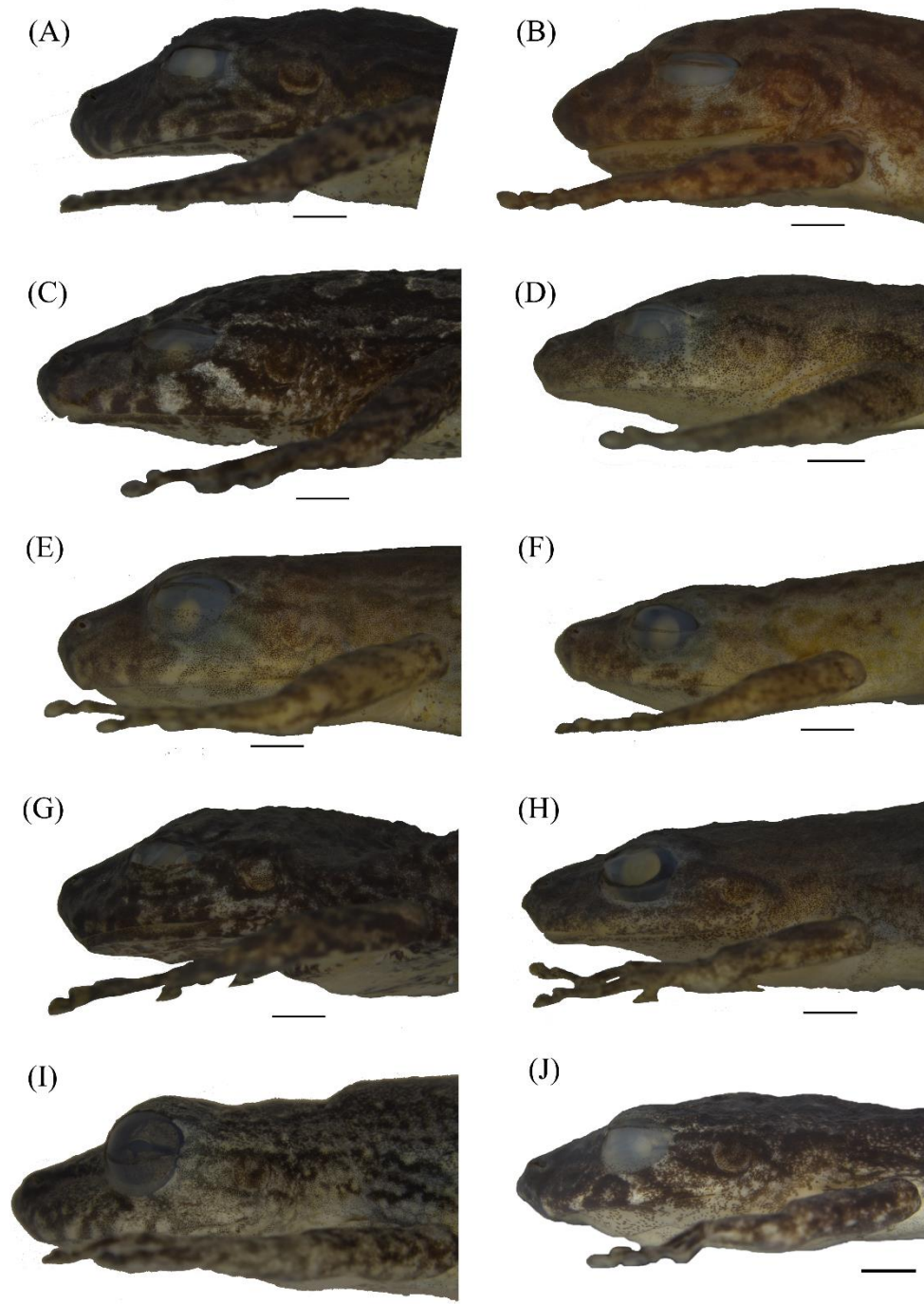
Figure 3. Lateral head view of populations of *Scinax skaios*. Females on the left and males on the right. Populations from: Santa Rita do Novo Destino (type locality) (A); Niquelândia (B); Pirenópolis (C-D); Caiapônia (E-F); Brasília (G-H); Paracatu (I-J). Scale bar = 2mm.
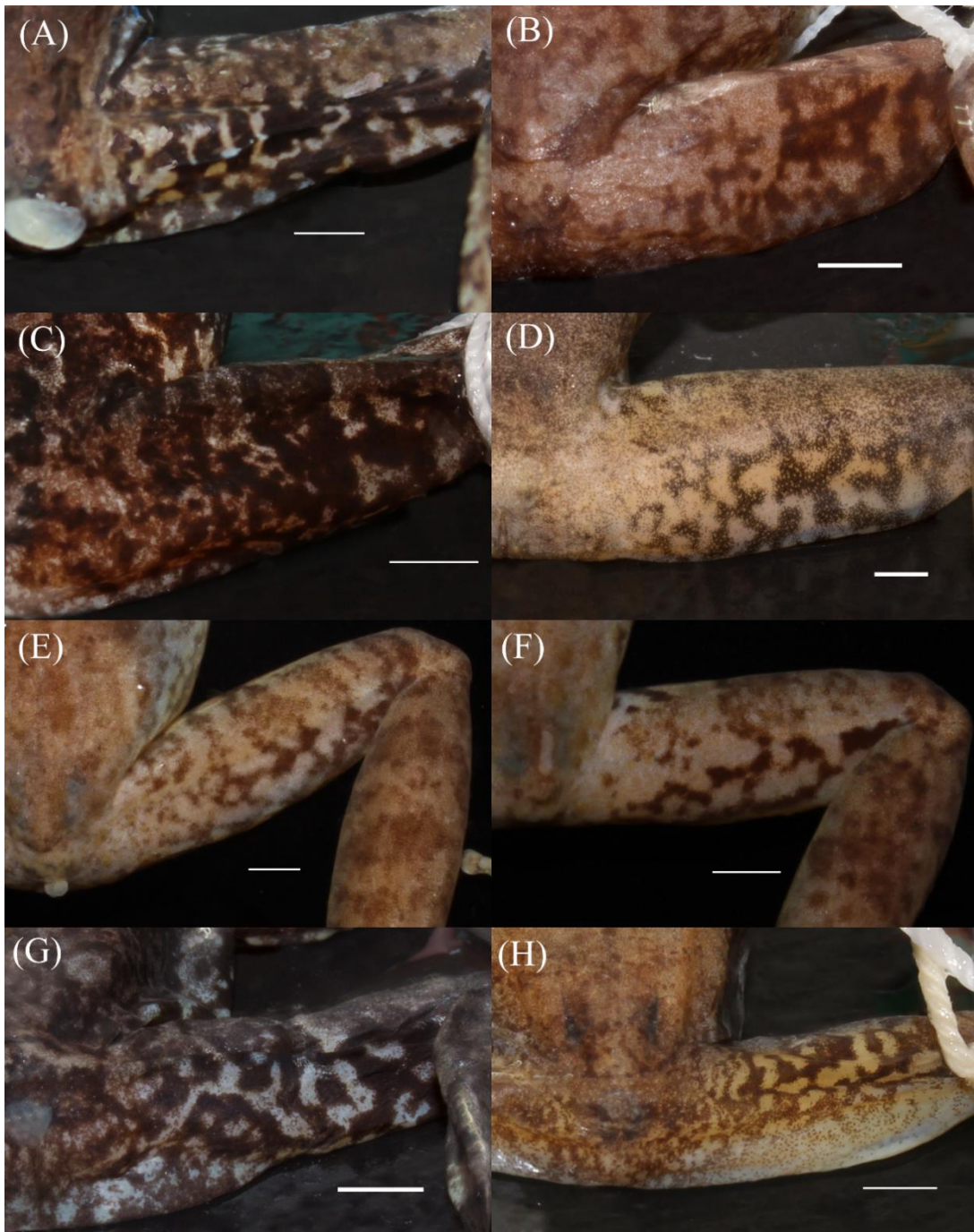
Figure 4. Hidden areas of thighs of *Scinax skaios*. Females on the left and males on the right. Populations from: Santa Rita do Novo Destino (type locality) (A); Niquelândia (B); Pirenópolis (C-D); Caiapônia (E-F); Brasília (G-H). Scale bar = 2mm.

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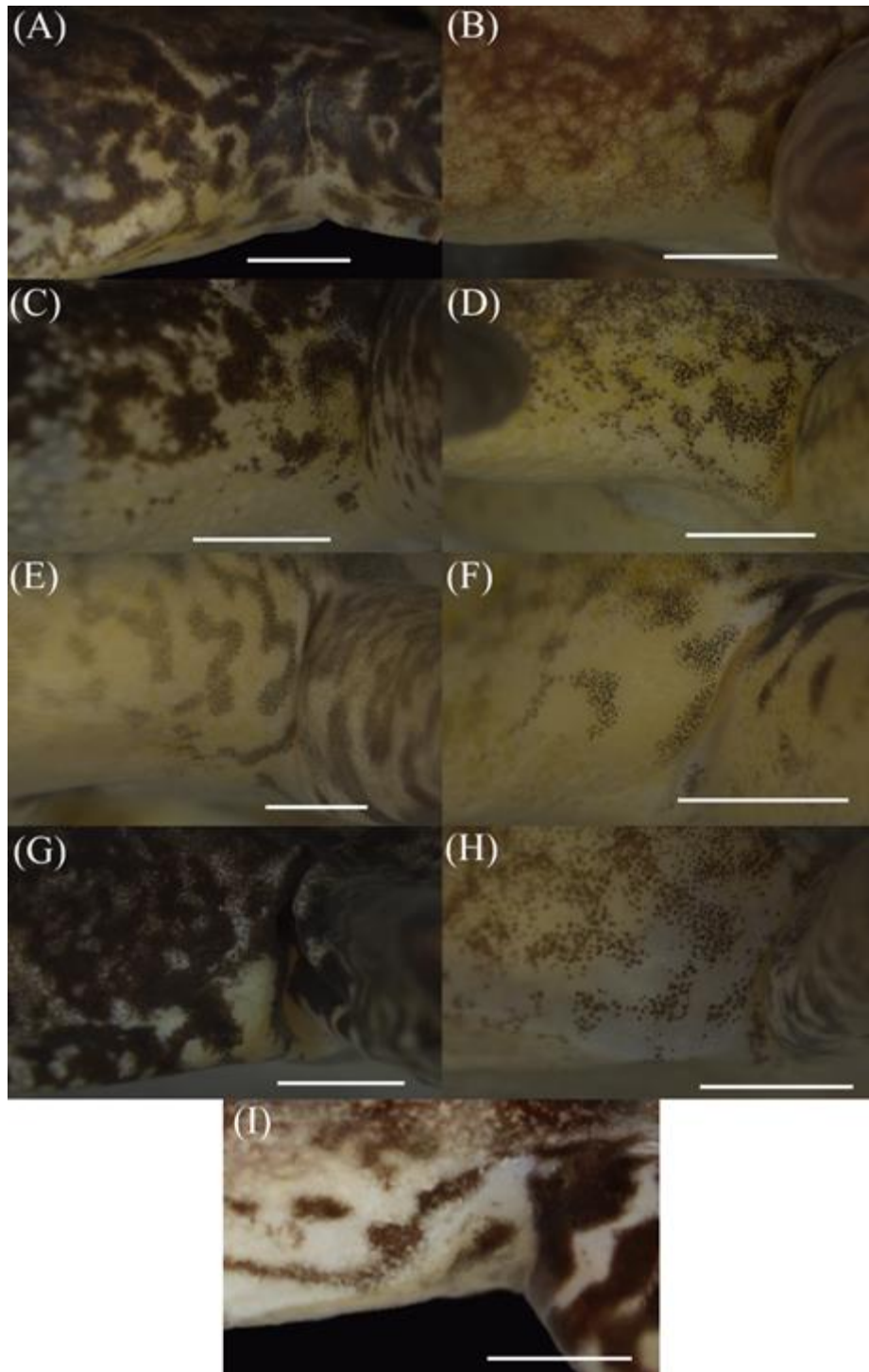
Figure 5. Inguinal region of *Scinax skaios*. Females on the left and males on the right. Populations from: Santa Rita do Novo Destino (type locality) (A); Niquelândia (B); Pirenópolis (C-D); Caiapônia (E-F); Brasília (G-H); male from Paracatu (I). Scale bar = 2mm.

Table 1. Morphological traits (mm) of adult specimens of eigth populations of *Scinax skaios*. Mean ± SD. N = number of specimens measured.

Trait	Santa Rita do Novo Destino (type locatity) (N=5)	Alto Paraíso de Goiás (N=9)	Caiapônia (N=33)	Luziânia (N=8)	Niquelândia (N=2)	Pirenópolis (N=13)	Brasília (N=18)	Paracatu (N=36)
Snout-vent length	28.37±3.82	23.47±3.16	27.50±3.05	28.16±4.97	26.77±1.65	26.61±3.47	29.01±4.08	25.59±3.77
Head length	10.82±1.21	9.78±0.86	10.48±1.10	10.58±1.66	10.39±0.50	9.69±0.98	10.56±1.59	9.61±1.26
Head width	9.79±1.30	9.00±0.85	9.91±1.07	10.00±1.65	9.64±0.02	9.21±0.94	10.26±1.45	9.14±1.23
Internarial distance	2.29±0.33	2.15±0.28	2.28±0.28	2.21±0.42	2.23±0.01	2.07±0.22	2.41±0.41	2.11±0.30
Eye-nostril distance	2.52±0.39	2.81±0.72	3.14±0.48	3.02±0.58	3.40±0.57	2.84±0.37	3.36±0.55	2.90±0.48
Nostril-snout distance	1.16±0.30	1.51±0.15	1.74±0.24	1.62±0.32	2.01±0.14	1.55±0.34	1.74±0.37	1.40±0.22
Eye diameter	3.32±0.26	3.79±0.52	4.16±0.42	3.70±0.59	4.40±0.41	3.64±0.30	4.29±0.75	3.59±0.53
Upper eyelid width	2.57±0.41	2.37±0.29	2.37±0.51	2.44±0.55	2.62±0.14	2.29±0.31	2.53±0.42	2.14±0.35
Interorbital distance	3.02±0.29	5.21±0.64	5.97±0.60	5.55±0.91	6.20±0.54	5.36±0.66	5.98±0.91	5.23±0.68
Tympanum diameter	1.42±0.24	1.47±0.19	1.46±0.22	1.26±0.10	1.17±0.09	1.26±0.21	1.31±0.39	1.38±0.68
Hand length	9.02±1.10	8.28±0.59	8.56±1.17	8.56±0.88	8.71±0.26	8.92±1.10	9.04±1.42	8.06±1.15
Forearm length	4.82±0.73	4.16±0.61	4.30±0.74	4.61±0.92	3.98±0.60	4.39±0.37	4.54±0.94	4.31±0.82
Arm length	3.88±0.81	3.29±0.44	3.40±0.64	3.77±3.78	3.63±0.61	3.17±0.45	3.60±0.81	3.08±0.49
Thigh length	14.63±1.96	13.53±1.42	14.32±1.78	14.43±1.80	14.08±1.30	13.65±1.41	14.95±2.33	13.65±1.84
Tibia length	16.65±2.05	12.46±1.51	13.01±1.85	13.38±2.25	12.55±0.76	12.49±1.27	13.88±2.24	12.39±1.69
Tarsus length	7.85±0.99	6.43±0.65	7.01±0.97	6.68±0.98	6.7±0.19	6.69±0.92	7.42±1.14	6.47±1.04
Foot length	12.78±1.42	12.09±0.99	12.26±1.61	12.6±2.03	12.03±1.48	11.97±1.15	13.31±2.24	11.80±1.59
Third finger disk diameter	1.05±0.12	1.05±0.12	1.04±0.21	1.16±0.17	1.28±0.08	1.07±0.15	1.23±0.34	1.06±0.21
Fourth toe disk diameter	1.1±0.24	0.97±0.11	0.93±0.24	1.08±0.12	1.14±0.06	0.90±0.14	1.14±0.30	0.90±0.19

53

Principal component analysis (PCA) for correlation matrix showed that the first two major components explain 44% of the data variation, with component 1 retaining 25.9% of the variation (explaining most of the variation) and component 2 retaining 18.1% of the data variation (Table 2; Appendix 2). The eigenvectors indicated that HW, IOD, and TL were the variables that contributed most to the first component and indicated the ED, NSD, and IOD variables to the second component (Figure 6). In the multivariate space we observed visually two groups directioned by IOD. The first group concentrated the populations Caiapônia (GO), Poço Azul (Brasília-DF) and Brasília (DF) with high IOD, while the second populations, Barro Alto (GO), Santa Rita do Novo Destino (GO), and Pirénopolis (GO) exhibiting lowest IOD together with the population of *S. goya* (Figure 6A-B). The population of Paracatu (MG) shows high morphometric variation across the multivariate space. The population of Alto Paraíso de Goiás (GO) clustered separated from other populations of *S. skaios* exhibiting differences in HW, TL and FL (Figure 6A-B).

Table 2. Scores (with contribution in parenthesis), eigenvalues, and explained variation of the first three principal components retained from a PCA on a morphometric dataset of eight populations of *Scinax skaios*: Alto Paraíso de Goiás, Caiapônia, Luziânia, Niquelândia, Pirenópolis, and Santa Rita do Novo Destino (type locality), State of Goiás; Brasília, Federal District, and Paracatu, State of Minas Gerais, Brazil.

Trait	PC1	PC2	PC3
Snout-vent length	13.92	0.0001	21.985
Head width	17.768	0.441	0.322
Internarial distance	6.392	5.418	6.7
Eye-nostril distance	4.299	0.021	25.598

Nostril-snout distance	3.007	19.037	3.734
Eye diameter	4.087	11.571	15.325
Interorbital distance	2.963	19.039	2.167
Tympanum diameter	7.445	3.468	12.939
Forearm length	4.711	17.59	0.678
Arm length	3.511	9.918	7.025
Thigh length	13.089	6.826	0.14
Tarsus length	6.408	1.218	3.333
Foot length	12.393	5.446	0.048
Eigenvalues	3.371	2.349	1.271
Percentage of variation	25.935	18.073	9.78
Cumulative percentage of variance	25.935	44.008	53.788

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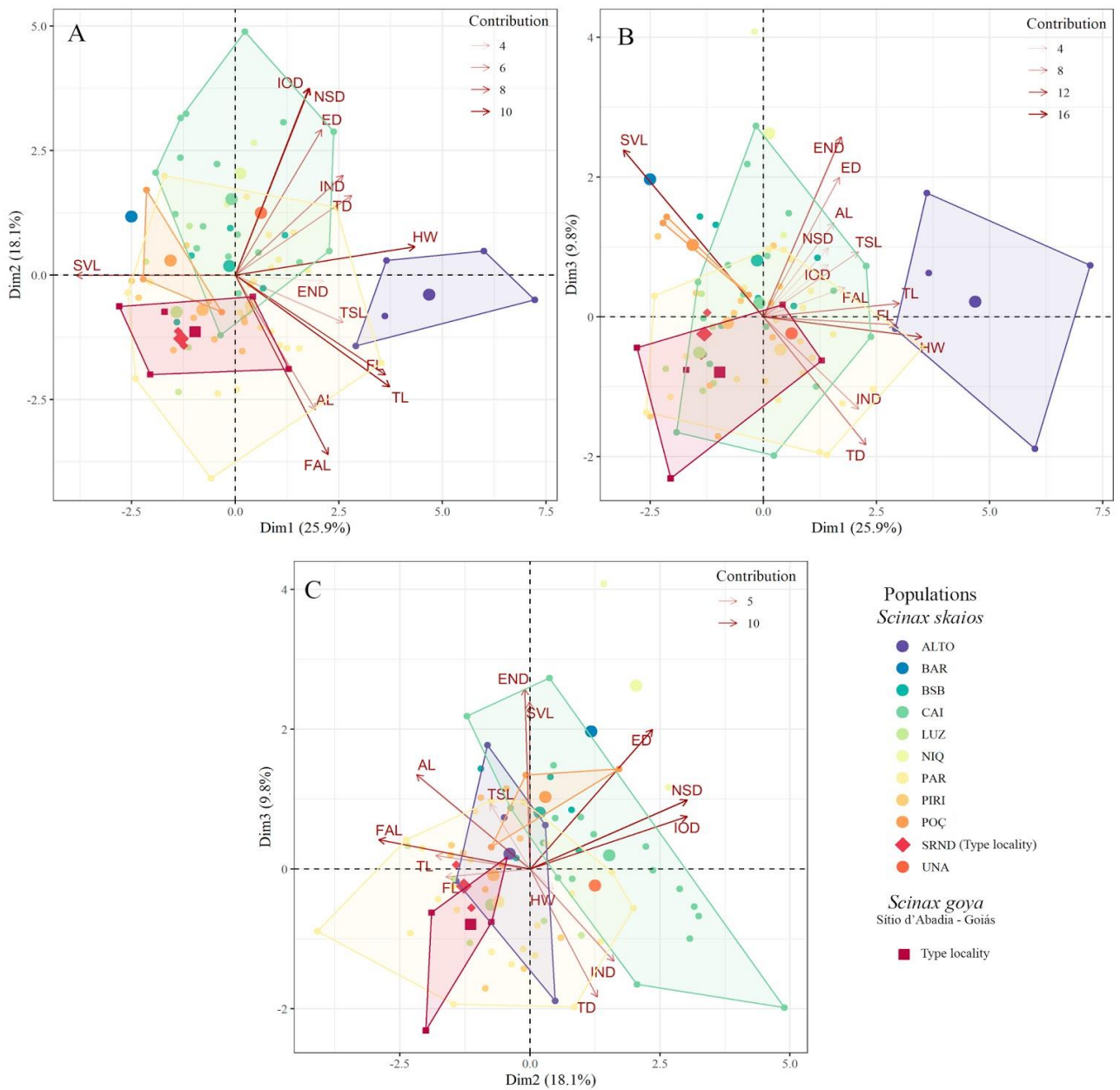
Figure 6. Multivariate morphometric space of *Scinax skaios*. Scatterplots of the three principal component scores for *S. skaios* from 11 populations of the Brazilian Cerrado plus *S. goya* from Sítio d'Abadia (Type locality). A) Dim1 and Dim2; B) Dim1 and Dim3; and C) Dim2 and Dim3. Polygon shows the localities of *S. skaios*: Alto Paraiso de Goiás-GO (ALTO), Poço Azul-Brasília, DF (POÇ), Caiapônia (CAI), Paracatu-MG (PAR); and *S. goya* in Sítio d'Abadia. Arrows indicating the percentage of contributions of morphometric variables to principal component scores.

Acoustic descriptions

The advertisement calls of four populations of *S. skaios* (Figure 7) have stereotyped temporal and spectral structure consisting of pulsed notes, that in accordance with Hepp et al. (2017), we classified as short calls.

Advertisement call of *S. skaios* from original description, by Pombal et al. (2010): They analyzed two calls, lasting 4.42 s and 7.9 s, with 42 and 73 notes. Average note duration was 0.27 ± 0.012 s (0.013-0.056, N=10 notes), emitted on an interval of 0.087 ± 0.053 s (0.036-0.168 s, N=10 intervals). The calls presented 312 or 612 pulses and notes had 9.2 ± 3.33 pulses (5-16, N= 10 notes). The dominant frequencies were 2.205 and 2.243 Hz. The equipment used by the authors for recording the vocalizations was Panasonic RQ-L309 portable tape recorder, and they were analyzed with Avisoft-Sonagraph Light and Cool Edit 96 softwares (here we used different equipments and analysis softwares).

The calls of *S. skaios* of three analysed populations (Caiapônia, Pirenópolis, and Santa Rita do Novo Destino) are very similar, with few differences in some parameters, mostly on temporal traits, as call duration, and number of notes, and subtle variation on the spectral parameter, dominant frequency (see Table 3). The call duration of males from type locality was longer than others. The number of notes of *S. skaios* calls are composed of a large number of pulsed notes, considering reaching up to 84 notes in the advertisement call of the Caiapônia population. The dominant frequency in all analyzed populations has a similar range, between 2.2 to 3.0 Hz, except for the Paracatu population, which has a frequency that can reach up to 3.5 Hz.

Males calling close to the rivulets in the gallery forests or in the cerrado near gallery forests. They used branches of marginal vegetation, rocks or on the ground ate the water borders to vocalizing. In both Santa Rita do Novo Destino and Pirenópolis

57

localities, the males started to vocalize around 9:00 p.m. Males from Caiapônia start calling later in the night, around 11:00 p.m to midnight (personal observation), and males from Brasília, although they were not recorded, they were heard calling only after midnight (R. Brandão personal observations). Unlike other *S. skaios* populations, males from Paracatu start their vocalization activities begin at sunset, around 5:30 pm.

Table 3. Acoustic parameters of advertisement call of males from Caiapônia, Santa Rita do Novo Destino (type locality), Pirenópolis (State of Goiás), and Paracatu, (State of Minas Gerais) Brazil. Abbreviations: CD = call duration; NN = notes number; ND = Note duration; NP = number of pulses; PD = pulse duration; NI = Notes intervall; CR = call rate per minute; DF = dominant frequency. N = call/males.

Population	CD	NN	ND	NP	PD	NI	CR	DF
Caiapônia (N=7/1)	2.2 ± 2.1 (0.69–5.53)	34.5± 31.3 (12–84)	0.02±0.00 (0.01–0.03	5.7±1.3 (4–7)	0.002±0.00 (0.002–0.003)	0.03±0.00 (0.02–0.04)	3±0	2.9±446.1 (2.3–3.0)
Santa R N Destino (N=1/1)	4.38	42	0.028	7	0.002	0.051	1	2.2
Pirenópolis (N=17/2)	3.7 ± 2.3 (1.94–10.4)	26.8 ± 10.3 (12–56)	0.02 ± 0.00 (0.01–0.03)	5.2 ± 1.2 (3–7)	0.002±0.000 (0.002–0.003)	0.06±0.01 (0.04–0.09)	5±0	2.6 ± 237.8 (2.2–3.0)
Paracatu (N=53/7)	0.26±0.06 (0.17–0.41)	3.49±0.63 (3–5)	0.03±0.01 (0.01–0.06)	7.15±1.73 (4–10)	0.002±0.000 (0.002–0.005)	0.05±0.01 (0.02–0.08)	4.42±2.43 (1–8)	3.1±0.21 (2.5–3.5)

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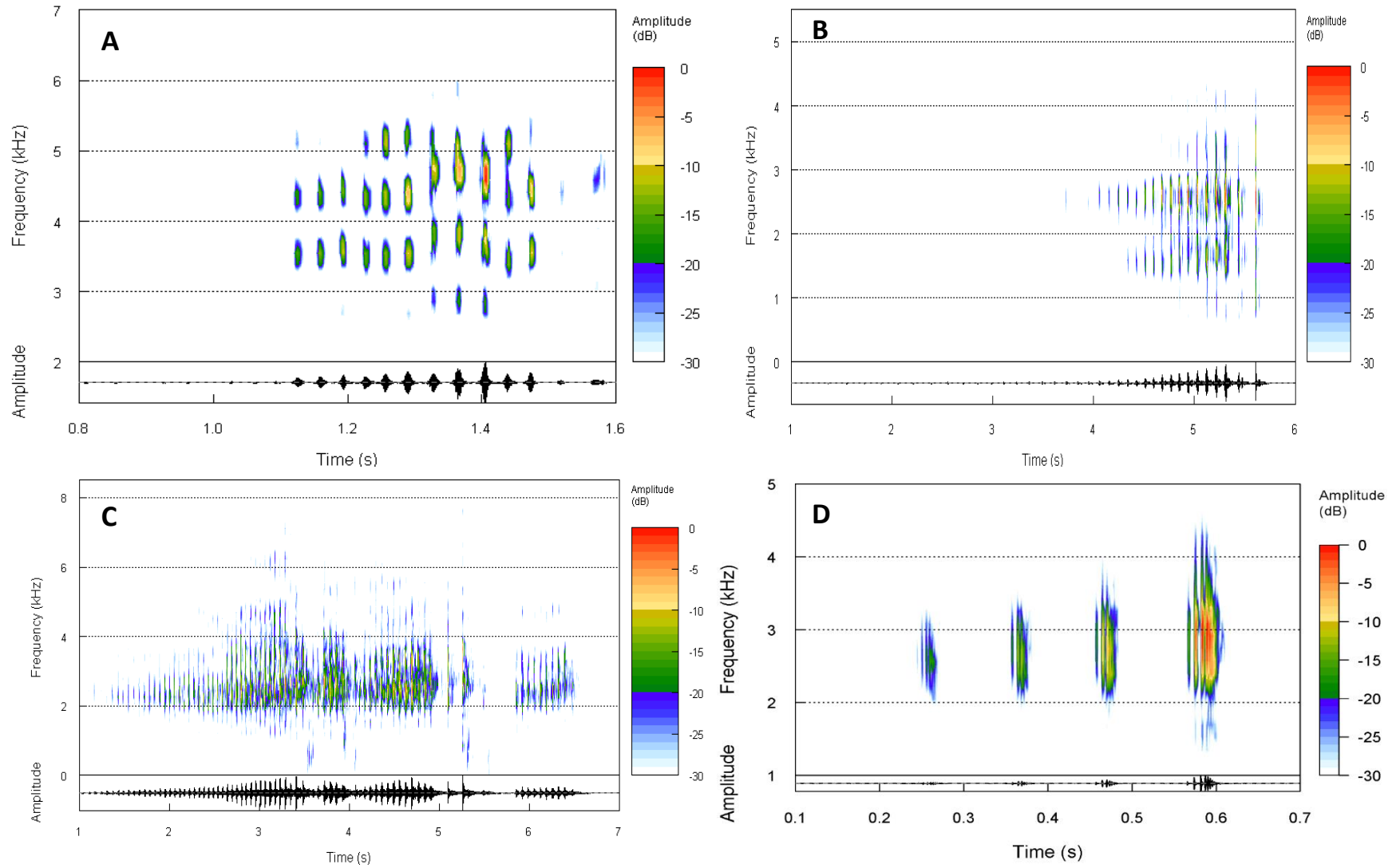
Figure 7. Spectrogram (above) and oscillogram (below) of advertisement call of *Scinax skaios* from Santa Rita do Novo Destino (type locality) (A), Pirenópolis (B), Caiapônia (C), and Paracatu (D).

Molecular assessment

Regarding 16S rRNA, *Scinax skaios* from Paracatu presents high genetic distance among all populations analyzed ranging 3.1% to 4.5%, differing 4.4%-4.5% from Brasília, 3.1% from type locality, 3.2% from Alto Paraíso de Goiás, 3.1% from Niquelândia, and 3.0%-3.2% from Caiapônia; the population from Caiapônia differs 3.2%-3.5% in relation to Brasília, 3.0%-3.2% from Paracatu (MG), 0.9-1.0% in relation to topotypical population, and Alto Paraíso de Goiás, and 0.6% in relation to Niquelândia. *Scinax skaios* from Brasília also showed high genetic divergence in relation to other populations, differing 4.4%-4.5% from Paracatu (MG), 3.3%-3.4% from Niquelândia, 3.2-3.5% from Caiapônia population, 3.3-3.4% from topotypical population, and 3.2%-3.3% from population of Alto Paraíso de Goiás. (Table 4). The *S. skaios* from Paracatu presents smaller genetic distances in relation to other species of the group *S. catharinae* than with its until then considered conspecific of other populations, as *S. pombali*, *S. longilineus*, and *S. centralis* (p-distances values of 3.9%, 2.9%, and 4.2%, respectively). It presents genetic distance similar in relation to *S. centralis* (uncorrected p-distance of 4.2%) of the distances presented in relation to other populations of *S. skaios* compared, and showed greater genetic distance in relation to *S. goya*, differing from this by 4.9%. Among the populations of *S. skaios* analyzed, a population in Brasília has a greater genetic distance among other species in the group, being closer genetically to *S. canastrensis* (uncorrected p-distance of 3.8-3.9%), differing from *S. longilineus*, *S. centralis*, *S. pombali*, and *S. goya* by 4.1-4.2%, 4.3-4.4%, 4.6%, and 4.8-4.9%, respectively. Individuals from Brasília are more genetically distant from *S. goya*.

The COI sequencing shows Paracatu population diverging 9.6% from toward to Caiapônia population, 8.2%-9.2% from Alto Paraíso de Goiás, 8.8% from topotypical population, and a genetic distance of 8.5% from toward to Niquelândia population. This

is remarkably higher than that within-species variation. The population of Caiapônia also presents high genetic distance in relation to other populations, differing 4.1-4.6% from Alto Paraíso de Goiás, 4.3% from Santa Rita do Novo Destino (type locality), and diverging 4% in relation to population of Niquelândia. There was no COI sequencing for

1160 *S. skaios* from Brasília (Table 5). In COI, the population of Paracatu shows smaller divergences also in relation to other species of the *S. catharinae* group (as well as for the 16S), the genetic distances were 7.9%, 7.8%, 7.1%, and 7% from toward to *S. longilineus*, *S. centralis*, *S. canastrensis*, and *S. pombali*, respectively. The genetic distances among *S. skaios* from Caiapônia and the other species of the *S. catharinae* group, were greater

1165 than the distances among it and the other populations of *S. skaios*, diverging 10.5% from *S. centralis*, 9% from *S. pombali*, and 8.9% from *S. canastrensis*, and *S. longilineus*.

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Table 4. Genetic distances of 16S rRNA fragment gene of the individuals from different populations of *Scinax skaios* and among the closest species of the *S. catharinae* group.

16S	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 *S. skaios_* ZUFG9644_Paracatu														
2 *S. pombali*_UFMG10610	0.039													
3 *S. longilineus*_CFBH17327	0.029	0.029												
4 *S. centralis*_ZUFG3242	0.042	0.039	0.033											
5 *S. canastrensis*_CFBH9153	0.028	0.029	0.009	0.032										
6 *S. goya*_MNRJ89024	0.049	0.042	0.041	0.048	0.042									
7 *S. skaios*_PHV4034_Niquelândia	0.031	0.045	0.034	0.039	0.031	0.053								
8 *S. skaios*_RAB1039_Santa Rita do N. Destino	0.034	0.046	0.035	0.036	0.030	0.057	0.006							
9 *S. skaios*_PHV4035_Niquelândia	0.031	0.045	0.034	0.039	0.031	0.053	0	0.006						
10 *S. skaios*_UFMG13862_Alto Paraíso de Goiás	0.032	0.045	0.035	0.040	0.032	0.050	0	0.007	0					
11 *S. skaios*_ZUFG6228_Caiapônia	0.030	0.042	0.038	0.039	0.033	0.050	0.008	0.009	0.008	0.009				
12 *S. skaios*_ZUFG6237_Caiapônia	0.032	0.044	0.040	0.041	0.035	0.051	0.009	0.010	0.009	0.010	0			
13 *S. skaios*_RAB1040_Brasília	0.044	0.046	0.041	0.043	0.038	0.048	0.033	0.033	0.033	0.032	0.032	0.034		
14 *S. skaios*_RAB1042_Brasília	0.045	0.046	0.042	0.044	0.039	0.049	0.034	0.034	0.034	0.033	0.033	0.035	0	

Table 5. Genetic distances of COI gene of the individuals from different populations of *Scinax skaios* and among the closest species of the *S. catharinae* group.

COI	1	2	3	4	5	6	7	8	9	10	11	12
1 *S. canastrensis*_CFBH9153												
2 *S. pombali*_UFMG10610	0.059											
3 *S. skaios*_ZUFG9644_Paracatu	0.071	0.07										
4 *S. longilineus*_CFBH17327	0.023	0.064	0.079									
5 *S. centralis*_ZUFG3242	0.078	0.072	0.078	0.091								
6 *S. skaios*_RAB1039_Santa Rita N. Destino	0.086	0.084	0.088	0.093	0.1							
7 *S. skaios*_PHV4034_Niquelândia	0.083	0.08	0.085	0.084	0.097	0.013						
8 *S. skaios*_PHV4035_Niquelândia	0.083	0.08	0.085	0.084	0.097	0.013	0					
9 *S. skaios*_ZUFG6228_Caiapônia	0.089	0.09	0.096	0.089	0.105	0.043	0.04	0.04				
10 *S. skaios*_ZUFG6237_Caiapônia	0.089	0.09	0.096	0.089	0.105	0.043	0.04	0.04	0			
11 *S. skaios*_RAB3699_Alto Paraíso de Goiás	0.083	0.081	0.082	0.087	0.098	0.014	0	0	0.041	0.041		
12 *S. skaios*_RAB3629_Alto Paraíso de Goiás	0.088	0.085	0.092	0.096	0.104	0.015	0.003	0.003	0.046	0.046	0.004	

Ecological Niche Modelling

The models based on the four ENMs-methods did not exhibit high variation between areas of greatest suitability (S2). Our model indicates highly suitable habitats (Suitability > 0.7) in 10% of the municipalities of Goiás, across the East and Northeastern of the state of Goiás, including areas in the Central Plateau, and around Federal District (Figure 9). The projection of the distribution models showed that predicted occurrence included the actual distribution of *S. skaios*, but also suggested that nearby areas with appropriate environmental requirements could be colonized by the species. *Scinax skaios* was until then known only for the type locality, municipality of the Santa Rita do Novo Destino, state of Goiás (Pombal et al., 2010; Frost, 2020) and a few other locations registered in museums and herpetological collections (Table 6, Figure 8). Of these records, only a few are in the literature, such as the species registration in Chapada dos Veadeiros, in the municipality of Alto Paraíso de Goiás (Santoro & Brandão, 2014). According to our models, *S. skaios* are unlikely to occur in the south region of the state of Goiás, as Caiapônia (Suitability < 0.6), and there is also a low probability of occurrence in the northwest of Minas Gerais State (Suitability < 0.7).
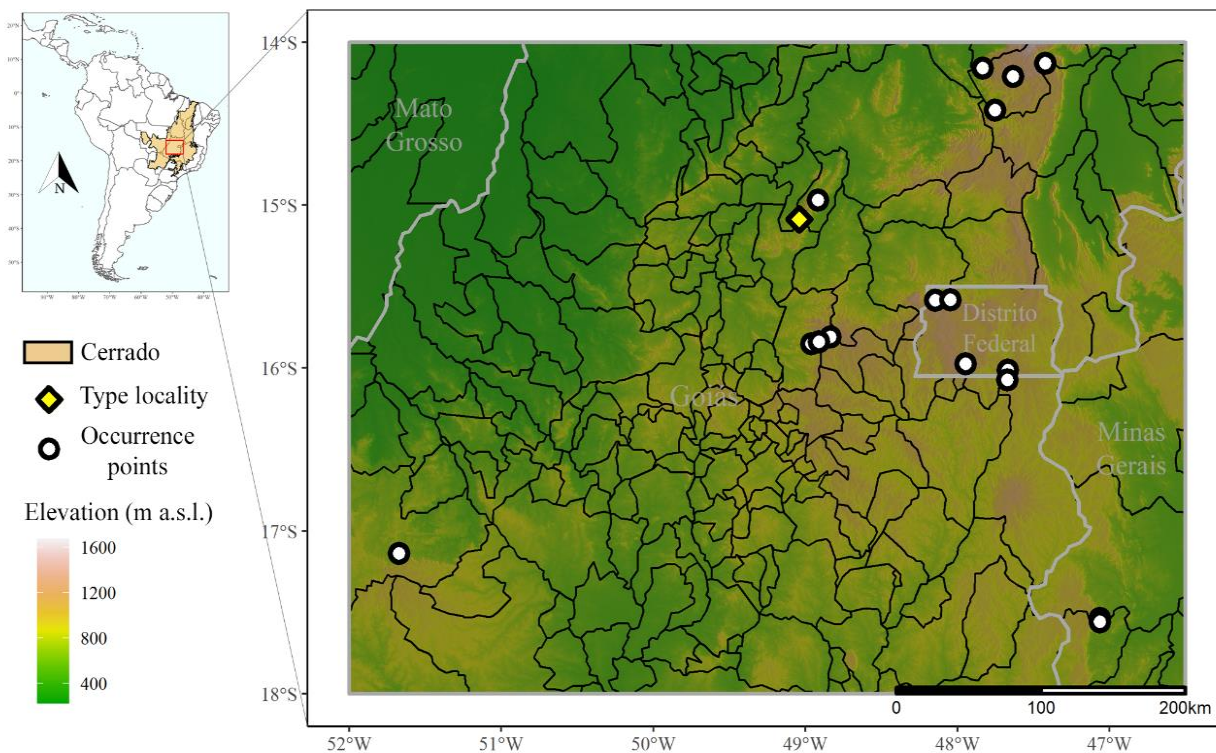
Figure 8. Known occurrence records of *Scinax skaios* (Only georeferenced records): Poço
Azul, Mumunhas, Brasília (Federal District), Alto Paraíso de Goiás (Chapada dos
Veadeiros), Barro Alto, Caiapônia, Luziânia, Pirenópolis, São João d' Aliança, Santa Rita
do Novo Destino (type locality) (State of Goiás), and Paracatu (Minas Gerais State).

Table 6. Georeferenced occurrence points of *Scinax skaios* with their respective
suitability values. Brazilian States abbreviations: DF = Distrito Federal; GO = Goiás; MG
= Minas Gerais.

Occurrence points	State	Suitability
Brasília	DF	0.64
Brasília	DF	0.66
Mumunhas	DF	0.69
Poço Azul (Brasília)	DF	0.65
Alto Paraíso de Goiás	GO	0.73

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Alto Paraíso de Goiás	GO	0.64
Alto Paraíso de Goiás	GO	0.7
Barro Alto	GO	0.59
Caiapônia	GO	0.54
Luziânia	GO	0.76
Pirenópolis	GO	0.65
Pirenópolis	GO	0.63
Pirenópolis	GO	0.63
Pirenópolis	GO	0.68
Santa Rita do Novo Destino	GO	0.72
São João d'Aliança	GO	0.66
Paracatu	MG	0.61
Paracatu	MG	0.65

1215   We identified in ensemble model potential areas of occurrence in 26 municipalities in Goiás, Federal District and Minas Gerais, within the Brazilian Cerrado domain (Table 7, Figure 9). Note that the expected suitable areas are concentrated in the central, eastern and northern regions of Goiás, including areas around the Federal District (Figure 9).

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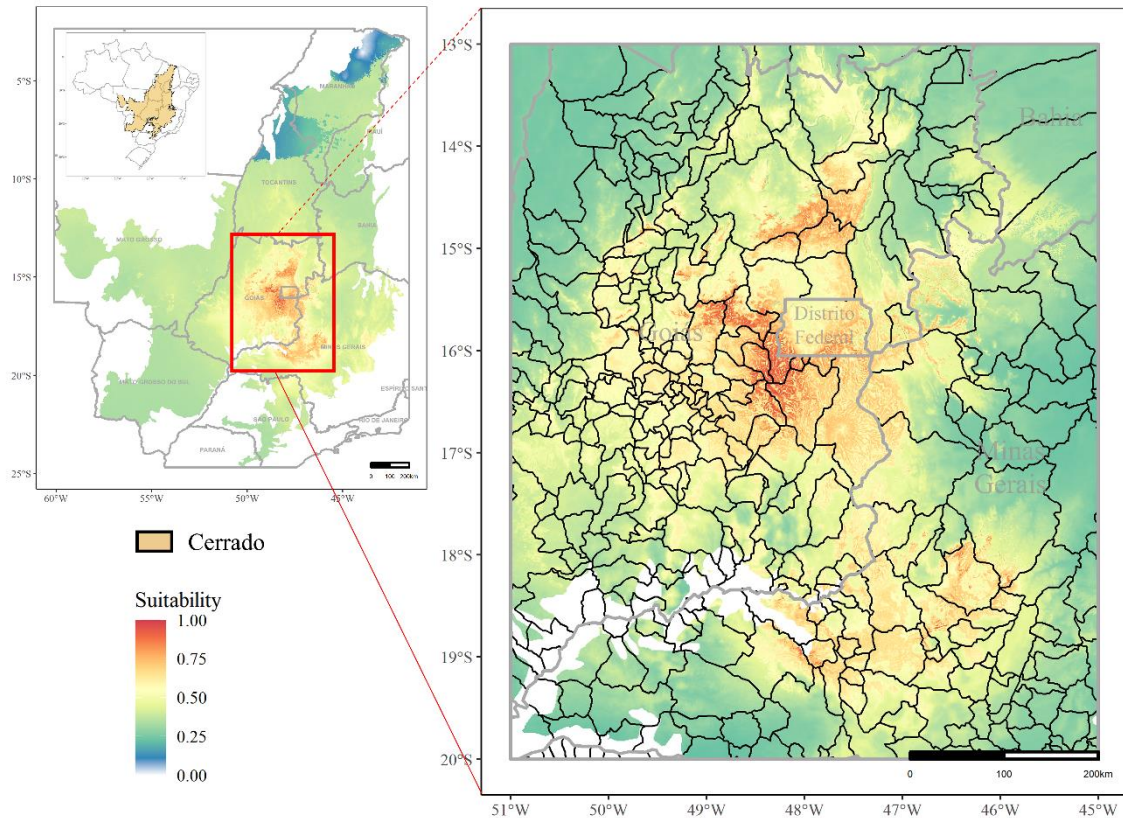
Figure 9. Consensus map built from the Ensemble approach, showing the predicted areas of all simulations with AUC > 0.7.

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Table 7. Summary of suitability by municipalities. The municipalities are in decreasing order of AUC and here are presented only those that presented suitability equal to or greater than 70%.

Municipality	State	Number of cells (> 0.7 suitability)	~Area (km^2) (> 0.7 suitability)	Suitability
Santo Antônio do Descoberto	Goiás	977	834.44	0.85
Novo Gama	Goiás	179	152.88	0.82
Alexânia	Goiás	844	720.85	0.81
Valparaíso De Goiás	Goiás	71	60.64	0.81
Cocalzinho De Goiás	Goiás	1494	1276	0.78
Águas Lindas De Goiás	Goiás	203	173.38	0.78
Cidade Ocidental	Goiás	388	331.39	0.77
Luziânia	Goiás	3758	3209.64	0.77

Silvânia	Goiás	2000	1708.17	0.75
Água Fria De Goiás	Goiás	1795	1533.08	0.74
Corumbá De Goiás	Goiás	987	842.98	0.74
Pirenópolis	Goiás	1494	1276	0.73
Abadiânia	Goiás	716	611.52	0.73
Gameleira De Goiás	Goiás	495	422.77	0.73
Vianópolis	Goiás	736	628.61	0.72
Cristalina	Goiás	4470	3817.74	0.71
Leopoldo De Bulhões	Goiás	414	353.59	0.71
Brasília	Federal District	3254	2779.17	0.7
Campo Limpo De Goiás	Goiás	105	89.68	0.7
Nerópolis	Goiás	150	128.11	0.7
Goianápolis	Goiás	148	126.4	0.7
Bonfinópolis	Goiás	127	108.47	0.7
Romaria	Minas Gerais	285	243.41	0.7
Pedrinópolis	Minas Gerais	269	229.75	0.7

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The overlay of the consensus map with shapefile of the municipalities and conservation units of the Brazilian Cerrado indicated suitable potential areas included in eight conservation units, considering only those with maximum suitability greater than

1235   80% (Appendix 2, Figure 10), are they: Área de Proteção Ambiental do Planalto Central (APA – Planalto Central), Reserva Biológica do Gama (REBIO do Gama), Área de Proteção Ambiental da Bacia do Rio São Bartolomeu (APA – Bacia do Rio São Bartolomeu), Área de Proteção Ambiental dos Pireneus (APA Pireneus), Área de Proteção Ambiental Pouso Alto (APA – Pouso Alto), Parque Distrital Salto do Tororó,

1240   Área de Proteção Ambiental da Bacia do Rio Descoberto (APA – Bacia do Rio Descoberto), Parque Nacional da Chapada dos Veadeiros (PARNA – Chapada dos Veadeiros).

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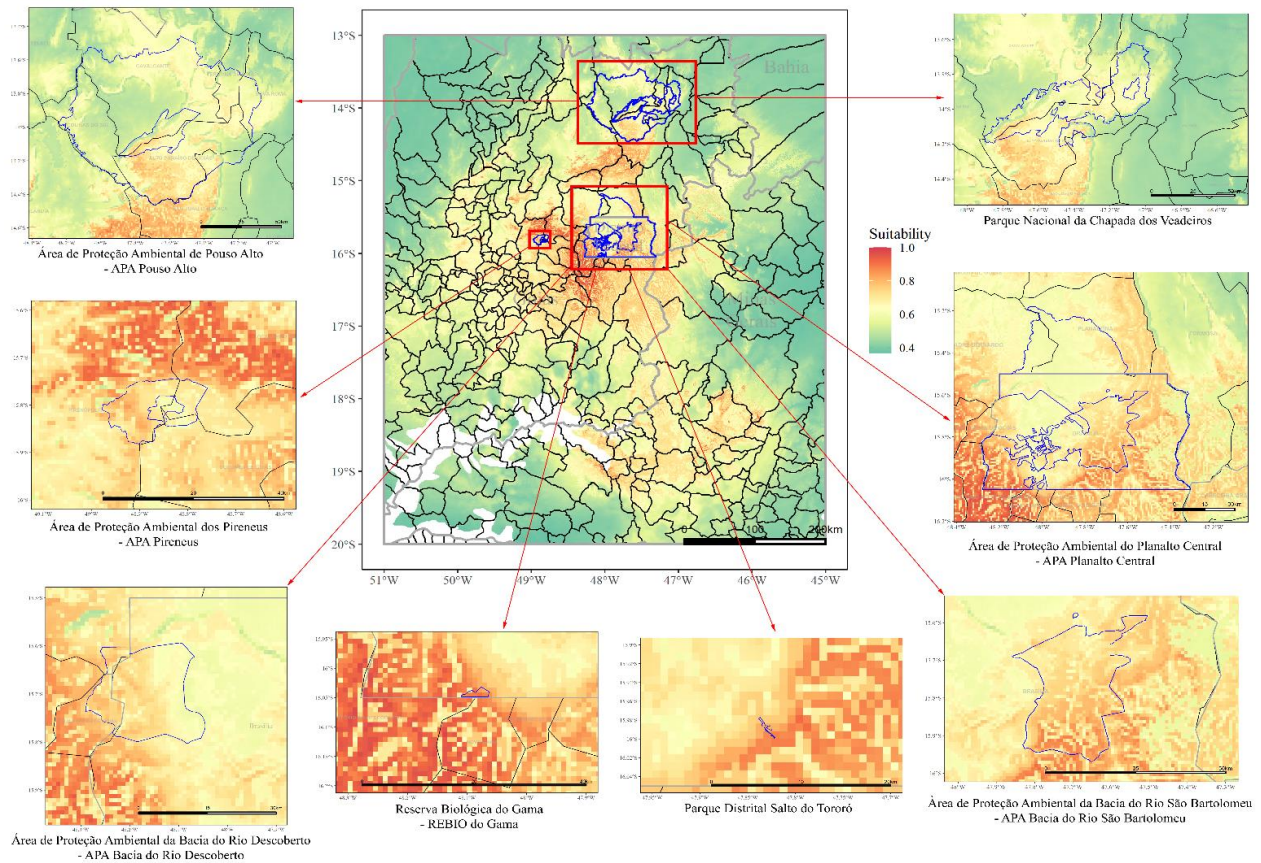
Figure 10. Conservation units within the Cerrado with a high probability of occurrence of *Scinax skaios* (maximum suitability > 80%).

Discussion

Through an integrative approach, we investigated the taxonomic identity and variation of a little known endemic Cerrado hylid, *Scinax skaios*. We evaluated the morphology of individuals from 11 different locations, material examined in museums and herpetological collections. After careful examination of the external morphology of the specimens, we observed some variations that are more qualitative than quantitative. Morphological variation is quite subtle, detected only when compared individuals of one population with another (mainly the topotypical population).

We were able to observe that three populations differ more in relation to the topotypes: Caiapônia (GO), Brasília (DF), and Paracatu (MG). Morphologically, the

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1260 features that most differentiate are the shape of the head, and the depth of the *canthus rostralis*, and morphometrically, the distance between nostrils and eyes, length of the head, and length of the tibia. Variation in coloration patterns has been widely recognized in amphibians (Hoffman & Blouin, 2000; Rudh & Qvarnstrom, 2013). It was possible to observe in individuals from different populations some variations in coloration and

1265 pattern of flanks and the hidden areas of the thighs (Figs. 1-6; 9-10). In addition to the morphological variations among populations, we also report variations in coloration between males and females, hitherto not mentioned in the literature, except for differences in size (Pombal et al., 2010). Individuals from Alto Paraíso de Goiás (Chapada dos Veadeiros), as well as those from Niquelândia, Barro Alto, Pirenópolis and Luziânia, do

1270 not present observable morphological differences, and the contribution of the morphometric variables was very small, overlapping all these groups.

When using acoustic traits in taxonomy, it is essential that the descriptions are detailed and correct to characterize the features and their variation, and thus provide a justification for the delimitation of a species (Köhler et al., 2017). Our results do not show

1275 substantial differences in the analyzed calls, except for the population of Paracatu, and some difference in the temporal and spectral features of the calls of Caiapônia. However, caution is necessary here, due to the low *N* sample in type locality, Caiapônia and Pirenópolis, it was not possible infer a call pattern that really represents those population, that is, we provide only an exploratory framework of the advertisement call pattern of

1280 few individuals from each analyzed population. The bioacoustic analysis showed that the temporal parameters such as the call duration, the number of notes, and pulses are the traits that most differentiate the populations, especially among the populations of Goiás and Minas Gerais, showing a divergence between the call pattern from the males of Paracatu with the others (Table 3). The calls of Paracatu have a much shorter duration

1285 and number of notes than other populations, with a greater number of pulses per note and a higher dominant frequency. The dominant frequency among the populations of Goiás analyzed are quite similar, however the population of Caiapônia showed slightly higher frequencies than the topotypical population and population of Pirenópolis.

The dominant frequency is considered a static trait, that is, there is little variation,
1290 however some males can change the amplitude of the call frequency during an interaction, but this change is relatively small (<5%) (Gerhardt, 1991). Considering the temporal and spectral (frequency) traits of the vocalizations, the results indicate the population of Paracatu as the most distinct. To obtain accurate taxonomic conclusions, it is necessary to correctly distinguish individual variation from intraspecific, this variation can be
1295 expected to be smaller among co-specifics of different populations, and greater between different species (Köhler et al., 2017). In this study we did not get a sufficient sample of calls by individual and from different males. Therefore, we suggest analyzing more calls from different males in the type locality (in this study only one call was used), Pirenópolis and especially analyzing more calls from Caiapônia, and Brasília that was not registered
1300 here, to better evaluate the interpopulation variations.

We had difficulties in getting vocalizations of the *S. skaios*, besides being a little known species, their reproductive period occurs in the middle of the year (in the driest and coldest months in the Cerrado), an unusual period for field expeditions to study frogs, because it is a period in which the rains have ceased and the temperature and humidity of
1305 the air drop a lot, with a low probability of finding anurans in reproductive activity. The period when our team went to the field to collect and record the vocalizations of the species, the males were no longer calling, probably that year they anticipated the reproductive period due to the early end of the rains.

Vences et al. (2005a, b) suggested that distance-based DNA barcoding could be a

useful tool reporting amphibian biodiversity. Pairwise divergences among populations of

S. skaios using the mitochondrial genes 16S-rRNA and COI revealed higher degrees of

differentiation mainly between Paracatu and Brasília in relation to topotypical population.

For 16S, the population of Paracatu differed from 3.1% to 4.5% of the other populations

of *S. skaios* and is closer to *S. canastrensis*, with a genetic distance of 2.8%. Despite being

morphologically more similar to *S. goya*, among the species compared, population of

Paracatu was the most genetically distant species (4.9%). *Scinax skaios* from Brasília also

showed high genetic divergence in 16S, differing from 3.2 to 4.5% of other populations.

The divergences over 3% can be considered as deep, and this threshold is a reasonable

predictor of lineages describing potential candidate species (Fouquet et al., 2007b). The

population from Brasília, as well as Paracatu population, also is more closer to *S.*

canastrensis, differing from this in 4.3-4.4%.

Regarding COI sequences, the population from Paracatu shows the greatest

divergence among the populations of *S. skaios*, differing from 8.2% to 9.6% of the other

populations (nucleotide divergence greater than the minimum of 3% threshold, proposed

by Fouquet et al. (2007b) to discriminate anuran species), and being genetically closer to

S. canastrensis (5.9%). The population of Caiapônia also presents high degrees of

divergence, differing from 4.0% to 9.6% of other populations. *Scinax skaios* of Caiapônia

seems to be closer to *S. canastrensis* and *S. longilineus* (8.9%). The COI gene has been

elected as an universal DNA barcode in animals (Hebert et al., 2003), however 16S gene

seems more effective to discriminate amphibian species (Vences et al., 2005a). Here we

used both genes for better data accuracy.

In recent years, molecular data using DNA barcoding has been incorporated into

research more frequently to investigate taxonomic status of Neotropical amphibian

species (Vences et al., 2005a; b; Fouquet et al., 2007a, b; 2013; Peloso et al., 2014; 2016; 2018; Ferrão et al., 2016; Nogueira et al., 2016; Correa et al., 2017; Orrico et al., 2017; Vaz-Silva et al., 2018; Carvalho et al., 2019; Escalona et al., 2019; Chasiluisa et al., 2020; Sturaro et al., 2020). Fouquet et al. (2007a), for example, found pairwise divergence among 16S sequences of specimens of *Scinax ruber* species group from Northeast of Brazil and French Guiana suggesting the presence of two different species. Nogueira et al. (2016) also found nucleotide divergence in *Scinax* sp. of the *S. catharinae* group from Chapada dos Guimarães for the 16S, with intraspecific divergence of 0.2%, as expected for the same species (Fouquet et al., 2007b), and they observed a high divergence of this *Scinax* sp in relation to other species of the group (6% to 13%), indicating a possible new species of the *S. catharinae* group for the region of Chapada dos Guimarães, in the Cerrado. High genetic divergences between two groups of *Boana crepitans* (Wied-Neuwied, 1824) was found by Orrino et al. (2017), with 4% in mtDNA 12S and 13% in COI, concordant with the recognition of the populations of two distributional groups as distinct species. In another recent study, Escalona et al. (2019) also using 16S and COI as markers, revealed genetic differentiation of populations of *Boana pugnax* (Schmidt, 1857) from opposite sides of Cordillera de Mérida, Northern Andes, with p-values distance of 1.3% (16S) and 4.4% (COI).

As well as in the studies cited above, in our results, we notice a difference in the p-distances values between the different markers, 16S and the COI. Just as some phenotypic characters are more conserved than others (Cherry et al., 1977), so are genes. Some genes outperform others regarding discriminatory power and universal applicability, and these characteristics may also vary among organism groups (Vences et al., 2005a). The 16S gene is a highly conserved mitochondrial marker, more than COI,

but mutations are common in some variable regions, corresponding to loops in the ribosomal RNA structure (Vences et al., 2005a).

1360   DNA barcoding alone is insufficient to provide a fully reliable species identification in anurans, especially when reference sequences do not cover the entire genetic variability and geographical distribution of a species. In addition to this genetic information gap for many Neotropical anuran lineages, attention must be taken, due the possibility of incomplete lineage sorting, and introgression that can cause gene 1365 heterophyly, especially in mitochondrial genes (Fouquet et al., 2007b).

Even following a minimum threshold for genetic distances, such as proposed by Fouquet et al. (2007b) for the delimitation of amphibian species, it is necessary to exercise caution when making a decision based only on this threshold, as it is not an absolute value that serves for all anuran clades, so it is recommended to use, in addition to other 1370 approaches (morphological, acoustic, ecological), more molecular markers to obtain the most accurate information possible. The distance-based DNA barcoding methods although very useful, they do not take into consideration fixed time span needed for speciation, there should be a continuum of pairwise genetic divergences of sister species, down to 0% in cases of rapid adaptive speciation, and any threshold will therefore miss a 1375 proportion of very young species (Vieites et al., 2009).

Some studies were able to identify candidate species with p-distances values below 3% (Coloma et al., 2012; Chasiluisa et al., 2020). Our results showed a great difference in the p-distances values in 16S and COI for the population of Caiapônia in relation to the others ($\leq 3\%$ in 16S and $> 4\%$ in COI), this may be due to greater variability 1380 of the sequenced fragment or some introgression. A greater share of haplotypes between the population of Caiapônia with the others, mainly in relation to the topotypical population (genetic distance of 0.9-1.0%), and subtle differences in the advertisement

call, may indicate that there was at some point an occasional hybridization along contact zones (Vences et al., 2005a).

1385   The genetic distances greater than the minimum value 3% proposed by Fouquet et al. (2007b) presented by the populations of Paracatu, and Caiapônia for COI, and Paracatu, and Brasília populations for 16S in relation to other populations of *S. skaios* suggest that these three populations may indicate potential new species. According to the nomenclature rules established by Vieites et al. (2009), these populations could be 1390   classified as an "confirmed candidate species (CCS)".

The ensemble approach model is a parsimonious way of dealing with variation based on the variation of the ENMs, avoiding the decision to choose one model over the other (Araujo & Newt, 2007). Our ENMs models indicated highly suitable areas of *S. skaios* in 10% of the municipalities of Goiás, in the eastern and northeastern regions of 1395   the state, including areas in the central highlands. According to the ENM, *S. skaios* is unlikely to occur in the southern region of Goiás, where Caiapônia is located (0.54 of suitability), due environmental conditions in this region are different from the conditions required by the species. They also indicate that it is unlikely to occur in the northwest region of Minas Gerais (Suitability values less than 0.7 for Paracatu). We can observe 1400   that the areas with the highest suitability suggested by the models are higher areas, in general, with an altitude higher than 800m (Fig. 15). Even with the topotypical population occurring in a region below that altitude (between 700 and 800m), and a few populations in the nearby municipalities, it can suggest that these populations may be on a threshold of the lowest altitudes where the species can occur. Ramos et al. (2018) associated genetic 1405   and geographical distribution data for *Pithecopus megacephalus*, an endemic species of hylid from Espinhaço Mountain Range, and they discuss the importance of high areas in

differentiating populations. Some researchers are already concerned with the biota of the sky islands, which are typically adapted to colder climates (Galbreath et al., 2009).

The overlapping of the consensus map with the shapefile of the conservation units in the Cerrado, showed suitable areas for the occurrence of *S. skaios* in some conservation units in State of Goiás and the Federal District. Of these protected areas, *S. skaios* had already been found only in the Chapada dos Veadeiros National Park. The species has also been found around the APA dos Pirineus, in the Serra dos Pirineus State Park. Despite the type locality of *S. skaios* being below 800 m in altitude, as well as the locations of Niquelândia and Barro Alto, the species also occurs in areas with high altitudes, such as the peak of the Serra dos Pirineus and Chapada dos Veadeiros, with more than 1200 m altitude.

According to the models, several high areas in the Cerrado, including conservation units, such as APA do Planalto Central, APA dos Pirineus, APA Pouso Alto, Tororó District Park, meet the species' environmental requirements and possibly it occur in these predicted areas. Under ideal conditions, the species will occupy a continuous portion of the environmental space that reflects its fundamental ecological niche (Soberón & Nakamura, 2009).

In summary, our results showed the phenotypic and molecular variations among different populations recognized as *S. skaios*, suggesting the occurrence of species complex composed by cryptic species, providing evidence of three candidates species of the *S. catharinae* group occurring in Brazilian Cerrado. They also showed the importance of using multiple lines of evidence in taxonomic studies, mainly in taxonomically complex groups, as is the case of the *Scinax catharinae* group. Species delimitation is fundamental for conservation of biodiversity, especially in the tropics, where indicators

such as species richness or degree of endemism are efficient indicators of biota (Fouquet et al. 2007).

We also hope that the geographic distribution provided here will provide insights to improve sampling efforts in these areas in the reproductive period of the species, because collecting and inventory involving anurans are usually carried out in the rainy months here in the Cerrado, between October and March, and there are rarely expeditions in the driest and coldest periods of the year. It is important to pay more attention to the winter species in the Cerrado, to know their habits and behaviors related to abiotic factors such as low temperature, and to understand the evolutionary patterns and processes that shaped these lineages, as well as the role of high areas in differentiating species. The recognizing and identification of new species, and its potential distribution provide a chance to set effective conservation priorities, especially when they occur in a conservation hotspot such as the Cerrado.

Ackownledgment

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Appendices

Appendix I. List of examined specimens

Scinax skaios. BRAZIL: Distrito Federal: Brasília, CHUNB 33786, 37348, 40939, 47607, 47608, 47615, 47621, 47622, 47632, ZUFG 9093, 9097, 9098, 14459; Poço Azul, ZUFG 15205-15209; Goiás: Alto Paraíso de Goiás, Chapada dos Veadeiros, CHUNB 16907-16913; 17546; 47575; Barro Alto, ZUFG 3662; Caiapônia, ZUFG 5867-5869; 5871-5872; 5874-5875; 5877-5880; 5882-5884; 6226-6239; 9088-9092; Luziânia, CHUNB 40889, 40894-40896, 40899, 43437, 43462, 43463; Niquelândia, ZUFG 8471, 8472; Pirenópolis, ZUFG 15183-15195; Santa Rita do Novo Destino, MNRJ 54471 (holotype), 54472-54474 (paratopotypes), ZUFG 15203 (topotype); Minas Gerais: Paracatu (36 specimens, not yet deposited in a scientific collection); Unaí, CHUNB 30908.

Appendix II. Overlay of the consensus map with shapefile of the municipalities and conservation units of the Brazilian Cerrado indicating suitable potential areas.

Conservation units	State	Number of cells (> 0.7 suitability)	~Area (km²) (> 0.7 suitability)	Suitability Mean	SD	Min	Max
Área De Proteção Ambiental Do Planalto Central	DF	2217	1893.49	0.69	0.09	0.49	0.97
Reserva Biológica Do Gama	DF	7	5.98	0.87	0.03	0.81	0.9
Área De Proteção Ambiental Da Bacia Do Rio São Bartolomeu	DF	713	608.96	0.74	0.06	0.62	0.89
Área De Proteção Ambiental Dos Pireneus	GO	151	128.97	0.72	0.05	0.61	0.84
Área De Proteção Ambiental Pouso Alto	GO	1018	869.44	0.59	0.08	0.45	0.82
Parque Distrital Salto Do Tororó	DF	2	1.71	0.79	0.03	0.76	0.81
Área De Proteção Ambiental Da Bacia Do Rio Descoberto	DF/GO	252	215.23	0.69	0.05	0.59	0.8
Parque Nacional Da Chapada Dos Veadeiros	GO	160	136.65	0.6	0.08	0.44	0.8
Floresta Nacional De Silvânia	GO	7	5.98	0.76	0.02	0.73	0.79
Apa Da Bacia Dos Ribeirões Do Gama e Cabeça De Veado	DF	86	73.45	0.68	0.04	0.6	0.78
Área De Proteção Ambiental Uruana De Minas	MG	9	7.69	0.52	0.06	0.42	0.78
RPPN Flor Do Cerrado III	GO	13	11.1	0.68	0.06	0.56	0.77
Parque Estadual Dos Pirineus	GO	10	8.54	0.65	0.05	0.6	0.77
Área De Protecao Ambiental Municipal Piracanjubinha	GO	9	7.69	0.74	0.03	0.67	0.76
Parque Estadual Altamiro De Moura Pacheco	GO	32	27.33	0.71	0.03	0.62	0.76
Apa Do Lago Paranoá	DF	18	15.37	0.66	0.04	0.6	0.76
Área De Proteção Ambiental João Leite	GO	551	470.6	0.7	0.02	0.6	0.75
Estação Ecológica De Águas Emendadas	DF	53	45.27	0.69	0.03	0.63	0.75
Área De Preservação Ambiental Do Córrego Da Lagoa	GO	4	3.42	0.65	0.04	0.59	0.74
Parque Estadual De Paracatu	MG	5	4.27	0.61	0.04	0.55	0.74
Arie Da Granja Do Ipê	DF	7	5.98	0.71	0.02	0.67	0.73

1815

Supplementary Material Data

S1 - The principal component axes from a PCA applied to a morphometric dataset of *Scinax skaios.*
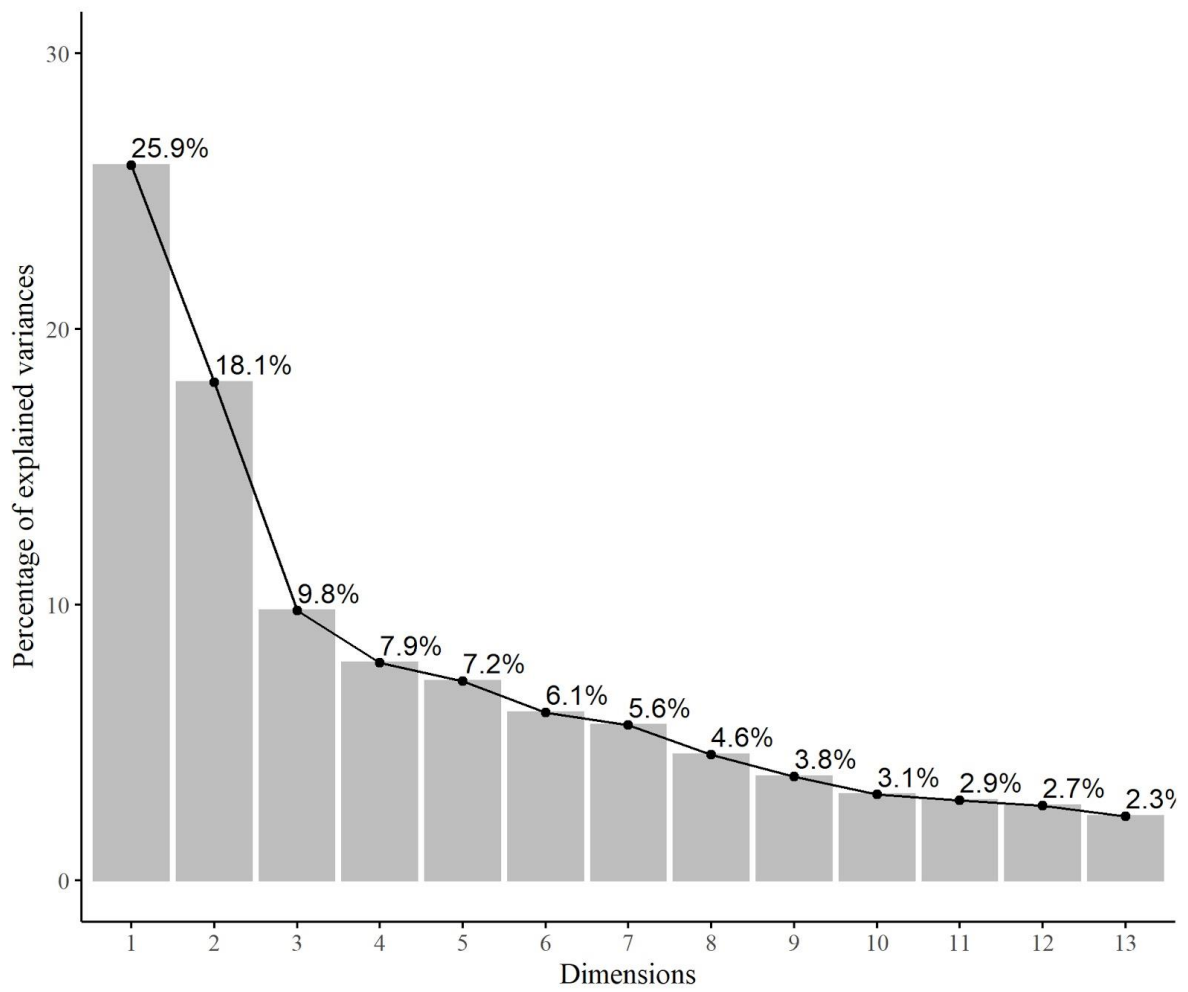
1820

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S2 - Mean square models maps showing the locations where there is the greatest variation between models.
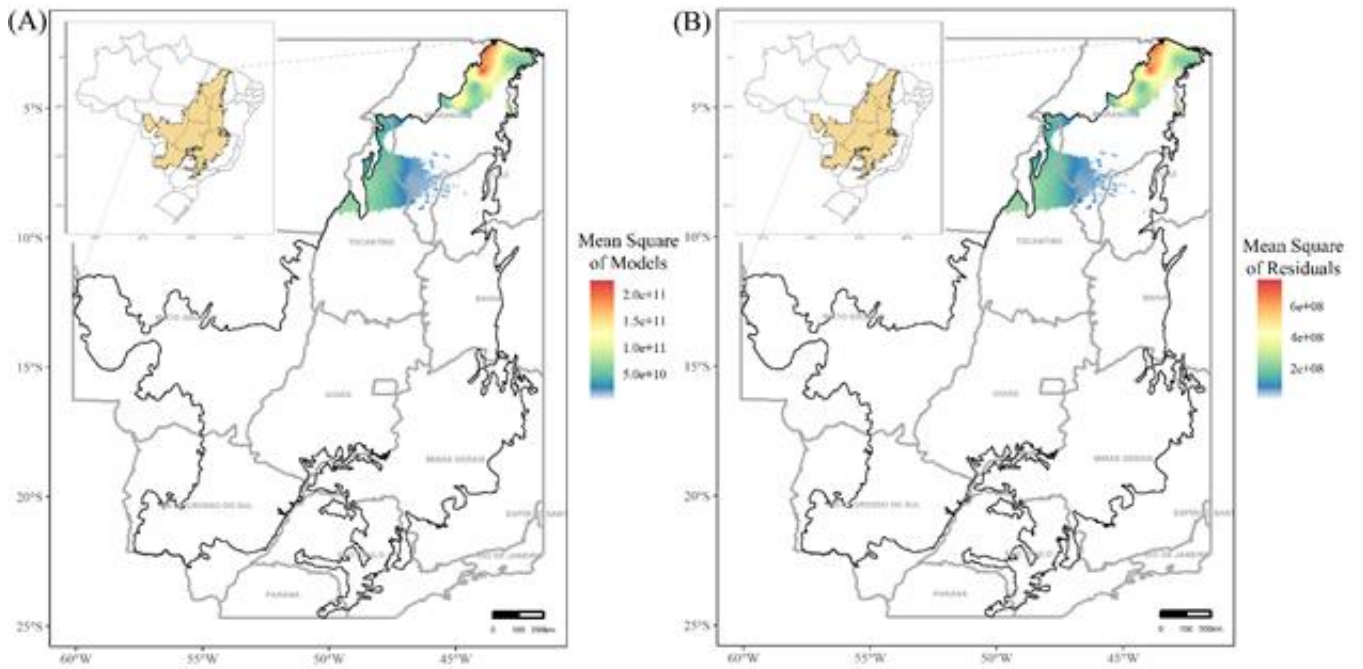
1835

1840

CAPÍTULO II

1845 **A New Species of the *Scinax catharinae* Group (Anura: Hylidae) From Cerrado of Minas Gerais State, Brazil**

Authors: Daniele Carvalho, Alejandro Valencia-Zuleta, Natan Medeiros Maciel and Reuber Albuquerque Brandão

1850

Abstract. A new species belonging to the *Scinax catharinae* group is described from
1855 the northwest of the State of Minas Gerais, Cerrado biome, Brazil. The new species is characterized by its snout subovoid in dorsal view, *canthus rostralis* remarkably concave, absence of vocal sac expanded in males, presence of nuptial pads, presence of interorbital blotch in form of inverted triangle in the head, absence of glandular acini on the medial region of forearms, mental region and pectoral area, and absence of
1860 externally differentiated glands on the inguinal region. The new species is only known from the type locality, in the municipality of Paracatu, Minas Gerais. We also described its vocalizations, tadpole, and habitat. The description of a new species suggests that the diversity of the *Scinax catharinae* clade for the Cerrado is greater than previously imagined.

1865

INTRODUCTION

1870   Several phylogenetic analyses of hylids have been done in the last two decades, and the major results founded by Faivovich et al. (2005) has been repeatedly corroborated (Wiens et al., 2006; Smith et al., 2007; Wiens et al., 2010; Pyron and Wiens, 2011; Pyron, 2014; Duellman et al., 2016; Faivovich et al., 2018). The monophyly of *Scinax* is well supported, having two well-defined clades, *Scinax*

1875   *catharinae* and *Scinax ruber* clades (Faivovich, 2002; Faivovich et al., 2005; Wiens et al., 2010; Duellman et al., 2016; Faivovich et al., 2018). The first clade is formed by the *Scinax catharinae* and the *Scinax perpusillus* groups (Faivovich et al., 2005), and it is supported by three synapomorphies suggested by Faivovich (2002): posterior portion of the cricoid ring extensively elongated and curved, partial mineralization of intercalary

1880   elements between ultimate and penultimate phalanges, and the laterodistal origin of the *m. extensor brevis distalis digiti III*.

Many changes in the taxonomic arrangement involving families, subfamilies, erection of new genera, and resurrection of available names was proposed by Duellman et al. (2016). One of these changes was the partition of *Scinax* into three genera: *Julianus*

1885   (encompassing the *S. uruguaius* species group, as defined by Faivovich et al., 2005), *Ololygon* (encompassing the *S. catharinae* clade, as defined by Faivovich, 2002 and Faivovich et al., 2005) and *Scinax* (encompassing all remaining species from *S. ruber* clade, as previously defined by Faivovich et al., 2005). However, the nomenclature proposed by Duellman et al. (2016) has not been widely accepted (e.g. Colaço and Da

1890   Silva, 2016; Conte et al., 2016; Lourenço et al., 2016; Faivovich et al., 2018; Ron et al., 2018; Lourenço et al., 2019) mainly due to the inconsistently applied methods and poor discussion by the authors. More recently, Faivovich et al. (2018) suggests to consider

Julianus and *Ololygon* as synonyms of *Scinax,* keeping the nomenclature previously defined by Faivovich et al. (2005).

1895   Currently, the *Scinax catharinae* group includes 37 species, occurring mainly in Brazil, especially in the Atlantic forest, with the exception of *Scinax aromothyella* Faivovich, 2005 and *Scinax berthae* (Barrio, 1962) which also occurs in Argentina, Uruguay and Paraguay (see Lourenço et al., 2016). Seven species of this group are currently found in Cerrado, all of them being restricted to wet gallery forests with fast

1900   flow streams: *Scinax machadoi* (Bokermann and Sazima, 1973), *Scinax canastrensis* Cardoso and Haddad, 1982, *Scinax luizotavioi* (Caramaschi and Kisteumacher, 1989), *Scinax centralis* Pombal and Bastos, 1996, *Scinax skaios* Pombal, Carvalho, Canelas and Bastos, 2010, *Scinax pombali* Lourenço, Carvalho, Baêta, Pezzuti and Leite, 2013, and *Scinax goya* (Andrade, Santos, Rocha, Pombal and Vaz-Silva, 2018) (Frost, 2019).

1905   Herein, we describe a new species of the *Scinax catharinae* group from Cerrado of the Minas Gerais State, Brazil, including data on its advertisement call, tadpole and natural history. We decided to follow the taxonomic arrangement of Faivovich et al. (2018) to tentatively maintain taxonomic stability until new evidence is raised for the use of the name *Ololygon*.

1910   **MATERIALS AND METHODS**

Specimens were collected in Paracatu municipality (17°32'19"S, 47°03'48"W; 17°31'46"S 47°03'41"W; 17°33'40"S 47°03'43"W; datum WGS84), northwest region of the State of Minas Gerais, Brazil. Type series are housed in follow Brazilian collections: Coleção Herpetológica da Universidade de Brasília (CHUNB), Brasília, Federal

1915   District; Célio F. B. Haddad Collection, Universidade Estadual Paulista Júlio de Mesquita Filho, Rio Claro, São Paulo (CFBH); Coleção Zoológica da Universidade Federal de Goiás (ZUFG), Goiânia, state of Goiás, and Museu Nacional do Rio de

Janeiro (MNRJ), Rio de Janeiro, state of Rio de Janeiro. Adults were euthanized by

absorption of 5% lydocaine, fixed in 10% formaldehyde and stored in 70% ethanol. Sex

was determined by the presence of nuptial pads and vocal slits in males, and oocytes in

females, directly observed through the translucent skin. We examined the muscles of

one paratype (ZUFG 15212). Small amounts of iodum/potassium iodine solution were

applied topically (Bock and Shear, 1972) to improve contrast. Measurements of adults

followed Duellman (1970): SVL (snout–vent length), HL (head length), HW (head

width), IND (internarial distance), END (eye–nostril distance), NSD (nostril–snout

distance), ED (eye diameter), UEW (upper eyelid width), IOD (interorbital distance),

TD (tympanum diameter), HDL (hand length), FAL (forearm length), AL (arm length),

THL (thigh length), TL (tibia length), TSL (tarsus length), FL (foot length), TFDD

(third finger disk diameter), and FTDD (fourth toe disk diameter). All measurements

were taken with a digital caliper (up to 0.01 mm). Webbing formulae followed Savage

and Heyer (1967) as modified by Myers and Duellman (1982). Description of snout

shape followed Heyer et al. (1990). Comparisons of adult specimens were based on

observations of museum specimens and literature information. Specimens used for

comparisons are listed in the Appendix, and are housed at the following Brazilian

collections: Coleção Herpetológica da Universidade de Brasília (CHUNB), Brasília,

Federal District; Coleção Zoológica da Universidade Federal de Goiás (ZUFG),

Goiânia, state of Goiás; Célio F.B. Haddad Collection, Universidade Estadual Paulista

Júlio de Mesquita Filho (CFBH), Rio Claro, state of São Paulo; Museu Nacional,

Universidade Federal do Rio de Janeiro (MNRJ), Rio de Janeiro, state of Rio de

Janeiro; Museu de Zoologia "Professor Adão José Cardoso", Universidade Estadual de

Campinas (ZUEC), Campinas, state of São Paulo; and Centro de Estudos e Pesquisas

Biológicas (CEPB), Pontifícia Universidade Católica de Goiás, Goiânia, state of Goiás.

We analyzed 53 advertisement calls of seven males and 16 aggressive calls of five males, recorded at the type locality on 23 June 2018, between 05:30 h p.m and 01:00 a.m, air temperature 15-18 C°. Vocalizations were recorded with Marantz PMD 660 digital recorder set at 44.100 Hz sample rate and 16 bits resolution coupled to directional microphone Sennheiser ME66. Calls were analyzed using Raven Pro 1.5 (Center of Conservation Bioacustics, 2014), with the following settings: window type: Hanning, window size = 256 samples, 3dB filter bandwidth = 270 Hz, brightness = 75%, overlap = 85% and DFT = 1.024 samples. The sound figures were obtained using Seewave v. 2.1.0 package (Sueur et al. 2008) on the R software v. 3.6.1. (R Development Core Team, 2019). Seewave settings were window type = Hanning, sampling rate = 44.100 Hz, overlap = 90%, window length = 256 points of resolution. The terminology used followed Köhler et al. (2017).

Tadpoles were collected with sieves and placed in plastic bags with creek water and taken to the laboratory. The tadpoles were euthanized by immersion in 5% lydocaine, preserved in 10% formalin and its stage of development was identified according to Gosner (1960). The description of the external morphology was based on 12 specimens from the type locality and measurements were taken in stages 25–34 (Gosner, 1960). The pictures were taken with stereomicroscope Leica DFC550 digital camera attached to a Leica M205A stereomicroscope (Leica Microsystems Co., Wetzlar, Germany), and the measurements were taken by Image J 1.x software (Schneider et al. 2012). Measurements and terminology followed Altig and McDiarmid (1999) for total length (TL), tail length (TAL), body length (BL), tail muscle height (TMH), tail muscle width (TMW), internarial distance (IND), and interorbital distance (IOD); Lavilla and Scroochi (1986) for body height (BH), body width (BW), eye diameter (ED), nare diameter (ND), nare–eye distance (NED), nares–snout distance

(NSD), and oral disc width (ODW); and Grosjean (2005) for dorsal fin height (DFH), ventral fin height (VFH), spiracle length (SL), spiracle width (SW). We also took the spiracle diameter (SD), and eye–snout distance (ESD). We determined the size of eyes, nostrils, and body shape in lateral view, oral disc size, height of dorsal fin, and tail muscle robustness from the ratios: ED/BH (small < 0.21 ≥ middle < 0.33 ≥ large); ND/BH (small < 0.06 ≥ middle < 0.33 ≥ large); and BW/BH (compressed < 1 ≥ depressed); ND/BL (small < 0.025 ≥ middle < 0.035 ≥ large); ODW/BW (small < 0.45 ≥ middle < 0.70 ≥ large); and DFH/TMH (low ≤ 1.0 < high) (adapted from Conte et al., 2007). Comparison of tadpoles from different species belonging to the *S. catharinae* species group was based on literature. The tadpoles were deposited in the Herpetological Collection of University of the Brasília, CHUNB), Brasília, Federal District, Brazil (CHUNB lot XXX).

RESULTS

Scinax sp. nov.

(Fig. 1)

Holotype

CHUNB 78790, adult male (SVL 25.33 mm), collected in Paracatu municipality (17°32'19"S, 47°03'48"W), State of Minas Gerais, Brazil on 23 June 2018, collected by Daniele Carvalho, Alejandro Valencia-Zuleta, and Natan M. Maciel.

Paratypes

Collected along with the holotype: CHUNB 78791-78799, 787803 (adult males), 787800-787802 (adult females) collected on 23 and 24 June 2018, ZUFG 15212, 15213, 15222 (adult females), 15214–15221, 15223 (adult males) collected on 23 and 24 June 2018; MNRJ 93626 (adult female), 93627–93630 (adult males) collected on 23 June 2018; CFBH 44959–44962 (adult males), 44963 (adult female) collected on 23 and 24

June 2018. All paratypes were collected by Daniele Carvalho, Alejandro Valencia-Zuleta and Natan Medeiros Maciel.

1995 **Diagnosis**

The new species has webbing between toes I and II that do not extend beyond the subarticular tubercle of the toe I, phenotypic synapomorphy pointed by Faivovich (2002) that support the inclusion of new species in the *Scinax* genus. Moreover, *Scinax* **sp. nov.** is assigned to the *Scinax catharinae* species group due to the laterodistal origin

2000 of the *m. extensores brevis distalis digiti III*, phenotip synapomorphy poitend by Faivovich (2002).

The new species is characterized by: (1) medium size for the group (SVL male 20.36–28.18 mm, n = 28, SVL females 29.16–35.22 mm, n = 8); (2) snout subovoid in dorsal view; (3) *canthus rostralis* well defined, remarkably concave; (4) absence of

2005 vocal sac expanded in males; (5) vocal slits present in males; (6) hypertrophied forearms in males; (7) presence of nuptial pads; (8) presence of interorbital blotch in form of inverted triangle in the head, exceeding the posterior margin of the eyes; (9) dorsum with smooth skin presenting scattered tubercles; (10) pectoral fold absent; (11) absence of glandular acini on the medial region of forearms, mental region and pectoral

2010 area; (12) absence of externally differentiated glands on the inguinal region; (13) flank and hidden areas of thighs with dark brown irregular blotches on a yellow pale pale background; (14) advertisement call with three pulsed notes.

2015
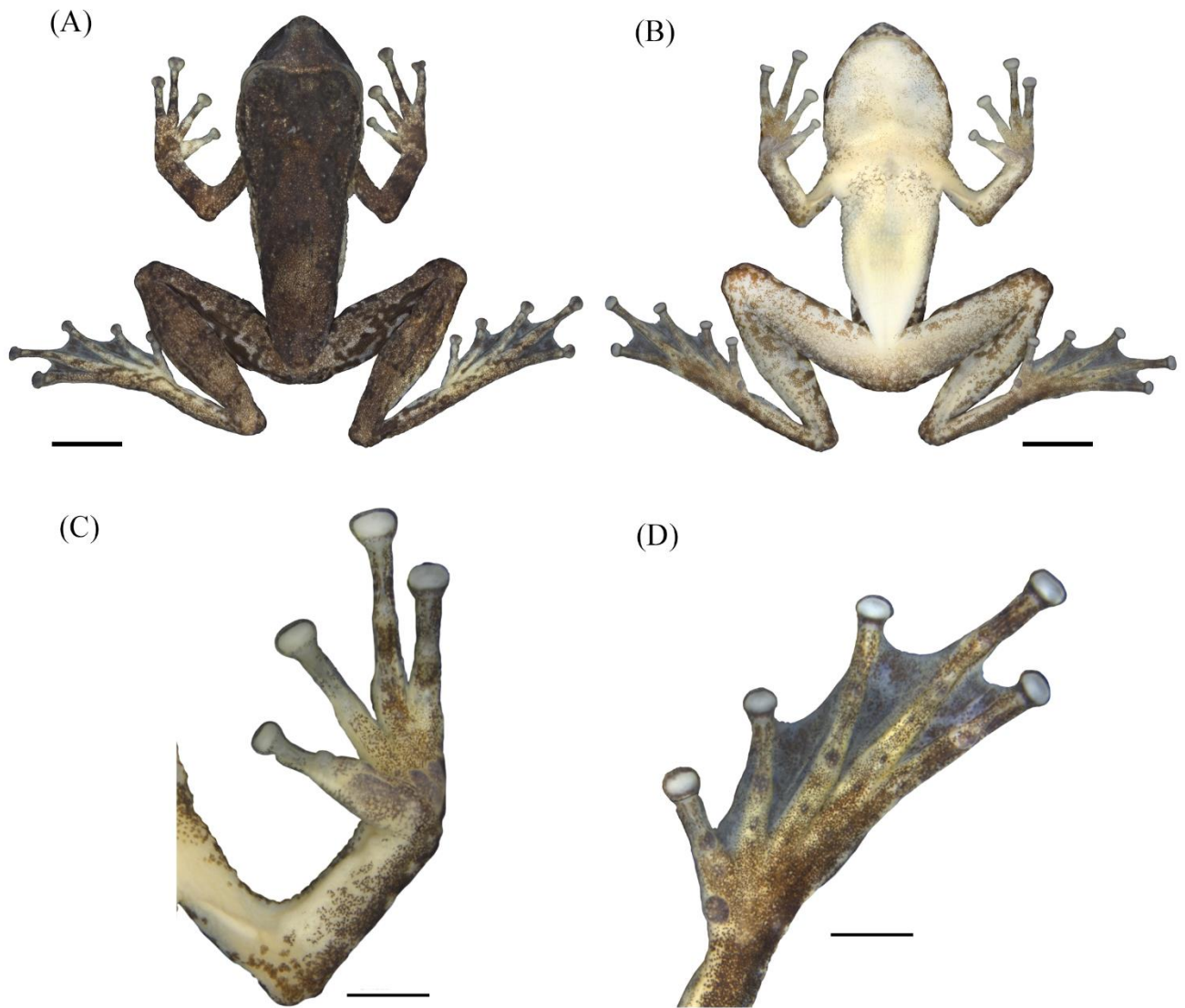
Figure 1. Dorsal (A) and ventral (B) views, palmar and plantar views of the right hand (C) and foot (D) of *Scinax* **sp. nov.** holotype (CHUNB 78790). Scale bar = 5 mm (A–B); 2 mm (C–D).

Comparison with other species

Scinax **sp. nov.** differs from *Scinax ariadne* (Bokermann, 1967a)*, Scinax catharinae* (Boulenger, 1888)*, Scinax longilineus* (Lutz, 1968), and *Scinax jureia*

(Pombal and Gordo, 1991) by its the smaller male SVL (20.36–28.18 mm; combined SVL of compared species 28.61–43.2 mm) and from *S. machadoi*, *Scinax melanodactylus* Lourenço, Luna and Pombal, 2014, *Scinax skuki* Lima, Cruz and Azevedo, 2011, *Scinax argyreornatus* (Miranda-Ribeiro, 1926), *S. berthae* by having larger males SVL (combined SVL of males in the compared species 12.0–18.0 mm). *Scinax* **sp. nov.** is distinguished from *S. ariadne*, *Scinax albicans* (Bokermann, 1967a), *Scinax flavoguttatus* (Lutz and Lutz, 1939), *S. catharinae*, *Scinax strigilattus* (Spix, 1824), *Scinax tripui* Lourenço, Nascimento and Pires, 2010, and *Scinax heyeri* (Peixoto and Weigoldt, 1986) by having smaller females (29.16–35.22 mm; combined SVL of females of the compared species ranging 36.84–46.11 mm). From *S. melanodactylus*, *Scinax caissara* Lourenço, Zina, Catroli, Kasahara, Faivovich, and Haddad, 2016, *S. luizotavioi*, *S. skuki*, *S. argyreornatus*, *S. berthae*, and *S. rizibilis* (Bokermann, 1964), *Scinax* **sp. nov.** differs by having larger females (13.2–32.4 mm in the females in the compared species).

Scinax **sp. nov.** differs from *Scinax agilis* (Cruz and Peixoto, 1983), *S. albicans*, *S. argyreornatus*, *S. aromothyella*, *S. berthae*, *S. machadoi*, *S. melanodactylus*, *Scinax ranki* (Andrade and Cardoso, 1987), *S. rizibilis*, and *S. skuki* by its well–defined *canthus rostralis* (poorly evident in the compared species). In *Scinax* **sp. nov.**, the *canthus rostralis* is extremely concave, more than any other species in the *Scinax catharinae* group.

Scinax **sp. nov.** differs from *S. melanodactylus* and *S. agilis* by the absence of compound pectoral fold. *Scinax* **sp. nov.** differs from all species of the *Scinax catharinae* group except *S. agilis*, *S. caissara*, *S. canastrensis*, *S. longilinius*, *S. rizibilis*, *S. skaios*, *S. goya*, and *S. melanodactylus* by its subovoid snout in dorsal view. *Scinax pombali*, *S. aromathyela*, *S. argyreornatus*, *S. berthae*, and *S. skuki* possess sub-

elliptical snout in dorsal view; *S. ariadne, Scinax garibaldiae* Lourenço, Lingnau,

Haddad, and Faivovich, 2019, and *Scinax obtriangulatus* (Lutz, 1973a) have snout

rounded in dorsal view; *S. albicans*, *S. angrensis* (Lutz, 1973b), *S. flavoguttatus*, *Scinax*

2055   *humilis* (Lutz and Lutz, 1954), *Scinax littoralis* (Pombal and Gordo, 1991), *S.*

strigilatus, *Scinax trapicheiroi* (Lutz and Lutz, 1954), and *S. tripui* have rounded with

and mucronate snouts, respectively; *Scinax carnevalli* (Caramaschi and Kisteumacher,

1989), *Scinax kautskyi* (Carvalho-e-Silva and Peixoto, 1991), and *Scinax muriciensis*

Cruz, Nunes and Lima, 2011 possess a mucronate snout; *Scinax luizotavioi* has

2060   mucronate with a pointed snout. *Scinax centralis* presents a snout slightly acuminate,

and *S. machadoi* have acuminate snout.

The new species does not have externally differentially inguinal gland,

differently from *S. ariadne*, *Scinax brieni* (De Witte, 1930), *S. caissara*, *S. canastrensis*,

S. catharinae, *S. centralis* (hypertrophied), *S. flavoguttatus*, *S. jureia*, *Scinax hiemalis*

2065   (Haddad and Pombal, 1987), *S. longilineus*, *S. luizotavioi*, *S. obtriangulata*, *S. rizibilis*,

and *S. tripui*.

Scinax **sp. nov.** differs from *S. agilis*, *S. albicans*, *S. angrensis*, *S. argyreornatus*,

S. berthae, *S. canastrensis*, *S. carnevallii*, *S. centralis*, *S. flavoguttatus*, *S. heyeri*, *S.*

hiemalis, *S. humilis*, *S. kautskyi*, *S. littoralis*, *S. machadoi*, *S. muriciensis*, *S. pombali*, *S.*

2070   *ranki*, *S. skuki*, *S. strigilatus*, *S. trapicheiroi*, and *S. tripui* by its hypertrophied forearms

in males (males of those species with non-hypertrophied forearms).

It differs from *S. aromothyella*, *S. berthae*, *S. garibaldiae*, and *S. rizibilis* by

absence of externally expanded vocal sac in males. It also differs from *S. aromothyella*,

S. berthae, *S. caissara*, and *S. garibaldiae* by having a smooth skin on the dorsum

2075   (rough skin in *S. ariadne*, *S. canastrensis*, *S. goya*, *S. longilineu*s, *S. pombali*, and *S.*

skaios; scattered tubercles distributed on dorsum in remaining species of the group).

Scinax **sp. nov.** presents the inguinal regions and hidden areas of the thighs with irregular dark brown blotches on pale yellowish background. This pattern is different from *S. ariadne* (light brown irregular blotches on violet or pink background; Lourenço et al., 2016); from *S. brieni* (pale bluish color in hidden surfaces; Lutz, 1973a); from *S. canastrensis* ("yellow" color; Cardoso and Haddad, 1982); from *S. agilis*, *S. caissara*, and *S. melanodactylus* by absence of flash color (Lourenço et al., 2014; 2016); from *S. catharinae*, *S. humilis,* and *S. trapicheiroi* (dark brown blotches on light blue or white background; Lourenço et al., 2016); from *S. carnevalli* (dark brown blotches on whitish background; Caramaschi and Kistemaucher, 1989); from *S. flavoguttatus* and *S. heyeri* (brown spots on orange background; Lourenço et al., 2016); from *S. hiemalis* (black blotches on green background; Haddad and Pombal, 1987); from *S. obtriangulatus* (dull grayish violet; Lutz, 1973a); from *S. kautskyi* because it presents white blotches on a dark brown background (Carvalho-e-Silva and Peixoto, 1991); from *S. luizotavioi* (light brown blotches on a pale background; Lourenço et al., 2013); from *S. ranki* (dark brown blotches on greenish background; Lourenço et al., 2016); from *S. skaios* (vermiculate dark brown spots on light green background; Pombal et al., 2010; Lourenço et al., 2013); from *S. strigulatus* (greenish color on hidden areas; Pimenta et al., 2007); from *S. tripui* (irregular brown blotches on light green background; Lourenço et. al., 2016); from *S. longilineus* and *S. machadoi* (vermiculate spots on yellow or pale background; Lourenço et al., 2016); from *S. centralis* (yellow blotches on a dark brown background; Pombal and Bastos, 1996).

The new species has a interocular blotch in form of inverted triangle that exceeds the posterior border of eyes as in *S. canastrensis*, *S. centralis*, *S. goya*, *S. hiemalis*, *S. jureia*, *S. longilineus*, *S. luizotavioi*, *S. ranki*, and *S. skaios*. However, it differs from *S. aromothyella*, and *S. berthae* (inverted triangle that does not exceed the

posterior border of eyes); from *S. argyreornatus*, *S. caissara, S. carnevalli*, and *S. kautskyi* (interocular blotch w-shaped not exceed the posterior border of eyes; Lourenço et al., 2019), from *S. trapicheiroi* (very large interocular blotch, Luna-Dias et al., 2009);

2105 from *S. agilis* and *S. melanodactylus* (black longitudinal line between eyes or with no defined markings, Lourenço et al., 2014); and *S. garibaldiae* (interocular trapezoid-shaped blotch, Lourenço et al., 2019).

The new species is most similar to *S. skaios* and *S. goya* but differs from them by having a most concave *canthus rostralis*, different color pattern of flanks and hidden

2110 areas of thighs (vermiculate dark brown spots on light green background in *S. skaios*; irregular dark brown spots and yellow stripes in flanks and irregular dark brown spots on pale yellowish background in *S. goya*. The new species has irregular dark brown blotches on pale yellow background with no stripes in flanks and hidden areas of thighs) (Figs. 2–3).

2115

2120
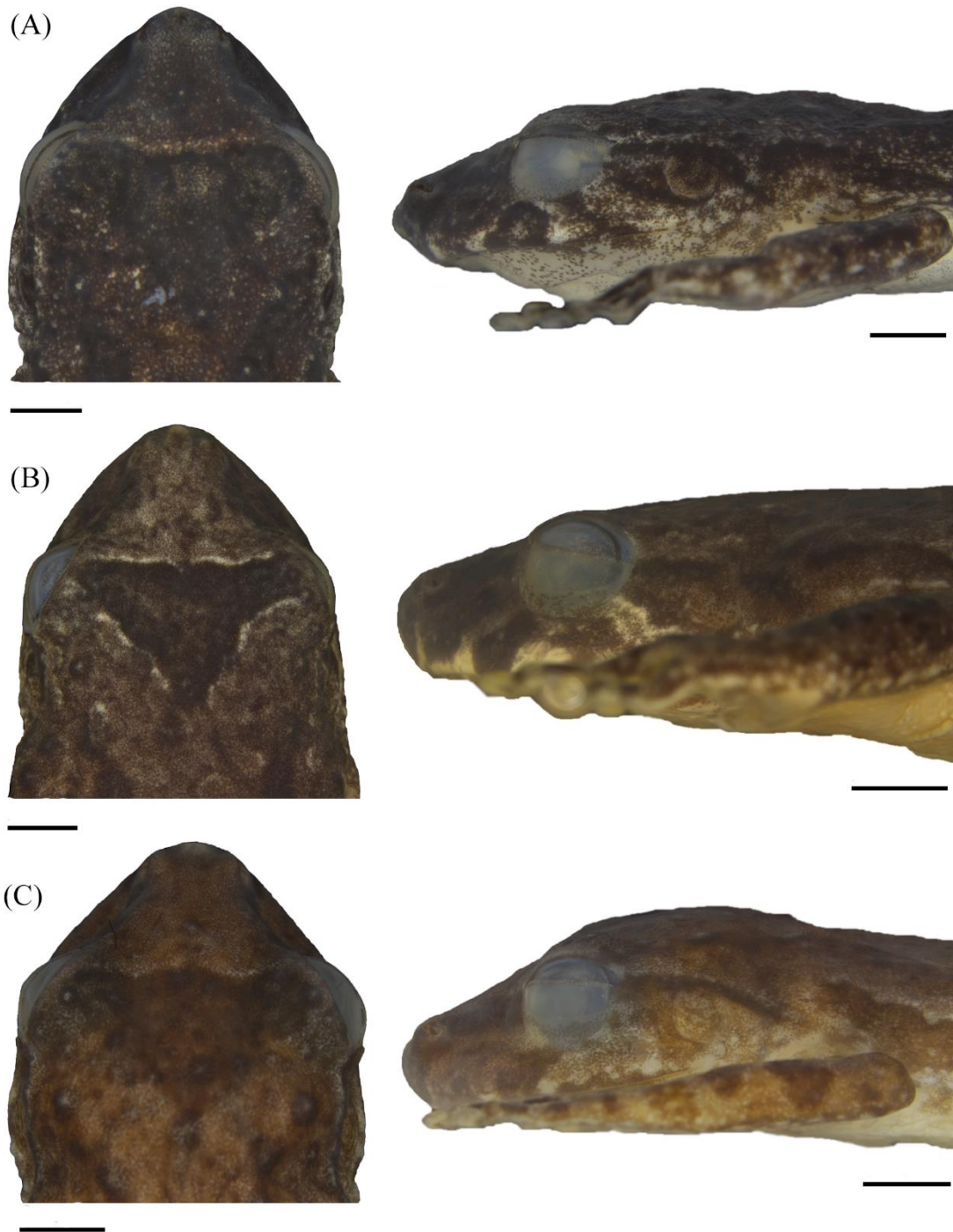
Figure 2. Dorsal and lateral views of head of three *Scinax catharinae* species group:
(A) *Scinax* **sp. nov.**; (B) *Scinax skaios*; and (C) *Scinax goya*. Scale bar = 2 mm.

2125
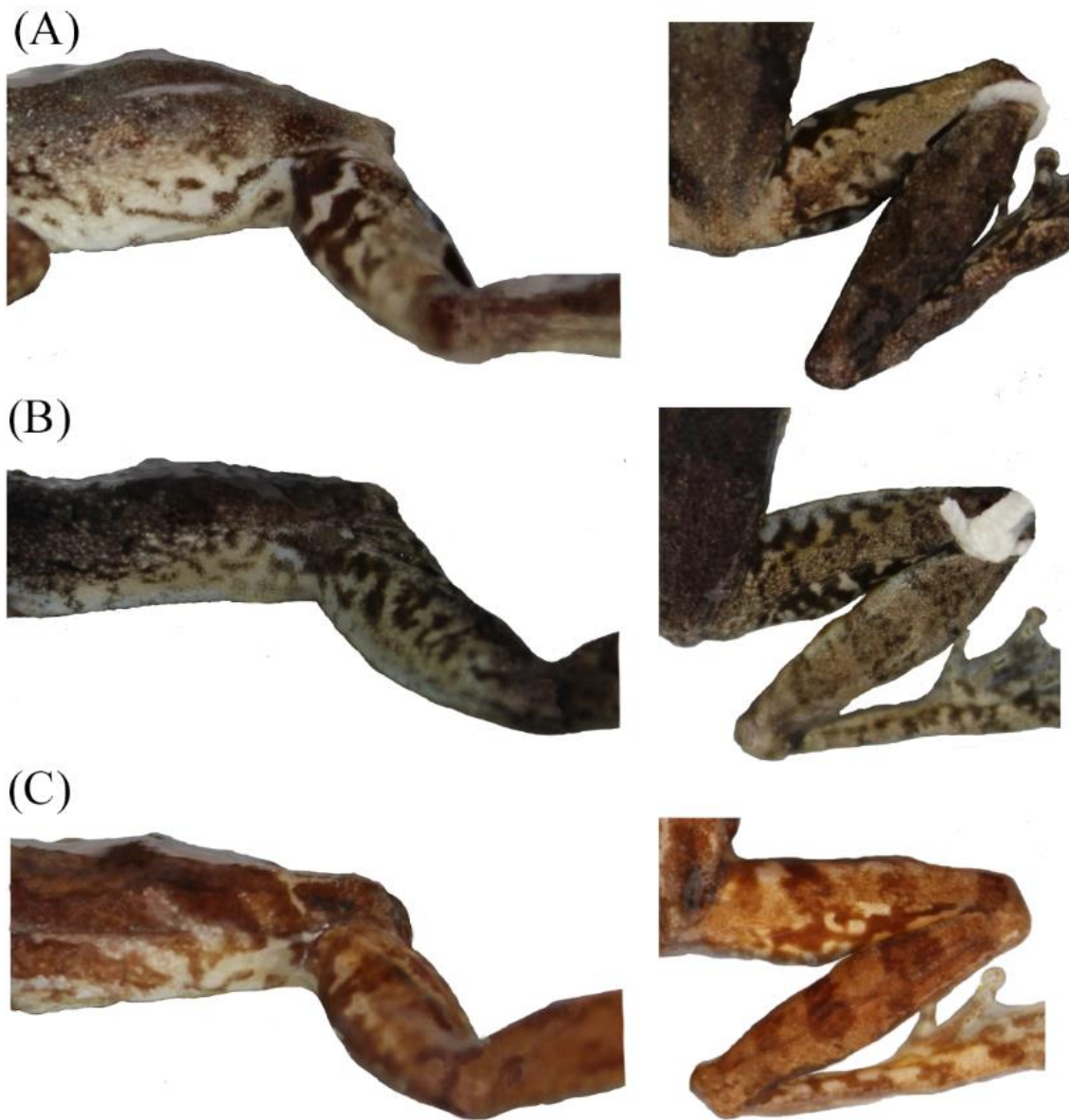
Figure 3. Hidden thigh and dorsal views of legs (left and right, respectively) of (A) *Scinax* **sp. nov.**; (B) *Scinax skaios*, and (C) *Scinax goya*. Scale bar = 2 mm.

2130

Description of holotype

Body slender. Head slightly longer than wide (HL 36% of SLV; HW 35% of SLV). Snout subovoid in dorsal view, protruding in lateral view; nostril elliptical, protruding, located laterally, nearby snout tip, opening dorsolaterally directed.

2135   Internostril distance 21% of head width. *Canthus rostralis* well marked, remarkably

concave. Loreal region concave. Eye large, protuberant, diameter 34% of head width. Interorbital distance 59% of head width. Tympanum rounded, *annulus tympanicus* well visible, diameter 42% of eye diameter. Supratympanic fold marked, evident, extending from posterior corner of eye to near the insertion of arms. Tongue oval, unattached posteriorly and laterally. Vocal slits present, extending from the lateral of tongue to the corner of the mouth. Choanae elliptical. Two contiguous series of four vomerine teeth, each located between the choanae. Vocal sac not externally expanded. Forearms hypertrophied, longer than arms (which are also slender), with small tubercles in outer margins. Hands 30% of SVL; inner metacarpal tubercle single, large and elliptical; outer metacarpal tubercle large and bilobed; subarticular tubercles rounded; supernumerary tubercles small and rounded; relative finger length II < III < V < VI; nuptial pad present in the medial outer margin on the finger II, unpigmented on finger II; webbing absent between fingers II and III and vestigial between other fingers; discs on fingers elliptical, wider than long. Legs slender, sum of THL and TL lengths slightly smaller than SVL; foot 42% of SVL; inner metatarsal tubercle single and elliptical, larger than inner metatarsal tubercle; outer metatarsal tubercle single, rounded, small and protruding; subarticular tubercles single and rounded; supernumerary tubercles single and rounded; relative toes length: I < II < III ≤ V < IV; webbing formula: $\mathbf{I}2^--1^+\mathbf{II}1-1\mathbf{III}1-2\mathbf{IV}2^--1\mathbf{V}$; discs elliptical, wider than long. Inguinal glands absent; acini glands absent in mental, pectoral and forearms regions. Pectoral fold absent; dorsal surfaces and flanks covered by scattered tubercles; skin on dorsum few granular; venter immaculate.

Measurements of holotype (in millimeters). SVL 25.33; HL 9.2; HW 8.98; IND 1.89; END 2.71; NSD 1.4; ED 3.13; UEW 1.93; IOD 5.37; TD 1.33; HDL 7.62; FAL 4.05; AL 3.09; THL 12.63; TL 11.81; TSL 5.41; FL 10.86; TFDD 0.99; FTDD 0.82.

2160   *Color of holotype in preservative:* General color of dorsum dark brown. Upper lip

cream with dark brown blotches and small dark brown dots. Interorbital blotch in form

of inverted triangle in the head dorsal view, bordered by a darker brown, almost black

discontinuous line. Iris gray. Dorsolateral region with dark brown blotches and dots on a

cream background. Sacral region with inverted v-shaped blotch dark brown. Hind limbs

2165   cream with dark brown bars dorsally. Dark brown dots on feet. Flanks and hidden areas

of thighs with dark brown on cream background. Venter is cream with several dark

brown dots scattered mainly in gular and pectoral regions.

Color in life. General coloration of dorsum yellowish. Blotches and dots on dorsum and

dorsal region of the limbs light brown. Upper lips and flanks pale yellow. Blotches on

2170   the hidden surface of thighs dark brown. Iris bronze. Venter whitish to pale yellow,

sometimes with several dots dark brown. Tympanum with the same coloration of

dorsum, yellowish.

Variation among paratypes

    Measurements of adult individuals are presented in Table 1. Dorsum skin varies

2175   in the amount and distribution of granules, being some specimens a little rougher than

others. Dots and spots patterns on the dorsum also vary in shape and quantity. The

general coloration of dorsum varies from yellowish to light brown. The color of

blotches, stripes and dots can vary from light to dark brown. Webbing formula can be

I2^-–2**II**1-1$^-$**III**1–1**IV**1$^+$–1**V** or **I**2^-–1$^+$**II**1–1$^-$**III**1–2$^-$**IV**2$^-$–1**V**.

2180

Etymology

    The noun chosen for the new species is in apposition meaning "good river" or

"strait river" in tupi-guarani language (Carvalho, 1987), due to the remarkable fishery of

Table 1. Measurements (in mm) of the type series of *Scinax* **sp. nov.** For abbreviations
see the text. Means and standart deviation followed by ranges in parentheses.

Measurements	Males (n= 28)	Females (n=8)
SVL	23.7±1.4 (20.3–28.1)	31.9±2.0 (29.1–35.2)
HL	9.0±0.4 (7.9–10.1)	11.7±0.7 (11.0–12.7)
HW	8.5±0.4 (7.8–9.3)	11.2±0.8 (10.2–12.4)
IND	2.0±0.1 (1.5–2.3)	2.5±0.3 (2.1–2.9)
END	2.7±0.2 (1.7–3.0)	3.5±0.4 (2.9–4.1)
NSD	1.3±0.1 (1.0–1.7)	1.6±0.2 (1.2–2.0)
ED	3.3±0.3 (2.5–3.7)	4.3±0.3 (3.8–4.7)
UEW	2.0±0.2 (1.7–2.3)	2.5±0.4 (1.6–3.0)
IOD	4.9±0.3 (4.2–5.7)	6.2±0.4 (5.7–6.9)
TD	1.1±0.1 (0.9–1.4)	1.3±0.1 (1.1–1.5)
HDL	7.5±0.5 (6.1–8.6)	9.7±1.0 (8.2–11.7)
FAL	3.9±0.51 (3.0–4.7)	5.5±0.5 (5.1–6.5)
AL	2.8±0.3 (2.2–3.5)	3.7±0.3 (3.3–4.3)
THL	12.8±0.8 (11.0–15.1)	16.6±1.1 (15.5–18.0)
TL	11.6±0.8 (9.8–13.7)	15.0±1.0 (15.0–16.6)
TSL	5.9±0.5 (5.2–7.7)	8.1±0.5 (7.3–9.0)
FL	11.1±0.7 (9.4–12.4)	14.2±1.3 (12.7–16.0)
TFDD	0.9±0.1 (0.7–1.2)	1.3±0.2 (1.1–1.6)
FTDD	0.8±0.1 (0.5–1.2)	1.1±0.1 (0.9–1.3)

the Paracatu river in the past and its feasibility for navigation. The specific name is an

homage to the Paracatu river, the main river of the Northwest region of Minas Gerais,

now threatened by mining activities and excessive water pumping for agricultural,

industrial and domestic uses. The conservation of the streams and rivulets where the

new species is found are essential for the maintenance and conservation of the Paracatu

river and its tributaries.

2195 **Distribution and natural history**

Known so far only in the type-locality in the municipality of Paracatu, Minas

Gerais State (Fig. 4). *Scinax* **sp. nov.** was found in small streams with stone beds in

gallery forest, within the Cerrado biome (Fig. 5). The reproductive period of the species

seems to occur in the driest and coldest season of the year in Cerrado, from June to July,

2200 when males were heard vocalizing, and there were tadpoles in the stream pools.
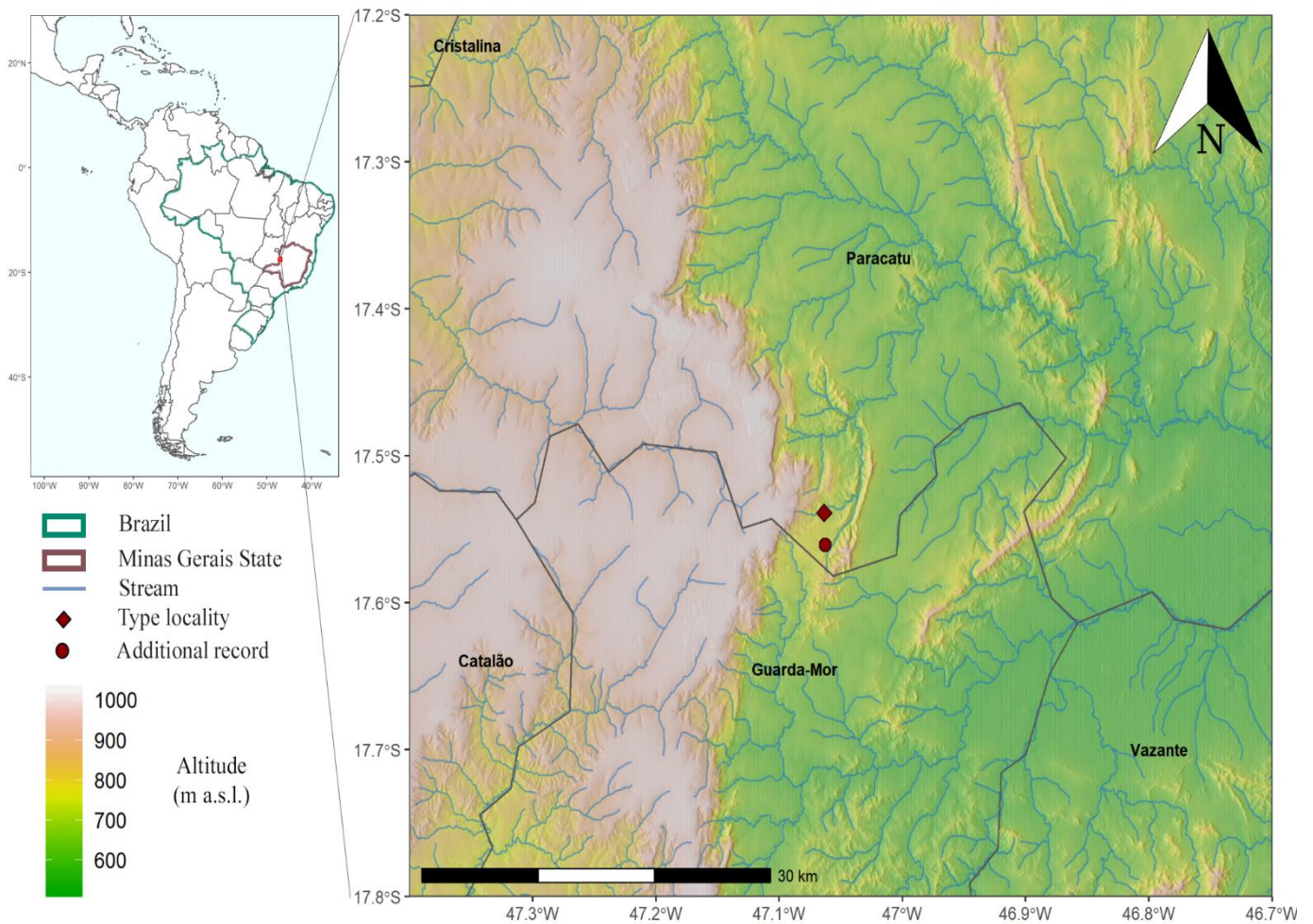
Figure 4. Known distribution of *Scinax* **sp. nov.** in the municipality of Paracatu,

northeast Minas Gerais state, Brazil.

2205 The species started its activities around 05:30 h p.m., the time when the stream,

shaded by the gallery forest, is getting dark, and is possible to hear some males calling,

and they stop vocalizing until about 11:00 p.m to midnight. The individuals were

associated with ground, on the rocks at the stream edge, and on shrubs around the

stream, up to 50 cm from the ground, and males were seen calling on all these substrates

2210   (Fig. 6). Females are larger than males (females average = 31.94 mm; males average =

23.78 mm), the amplexus is axillary, and the eggs are deposited directly in water.

Figure 5. Environment where *Scinax* **sp. nov.** was found, streams with stone beds in

gallery forest in the Paracatu municipality, State of Minas Gerais, Brazil (type locality)**.**

2215   Photo by Alejandro V. Zuleta
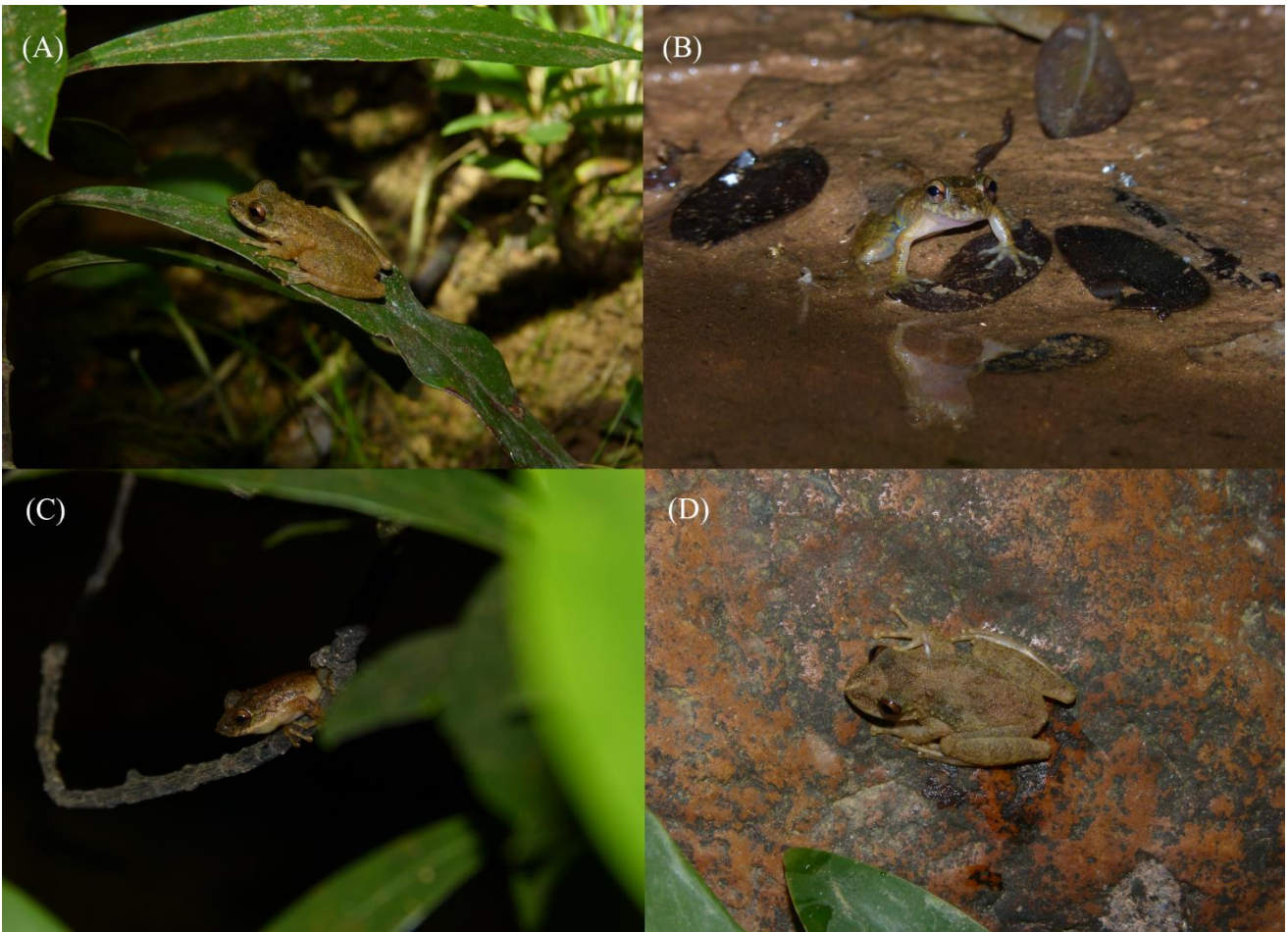
Figure 6. Substrates where *Scinax* **sp. nov.** are found: on scrubs leaves around the

stream (A), on the ground (B), on perched in the scrubs around the stream (C), and on

the rocks at the stream edge (D). Photo by Alejandro V. Zuleta.

2225

Vocalizations

The advertisement call of *Scinax* **sp. nov.** is composed by a short sequence of

multipulsed notes (N = 53 calls; seven males), characterized as short squawk-like notes

(showing intermediate duration and amplitude peak at the end of the note, as proposed

2230   by Hepp et al., 2017), with an ascending amplitude modulation. The call duration ranges

from 0.17 to 0.41 seconds (0.26 ± 0.06) and it is composed by three to five notes (3.49 ±

0.63), lasting from 0.0016 to 0.065 seconds, emitted with variable intervals from 0.022

to 0.079 seconds (0.05 ± 0.016). The notes have a pulsed structure, ranging from four to 10 pulses per note (0.002 ± 0.0007). These are spaced and range from 0.002 to 0.005

2235   seconds (7.15 ± 1.73). Dominant frequency varied from 2.5 to 3.5 kHz (3.12 ± 0.21) (Fig. 7A; Table 2).

We also recorded a different type of vocalization (territorial call), less frequent than the advertisement call, that seems to be employed to keep distance among calling males (observed in the field). This call was composed of only one multipulsed note (N =

2240   16 calls; emitted by five males), ranging from 0.185 to 0.447 seconds (0.315 ± 0.075). The number of pulses composing the note was higher than advertisement calls, ranging from 22 to 48 pulses (38.12 ± 7.80), with duration from 0.003 to 0.004 seconds (0.003 ± 0.000). The dominant frequency varied from 3.0 to 3.4 kHz (3.2 ± 0.14) (Fig. 7B).

2245   **Comparison with advertisement calls of other species of the *Scinax catharinae* group**

The calls of the *Scinax catharinae* species group are considered complex (Pombal et al., 1995; Pombal and Bastos, 1996) and so far, vocalizations of 23 species have been described (Table 2). The advertisement call of *Scinax* **sp. nov.** is

2250   distinguished from *S. canastrensis*, *S. longilineus* and *S. skaios* by having higher dominant frequency, and differs from *S. agilis*, *S. argyreornatus*, *S. aromothyella*, *S. berthae,* and *S. centralis* by presenting lower dominant frequency. It is distinguished from *S. agilis*, *S. argyreornatus*, *S. aromothyella*, *S. berthae*, *S. canastrensis*, *S. heyeri*, *S. hiemalis*, *S. longilineus*, *S. machadoi*, *S. ranki*, *S. rizibilis*, and *S. skaios* by the lower

2255   number of notes. The advertisement call of *S. goya* (the most morphologically similar species to the new species) shares many acoustic parameters with *Scinax* **sp. nov.**
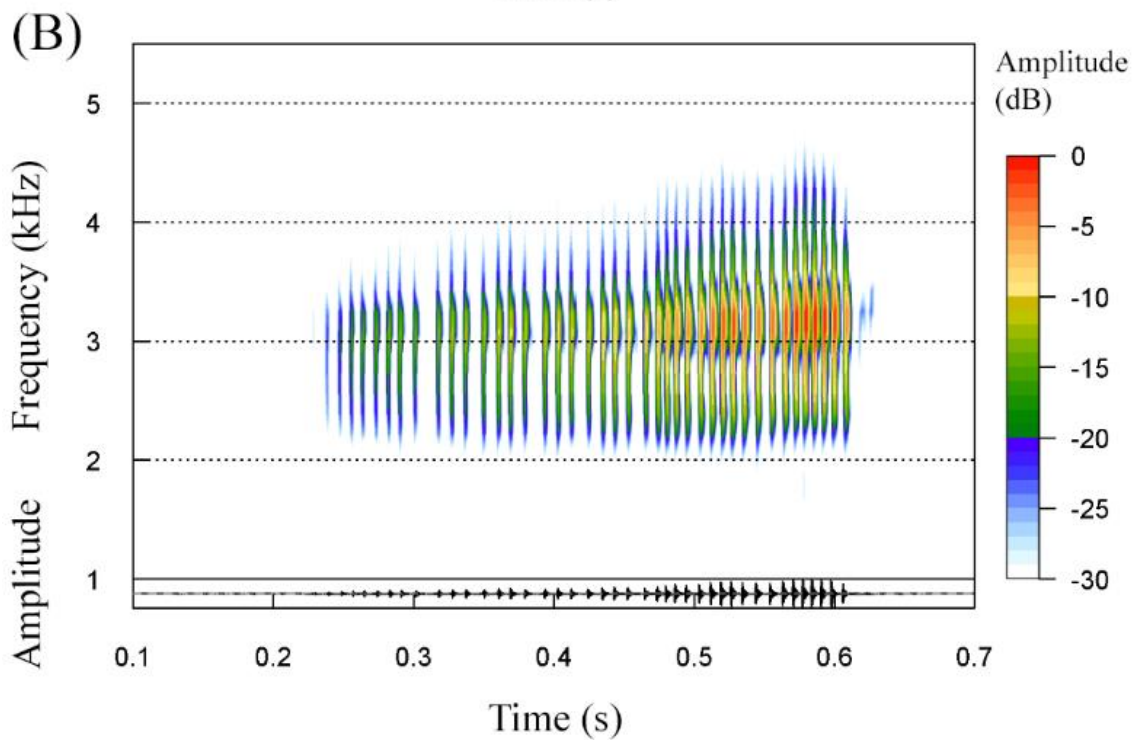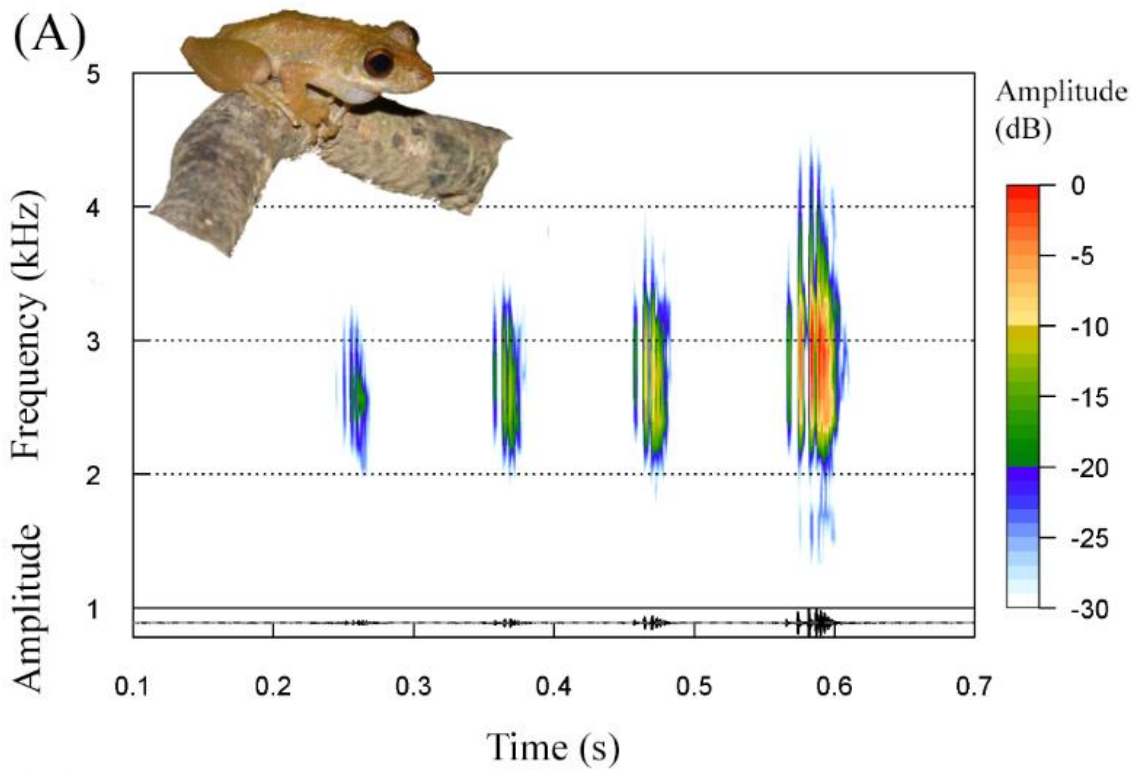
Figure 7. A) Spectrogram (above) and oscillogram (below) of the advertisement call; B) spectrogram (above) and oscillogram (below) of the territorial call of *Scinax* **sp. nov.** from type locality at municipality of Paracatu, State of Minas Gerais, Brazil.

Table 2. Acoustic parameters of the calls described of the species of the *Scinax catharinae* group. Abbreviations: CD–call duration; NN–number of notes; ND–notes duration; NP–number of pulses; PD–pulse duration; NI–notes interval; CR–call rate (number of calls per minute); DF–dominant frequency. Data presented in mean ± standart deviation (when available) followed by range.

Species	CD (s)	NN	ND (s)	NP	PD (s)	NI (s)	CR (calls/min)	DF (kHz)	References
S. agilis	–	1	0.36–0.40	–	–	–	–	7.45–7.92	Nunes et al. (2007) note a
	–	13–29	0.010–0.032	–	–	0.062–0.133	–	5.60–7.88	Nunes et al. (2007) note b
S. albicans	0.7	–	0.03	–	–	–	–	3.3–4.1	Heyer (1980)
S. angrensis	0.2–0.7	01–07	0.025 ± 0.012	–	–	0.023–0.076	–	1.11–5.75	Garey et al. (2012)
S. argyreornatus	0.8	5	0.02–0.04	–	–	–	–	5.0–6.5	Pombal et al. (1995) short call
	10–25	130–280	0.02–0.09	–	–	0.04–0.08	–	5.0–6.5	Pombal et al. (1995) long call
S. aromothyella	4.49±2.41 (1.04–20.76)	11.09 ± 12.37 (2–74)	0.11 ± 0.05 (0.04–0.22)	29.36 ±11.00 (18–49)	0.003 ±0.001 (0.001–0.005)	0.20 ± 0.09 (0.05–0.48)	–	5.12 ± 298 (4.7–5.4)	Pereyra et al. (2012) short note
	4.49±2.41 (1.04–20.76)	0.68 ±0.60 (0–3)	0.42 ± 0.17 (0.30–0.61)	74.57 ±20.17 (33–102)	0.004±0.002 (0.002–0.006)	0.20±0.09 (0.05–0.48)	–	5.0±311 (4.8–5.5)	Pereyra et al. (2012) trilled note
S. berthae	22.21±19.23 (3.2–52.04)	7.87 ±275 (0–52)	0.39 ±0.06 (0.13–0.59)	62 ±3.54 (40–88)	0.005 ±0.002 (0.002–0.007)	0.18 ± 0.04 (0.06–0.60)	–	4.9 ± 275 (4.4–5.3)	Pereyra et al. (2012) short note
	22.21±19.23 (3.2–52.04)	87.67 ±155.60 (4–620)	0.07 ±0.02 (0.03–0.14)	21.62 ± 5.58 (12–39)	0.003 ±0.001 (0.002–0.005)	0.18 ± 0.04 (0.06–0.60)	–	4.88 ±285 (4.4–5.2)	Pereyra et al. (2012) trilled note

Species									Reference
S. caissara	0.01–0.02	1	0.01–0.02	–	–	0.01–32.7	–	3.1–4.4	Lourenço et al. (2016)
S. canastrensis	0.8	6 a 7	–	–	–	< 0.1	–	–	Cardoso & Haddad (1982) nuptial call
	0.8	–	0.4	–	–	–	–	3	Cardoso & Haddad (1982) encounter call
	0.536±0.015 (0.445–0.628)	7 ± 1 (6–8)	0.026 ± 0.001 (0.016–0.036)	9 ± 3 (6–13)	0.024 ± 0.002 (0.02–0.04)	–	–	2.27 ± 0.42 (2.25–2.34)	Bang & Giaretta (2017)
S. catharinae	2.5	–	0.04	–	–	–	–	2.2–3.1	Heyer (1980)
S. centralis	2.39–0.91	1–10	0.02–0.03	–	–	–	–	3.49–4.89	Bastos et al. (2011)
S. goya	0.29–0.9	4–11	0.01–0.05	1–14	–	0.02–0.11	–	2.07–3.10	Andrade et al. (2018)
S. heyeri	0.34–0.56	6–9	0.002–0.01	–	–	0.55–0.66	–	2.84–3.87	Hepp et al. (2017)
S. hiemalis	0.191±0.413 (0.563–2.434)	9±2 (6–17)	0.052±0.015 (0.021–0.096)	8±2 (4–13)	0.002±0.005 (0.001–0.004)	–	–	2.81±0.44 (2.25–3.53)	Bang & Giaretta (2017)
S. humilis	0.109–0.345	3–5	0.002–0.006	–	–	0.003–0.055	–	3.0–3.9	Hepp et al. (2017)
S. littoralis	0.21–0.79	3–4	0.050±0.013	–	–	0.024–0.066	–	1.89–3.53	Hepp et al. (2017)
S. longilineus	0.613–1.418	8–19	0.016–0.067	–	–	0.024–0.141	–	2.0–2.7	Hepp et al. (2017)
S. luizotavioi	0.12–0.49	2–6	0.005–0.018	–	–	0.078–0.435	–	2.7–4.1	Hepp et al. (2017)
	0.08–0.54	2–4	0.003–0.008	–	–	0.063–0.075	–	3.0–4.0	Hepp et al. (2017)
S. machadoi	–	6–7	0.05	–	–	–	–	3.5	Bokermann & Sazima (1973)
S. ranki	0.73±0.32 (0.049–0.161)	8±2 (6–15)	0.03±0.010 (0.015–0.051)	8±2 (4–13)	0.023±0.005 (0.002–0.003)	–	–	2.89±0.32 (2.34–3.42)	Bang & Giaretta (2017)
S. rizibilis	0.74–2.95	7–23	1.00–4.70	15–72	–	–	–	2.8–4.0	Pombal et al. (1995)

S. strigilatus	0.01–0.02	1	0.01–0.02	–	–	–	–	2.6–3.4	Mendes et al. (2013)
S. skaios	4.4–7.9	42–73	0.01–0.05	–	–	0.04–0.2	–	2.2	Pombal et al. (2010) long call
	0.02–0.05	1	0.01–0.05	–	–	–	–	2.3	Pombal et al. (2010) short call
S. trapicheiroi	0.006–2.218	1–8	0.006–0.337	–	–	0.002–0.645	–	2.7–3.3	Hepp et al. (2017)
Scinax **sp. nov.**	0.26±0.06 (0.17–0.41)	3.49±0.63 (3–5)	0.032±0.01 (0.016–0.065)	7.15±1.73 (4–10)	0.002±0.000 (0.002–0.005)	0.05±0.01 (0.022–0.086)	4.42±2.43 (1–8)	3.12±0.210 (2.5–3.5)	Present study advertisement call
	0.31±0.075 (0.18–0.44)	1±0 (1)	0.31±0.075 (0.18–0.44)	38.12±7.80 (22–48)	0.003±0.000 (0.003–0.004)	–	2.4±1.94 (1–5)	3.231±0.140 (3.0–3.4)	Present study territorial call

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However, the call of the new species is composed by smaller number of notes (3–5; *S. goya*: 4–11 notes) and generally presents a higher dominant frequency (2.5–3.5 kHz; *S. goya*: 2.07–3.1 kHz). For comparisons and more information about the calls of the

2275 *Scinax catharinae* group, see Table 2.

Tadpole External morphology

Maximum total length 27.05 mm at stage 34 (Table 3). Body depressed (BW/BH = 1.19), elliptical in dorsal view (Fig. 8A–B); about one third of total length (BL/TL =

2280 0.29); snout rounded in dorsal and lateral views. Nares small (ND/BH = 0.03), rounded, dorsally located, dorsolaterally directed. Small-sized eyes (ED/BH = 0.20); dorsally positioned, dorsolaterally directed. Spiracle sinistral, short, wide, and posterodorsally projected, positioned on the lateral midline; inner wall fused to the body, with distal portion free and longer than the external wall; opening located at the posterior third of

2285 the body. Intestinal tube circularly coiled, switchback point located at the center of abdominal region. Vent tube with dextral opening, reaching the margin of the ventral fin. Oral disc ventral, not emarginated, with a marginal biseriate row of elongated papillae, interrupted by a dorsal gap; submarginal papillae aggregate in the laterals of the oral disc. Labial tooth row formula (LTRF) 2(2)/3, with row A–1 being slightly

2290 longer than A–2, P–1, P–2 and P–3 of the same length; jaw-sheaths narrow, darkly colored and finely serrated on the margins; upper jaw sheath slightly M-shaped and lower sheath V-shaped (Figs. 8C–D). Robust caudal musculature, segmented and gradually tapering; dorsal fin high (DFH/TMH = 0.34) and convex, originating in the posterior third of the body to the tail tip, higher than ventral fin; ventral fin parallel to

2295 the caudal musculature; tail tip rounded.

Figure 8. Tadpole of *Scinax* **sp. nov.** (CHUNB XXXX) at stage 34. A: Lateral view. B: Dorsal view. C and D: Oral disc. Photos: Natan M. Maciel. Scale bar = 5 mm (A–B); 2 mm (C); 1 mm (D).

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Table 3. Measurements (in mm) of *Scinax* **sp. nov.** from Paracatu, Minas Gerais State, Brazil (CHUNB lot xxx). Data presented as mean ± standart deviation (range). For stages 30, 31, and 34 only mean. For abbreviations, see text above.

Measurements	Stage 25-28 (n=9)	Stage 30 (n=1)	Stage 31 (n=1)	Stage 34 (n=1)
TL	22.4±1.5 (19.4–25.3)	27.3	27.1	27.5
TAL	15.5±1.1 (13.4–17.2)	19.2	19.3	17.5
BL	6.7±0.3 (6.0–7.2)	7.5	7.4	9.6
BH	4.0±0.2 (3.7–4.4)	3.6	4.6	5.0
BW	4.4±0.4 (3.8–5.0)	4.9	5.0	9.1
ED	0.8±0.8 (0.7–0.9)	0.8	0.9	0.5
ND	0.1±0.0 (0.0–0.1)	0.1	0.2	0.2
IOD	3.1±0.1 (2.9–3.3)	3.5	3.5	6.1
IND	2.1±0.1 (1.7–2.3)	2.4	2.5	4.2
NED	0.6±0.0 (0.5–0.7)	0.6	0.7	1.0
ESD	2.0±0.2 (1.7–2.3)	2.1	2.5	3.7
NSD	1.2±0.1 (1.1–1.6)	1.3	1.6	2.3
DFH	1.2±0.3 (0.4–1.5)	3.0	1.4	2.2
VFH	0.7±0.2 (0.2–0.9)	2.3	0.9	1.4
TMH	2.4±0.1 (2.2–2.6)	4.3	3.3	3.8
TMW	2.0±0.1 (1.8–2.2)	2.4	2.5	4.0
SL	0.6±0.0 (0.5–0.7)	0.7	1.0	1.2
SW	0.4±0.0 (0.3–0.6)	0.4	0.6	0.6
SD	0.2±0.0 (0.1–0.2)	0.2	0.2	0.2
ODW	2.2±0.1 (2.0–2.4)	2.5	2.4	3.9

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Tadpole morphological variation

Some individuals have an interocular brownish blotch in triangle-shaped, snout oriented, almost reaching the level of the nostrils, or M-shaped. Dark brown spots on the tail and fins can be more distributed. The body shape in some specimens may be

2315   ovoid.

Tadpole color in life. Body uniformly yellowish. Tail musculature background pale

yellow with dark brown melanophore agglomeration forming irregularly angular shaped

spots. Fins translucent, with large dark brown blotches scaretted. Iris black with golden

dots irregularly scattered and a golden rim around the pupil.

2320 ***Tadpole color in preservative.*** In 10% formalin, body light brown with scattered

irregular dark brown spots, tail musculature yellowish. The intestinal region, spiracle

and fins translucent. The tail musculature and the fins presented dark brown scattered

blotches. The iris loses its golden tones, becoming black.

Comparison with tadpoles of other species of the *Scinax catharinae* group

2325 Tadpoles of *Scinax* **sp. nov.** differ in snout shape in lateral view (rounded) from

S. argyreornatus, *S. hiemalis*, *S. humilis*, *S. machadoi*, and *S. skuki* (snout truncate in

lateral view in these species; Bokermann and Sazima, 1973; Haddad and Pombal, 1987;

Carvalho-e-Silva and Carvalho-e-Silva, 1998; Rodrigues et al., 2017), *S. obtriangulatus*

(snout sloped; Heyer et al., 1990), and *S. pombali* (snout sloped to truncated; Lourenço

2330 et al., 2013). The snout shape in dorsal view distinguished the larva of the new species

from *S. ariadne*, *S. flavoguttatus*, and *S. kautskyi* (snout truncate in these species;

Bokermann, 1967b; Heyer et al., 1990; Carvalho-e-Silva et al., 1995; Carvalho-e-Silva

and Carnaval, 1997), and from *S. melanodactylus* (rounded to slightly truncate; Abreu et

al., 2015).

2335 *Scinax* **sp. nov.** tadpoles differ from those of *S. ariadne*, *S. cardosoi*, *S.*

machadoi, *S. rizibilis*, *S. skuki*, and *S. trapicheiroi* in LTRF (2/3; 2(1,2)/3; 2(2)/3(1);

2(2)/3(3); and 2/3, respectively) (Bokermann, 1964; Bokermann and Sazima, 1973;

Rodrigues et al., 2017; Kirchmeyer et al., 2019). The interrupted anterior row of

papillae of *Scinax* **sp. nov.** distinguishes it from *S. albicans*, *S. angrensis*, *S. ariadne*, *S.*

2340 *flavoguttatus*, *S. heyeri*, *S. pombali*, *S. machadoi*, *S. rizibilis*, and *S. strigilatus*

(uninterrupted in these species) (Bokermann and Sazima, 1973; Peixoto and Weygoldt, 1987; Heyer et al., 1990; Carvalho-e-Silva and Carvalho-e-Silva, 1994; Carvalho-e-Silva et al., 1995; Carvalho-e-Silva and Carnaval, 1997; Camurugi et al., 2013; Lourenço et al., 2013). It was distinguished from tadpoles of *S. angrensis, S. argyreornatus, S. berthae, S. humilis, S. melanodactylus, S. littoralis, S. luizotavioi, S. machadoi, S. ranki, S. rizibilis, S. trapicheiroi, S. skuki*, and *S. tripui* by having biseriate marginal papillae (uniseriate marginal papillae in these species).

The submarginal papillae of *Scinax* **sp. nov**. tadpoles is different from *S. albicans*, *S. angrensis*, *S. argyreornatus*, *S. catharinae*, *S. humilis*, *S. machadoi*, *S. obtriangulatus*, S. *trapicheiroi*, and *S. tripui* (sparse submarginal papillae; Bokermann and Sazima 1973; Heyer et al., 1990; Carvalho-e-Silva and Carvalho-e-Silva, 1994; 1998; Carvalho-e-Silva et al., 1995; Conte et al., 2007; Lourenço et al., 2009a), *S. melanodactylus* (few and sparse or absent; Abreu et al., 2015), *S. luizotavioi*, *S. pombali*, and *S. rizibilis* (forming rows; Bokermann, 1964; Bertoluci et al., 2007; Lourenço et al., 2013), and *S. berthae* (absent submarginais papillae; De Sá et al., 1997).

Nostrils dorsally positioned distinguished *Scinax* **sp. nov.** tadpoles from *S. aromothyella*, *S. berthae*, *S. heyeri*, *S. longilineus*, *S. machadoi*, *S. melanodactylus*, and *S. strigilatus* (dorsolateral nostrils) and from *S. ranki* (lateral nostrils). It was separated from *S. berthae* and *S. kautskyi* by the dextral vent tube (medial vent tube in these species; Carvalho-e-Silva et al., 1995; De Sá et al., 1997), and also differs from *S. berthae* by having an M-shaped upper jaw sheath and the absence of the tail flagellum (arch-shaped and with tail flagellum in this species; De Sá et al., 1997).

DISCUSSION

Here we described a new species of the *Scinax catharinae* group from the northwest of the State of Minas Gerais, Cerrado biome, Brazil. The species recognition in this group is complex, and can be complicated (Pombal and Bastos, 1996), due to the various morphological similarities between adult specimens (Carvalho-e-Silva, 1986) resulting in a large number of unidentified specimens in collections (Lourenço et al., 2014; 2016).

Scinax **sp. nov.** is distinguished from the others species of the same group mainly by its well defined, remarkably concave, more than any species in this group; absence of glandular acini in body regions commonly seen in many species in the group, and flanks and hidden areas of thighs with irregular blotches patterns. Despite being morphologically quite similar to *S. skaios* and *S. goya*, the new species differs from them mainly in the depth of the curvature of *canthus rostralis*, and in the color pattern of flanks and hidden areas of thighs.

Scinax **sp. nov.** was found in the municipality of Paracatu, Minas Gerais State, and it is known so far only in the type-locality, near the border with the municipality of Guarda-Mor, state of Minas Gerais. The species of the *S. catharinae* group are typical of forested areas, and those that inhabit Cerrado areas occupy similar habitats (gallery forests), mostly in higher regions (Lourenço et al., 2009a; 2009b; 2013; 2014; 2016; Pombal et al., 2010) with fast-flowing streams or rivulets. None of the species of this group that occurs in the Cerrado were found in sympatry with the new species.

Most of the descriptions of *Scinax* vocalizations indicate the pulsed composition of the notes (Hepp et al., 2017), as observed in the new species. However, the calls emitted by the *S. catharinae* species group are more variable in amplitude, spectral and temporal features than the calls emitted by species of the *Scinax ruber* clade (Pereyra et al., 2012), composed by diverse types of notes, that may be directly related to the

119

functional diversity of the group (Bastos et al., 2011). The calls of *Scinax* **sp. nov**. presented, up to date, two different calls, the advertisement and other that seems to have territorial function, following the functional categorization proposed by Toledo et al. (2015) and Kölher et al. (2017). The territorial call is considered as a subcategory of

2395 aggressive calls (see Kölher et al., 2017) and are not always easy to differentiate from other types of calls. Here, we certify that this call described is in fact territorial call, due these signals were emitted during close contact between calling males. *Scinax* **sp. nov.** is morphologically more similar to *S. goya* and *S. skaios*, but its call is clearly different, mainly the number of notes and dominant frequency ranges (Table 2).

2400   Morphological and ecological studies of tadpoles of the *S. catharinae* group are still scarce, despite recent advances (Pezzuti et al., 2016). The larval characters, as call features, give us valuable information regarding taxonomy and phylogenetic relationships within the Scinaxini tribe (Carvalho-e-Silva and Carvalho-e-Silva, 1994; Conte et al., 2007; Kolenc et al., 2007). The tadpoles of *S. catharinae* group exhibit

2405 great variability in shape body, oral disc size and structure, patterns of coloration, and behaviour, but still are poorly studied (Pezzutti et al., 2016). Although there are many morphological and ecological variations of tadpoles in this group, there are certain similarities, which may be related to habitat use and/or vegetation (Conte et al., 2007).

        The finding of one more species of the *S. catharinae* group in the Brazilian

2410 Cerrado highlights its biodiversity importance for amphibians. However, despite of the knowledge gaps on the Cerrado role on the evolution and conservation of the Brazilian biota, the habitat loss in the Cerrado is twice those observed in the Amazon, suggesting that the biome will be restricted to its scarce protected system in few years (Françoso et al., 2015).

2415   We are unaware of the presence of *Scinax* **sp. nov.** in protected areas. However, very few and small sized protected areas are present in the known region of its distribution. Since the species is restricted to streams and rivulets with fast-flow associated to gallery forests, conservation actions aiming to the species conservation can be beneficial to the maintenance of the mistreated Paracatu river as to the water

2420 provision in the region.

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APPENDIX

Additional specimens examined

Scinax albicans (n=12): BRAZIL: **Rio de Janeiro:** Teresópolis, MNRJ 4037, 4038 **syntypes**; Mangaratiba, MNRJ 35863-35866; Nova Friburgo MNRJ 39887-39891.

2705 *Scinax ariadne* (n=11): BRAZIL: **São Paulo:** São José do Barreiro, Serra da Bocaina National Park, MNRJ 76658, 76660-76667; ZUEC 2023, 2024.

Scinax *canastrensis* (n=13): BRAZIL: **Minas Gerais:** Capitólio, MNRJ 49484; São Roque de Minas, Serra da Canastra National Park, CFBH 62254 **paratype**, MNRJ 4117 **holotype**, 4148 **paratype**, ZUEC 4188, 4189, 4191, 4193 **paratypes**; Perdizes, ZUEC

2710 8201-8204; Ribeirão das Moendas, MNRJ 49473.

Scinax carnevalli (n=9): BRAZIL: **Minas Gerais:** Botumirim, MNRJ 82433, 82434; Caratinga, ZUEC 6633, 6635; Grão Mogol, MNRJ 88767; Marliéria, Rio Doce State Park, MNRJ 4182 **holotype**, 4183, 4184 **paratypes**.

Scinax catharinae (n=12): BRAZIL: **Santa Catarina:** Rancho Queimado, MNRJ 72424-72429, 72431, 72434, 72435; **São Paulo:** Ubatuba, Itaguá Beach, CEPB 6504, 6506, 6516.

Scinax centralis (n=12): BRAZIL: **Goiás:** Caldas Novas, ZUFG 10369, 10371, 10374, 10382, 10937, 10940, 10941, 10943; Goiânia, Altamiro de Moura Pacheco Ecological Park, CEPB 26, 27; Silvânia, MNRJ 17465 **holotype**, ZUFG 11059.

Scinax flavoguttatus (n=10): BRAZIL: **Rio de Janeiro:** Cachoeiras de Macacu, MNRJ 68785-68794.

Scinax heyeri (n=6): BRAZIL: **Espírito Santo:** Santa Teresa, MNRJ 38367-38372.

Scinax humilis (n=8): BRAZIL: **Rio de Janeiro:** Duque de Caxias, MNRJ 1478 **paralectotype**; Nova Iguaçu, MNRJ 2248 **lectotype**; Pacarambi, MNRJ 76526-76531.

Scinax jureia (n=9): BRAZIL: **São Paulo:** Iguapé, Estação Ecológica da Jureia-Itatins, MNRJ 14202, 14203 **paratype**; ZUEC 8875 **holotype**, 8885, 8889, 8870, 8872, 8885, 8896 **paratypes.**

Scinax kautskyi (n=6): BRAZIL: **São Paulo:** Aracruz, MNRJ 39785-39788, 39792, 39794.

Scinax littoralis (n=10): BRAZIL: **São Paulo:** Iguapé, ZUEC 8892 **holotype**, 8876, 8880,8882, 8885, 8886, 8888, 8890, 8893, 8894 **paratypes**.

Scinax longilineus (n=3): BRAZIL: **Minas Gerais:** Poços de Caldas, CHUNB 57632-57634.

Scinax luizotavioi (n=17): BRAZIL: **Minas Gerais:** Santa Bárbara, MNRJ 4210 **holotype**, 4211-4215 **paratypes**; São Gonçalo do Rio Abaixo, MNRJ 4509-4516 **paratypes**; Viçosa, ZUEC 16149; Espírito Santo: Vargem Alta, ZUEC 20841, 20853.

Scinax machadoi (n=10): BRAZIL: **Minas Gerais:** Botumirim, MNRJ 82435, 82436; Cristália, MNRJ 32888; Jaboticatubas, CFBH 6244 **paratype**, MNRJ 57810, 87811, ZUEC 1926, 15904, 15912 **paratypes**.

Scinax obtriangulatus (n=10): BRAZIL: **Minas Gerais:** Cidade Azul, MNRJ 55844-55847; **Rio de Janeiro:** Alto Itatiaia, MNRJ 4036, 87592 **paratypes**; Petrópolis, ZUEC 14742; Resende, ZUEC 4082; **São Paulo:** Campos do Jordão, ZUEC 3819; São José do Barreiro, ZUEC 6476.

Scinax pombali (n=5): BRAZIL: **Minas Gerais:** Capitólio, MNRJ 49479 **holotype**, 49476-49478, 54986 **paratypes**.

 Scinax ranki (n=10): BRAZIL: **Minas Gerais:** Poços de Caldas, CFBH 6256, 6259 **paratypes**, MNRJ 91198-91200; **Rio de Janeiro:** Angra dos Reis, CHUNB 57649-57653.

Scinax skaios (n=92): BRAZIL: **Goiás:** Alto Paraíso de Goiás, CHUNB 16907-16913, 17546, 47575; Barro Alto, ZUFG 3662; Caiapônia, ZUFG 5867-5869, 5971, 5972, 5874, 5875, 5877-5880, 5882-5884, 6226-6239, 9088-9090; Formoso, CHUNB 73198, 73200; Luziânia, CHUNB 40889, 40894-40896, 40899, 43437, 43462, 43463; Niquelândia, ZUFG 8471, 8472; Pirenópolis, ZUFG 15183-15195; Santa Rita do Novo Destino, MNRJ 54471 **holotype**, 54472-54474 **paratopotypes**, ZUFG 15203 **topotype**; Distrito Federal: Brasília, CHUNB 33786, 37348, 40939, 47607, 47608, 47615, 47621, 47622, 47632, ZUFG 9093, 9097, 9098, 14459, 15205-15209; Minas Gerais: Unaí, CHUNB 30908.

Scinax skuki (n=10): BRAZIL: **Alagoas:** Maceió, MNRJ 70000 **holotype**, 70003-70011 **paratypes**.

Scinax strigilatus (n=8): BRAZIL: **Bahia:** Arataca, MNRJ 44988; Ibirapitanga, MNRJ 38098 **neotype**, 38093, 38094, 38096; Itapebi, MNRJ 38101-38103.

Scinax trapicheiroi (n=6): BRAZIL: **Rio de Janeiro:** Maricá, MNRJ 75041, 75043, 75045, 75046; Saquarema, MNRJ 79576, 79578.

Scinax goya (n=18): BRAZIL: **Bahia:** Sítio d'Abadia, CEPB 10015-10020, 10022-

2765   10033 **topotypes**.

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CONCLUSÕES GERAIS

O presente trabalho teve como objetivo investigar as populações conhecidas de *Scinax skaios*, fazendo uma avaliação da variação intraespecífica na morfologia, canto de anúncio e variação molecular para esclarecer a real identidade desta espécie e desvendar possíveis espécies novas. Além disso, uma modelagem de nicho ecológico foi feita para prever áreas adequadas de ocorrência da espécie.

Como conclusões gerais, podemos destacar:

- As variações morfológicas são mais qualitativas do que quantitativas. As variações na forma do corpo são sutis, algumas delas sendo perceptíveis apenas quando comparando indivíduos de uma população com outra. Apesar de sutis, podemos observar variação nos traços morfológicos/cromáticos (até então nunca registrados para a espécie), como forma do focinho, padrões de manchas e faixas nos flancos e parte oculta das coxas, formato da cabeça e profundidade do *canthus rostralis.*

- Populações de *S. skaios* que mostraram maior variação nos traços morfológicos e morfométricos avaliados, em comparação com a população topotípica, foram as populações de Caiapônia (Goiás), Brasília (Distrito Federal) e Paracatu (Minas Gerais).

- O canto de anúncio da população de Paracatu difere das demais analisadas, principalmente no número de notas (muito menor do que apresentado pelos machos da localidade-tipo, Pirenópolis e Caiapônia), no maior número de pulsos por nota e maior frequência dominante. Nosso *n* amostral para vocalizações das populações de *S. skaios* que ocorrem em Goiás foi baixo, e por isso, sugerimos a análise de um número maior de cantos por macho e de diferentes machos para obter uma informação mais robusta sobre os parâmetros acústicos das populações,

além de incluir dados acústicos da população de Brasília (o que não foi possível neste trabalho).

- As análises moleculares revelaram alto grau de divergência genética entre as populações de Paracatu, Brasília e Caiapônia em relação a população topotípica, sugerindo que essas populações sejam novas espécies sob o nome de *S. skaios*.

- A modelagem de nicho indicou áreas adequadas à ocorrência de *Scinax skaios* em 10% dos municípios do Estado de Goiás, em regiões mais a leste e nordeste do Estado, incluindo áreas altas no Planalto Central, e ainda mostrou uma baixa probabilidade de que a espécie ocorra na região sul de Goiás.

- Os resultados mostram variações fenotípicas e genéticas entre as diferentes populações reconhecidas como *S. skaios*, sugerindo que essas populações se referem a um complexo de espécies crípticas, e fornecem evidência de três espécies candidatas do grupo *Scinax catharinae* ocorrendo no Cerrado.

- Este trabalho evidencia a importância da utilização de múltiplas linhas de evidência em estudos taxonômicos.

- O reconhecimento e descrição de novas espécies é fundamental para a conservação da biodiversidade. Aqui, a descrição formal de uma das espécies candidatas confirmadas (população de Paracatu, Minas Gerais) é apresentada. A descrição de uma nova espécie do grupo *Scinax catharinae* para o Cerrado é muito importante, revelando que a diversidade deste grupo, até então pouco representado neste bioma, pode ser maior do que se imagina. Como é uma espécie restrita a córregos e riachos associados à matas de galeria, ações de conservação da espécie traria benefícios não somente a espécie, mas também ao rio Paracatu, muito mal tratado nos últimos anos, e ao abastecimento de água da região.