UNIVERSIDADE FEDERAL DO AMAZONAS – UFAM
INSTITUTO DE CIÊNCIAS BIOLÓGICAS – ICB

PROGRAMA DE PÓS-GRADUAÇÃO EM ZOOLOGIA – PPGZOOL

Avaliação do status taxonômico de uma linhagem de rãzinha-da-serrapilheira do complexo *Adenomera simonstuarti* das florestas de areia branca da Reserva de Desenvolvimento Sustentável do Rio Negro, Amazônia Central, Brasil

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Manaus, Amazonas

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“If the future's looking dark
We're the ones who have to shine
If there's no one in control
We're the ones who draw the line
Though we live in trying times -
We're the ones who have to try
Though we know that time has wings -
We're the ones who have to fly”
Everyday Glory (1993) - Rush

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SUMÁRIO

INTRODUÇÃO GERAL

A Amazônia é berço de inúmeras espécies da fauna e flora e abriga alta diversidade biológica (Tisseuil *et al.*, 2013; Hermes *et al.*, 2015; Vacher *et al.*, 2020). Ela ocupa aproximadamente metade do território brasileiro e sua área compreende cerca de 25% das florestas do planeta (Baccaro *et al.*, 2008). Surpreendentemente, o conhecimento sobre a diversidade de espécies Amazônicas permanece ainda subestimado. Sabe-se atualmente que, a proporção de espécies ainda não descritas pra região é alta em diversos grupos biológicos-Déficit Linneano (e.g., Machado *et al*., 2018; Vacher *et al*., 2020). Além disto, o conhecimento sobre a distribuição geográfica de diversas espécies possui ainda grandes lacunas a serem preenchidas-Déficit Wallaceano (Bini *et al*., 2006).

Dentre os vertebrados amazônicos, os anuros estão entre os que possuem provavelmente a riqueza de espécies mais subestimada. A cada ano, inúmeras espécies são descritas para a Amazônia (e.g. Ferrão *et al*., 2020a; Ferrão *et al*., 2020b; Lima *et al*., 2020; Moraes & Lima, 2021) e a taxa de descrição de novas espécies não parece diminuir, uma vez que foram descritas para a Amazônia brasileira 41 espécies nos dois últimos anos (16 espécies em 2019 e 26 em 2020) (Segalla *et al.*, 2021).

Um dos primeiros grandes estudos compilando o conhecimento sobre a riqueza de anuros na Amazônia foi feito por Azevedo-Ramos e Galatti (2002) revelando 163 espécies na Amazônia brasileira. O baixo número pode ter relação com as dificuldades enfrentadas para acessar áreas distantes dos centros urbanos. Entretanto, sabe-se hoje que pelo menos 370 espécies ocorrem na Amazônia brasileira (Hoogmoed e Galatti, 2021). Em se tratando da Amazônia em sua totalidade, Godinho e Silva (2018) registraram 577 espécies, evidenciando que o Brasil abriga 65% das espécies de anfíbios de toda Amazônia.

O estudo mais recente com base em dados moleculares obtidos em larga escala geográfica revelou que pelo menos 876 espécies, entre *taxa* descritos e não descritos, possam ocorrer na Amazônia (Vacher *et al*., 2020), uma estimativa aproximadamente duas vezes maior do que a apresentada pela Lista Vermelha da União Internacional pela Conservação da Natureza (IUCN Red List). A diferença entre estas duas últimas estimativas representam, em grande parte, espécies não descritas associadas a espécies nominais tidas como amplamente distribuídas (Vacher et al., 2020). O elevado número de espécies não descritas impacta diretamente na conservação dos anuros amazônicos, uma vez que a elaboração de medidas conservacionistas é baseada principalmente na distribuição de espécies nominais (Foden *et al.*, 2013; Jenkins *et al*., 2013; Caminer *&* Ron, 2014).

Vários grupos de anuros, principalmente os proximamente relacionados, possuem morfologia externa conservada e apresentam diferenças sutis entre espécies-espécies crípticas (e.g., Jorge et al., 2020; Lima et al., 2020). A cripticidade entre pares de espécies propiciou inúmeras delimitações errôneas de espécies em estudos baseados exclusivamente em caracteres morfológicos (Simões, 2010). Esta abordagem dificulta a descrição de espécies novas com morfologia mais conservada, visto que nem sempre o processo de especiação promove mudanças facilmente diagnosticáveis na morfologia das espécies (Bickford, 2007). Entretanto, este cenário vem mudando nos últimos anos. Taxonomistas têm integrado dados moleculares, morfológicos, filogeográficos, bioacústicos, ecológicos e de comportamento reprodutivo para incrementar as delimitações de espécies em estudos recentes-taxonomia integrativa (Padial *et al*., 2010). Com esta abordagem integrativa, muitas espécies crípticas anteriormente "escondidas" em espécies tidas como amplamente distribuídas estão sendo delimitadas e descritas (e.g., Ferrão et al., 2016, 2017, 2018a, 2018b; Lima et al., 2020; Carvalho et al., 2021; Fouquet et al., 2021a, 2021b).

As rãzinhas-da-serrapilheira do gênero *Adenomera* Steindachner, 1867 estão entre os anuros neotropicais com maior déficit Linneano. Fouquet et al. (2014) revelaram 31 espécies candidatas confirmadas em *Adenomera*, número que representava na época um aumento de 94% na riqueza de espécies para o gênero. Carvalho et al. (2021) revelaram algumas espécies candidatas a mais e descreveram seis novas espécies de *Adenomera*. Das 29 espécies nominais atualmente conhecidas de *Adenomera*, 14 foram descritas entre 2013 e 2021 (Carvalho e Giaretta, 2013; Carvalho et al., 2019a, 2019b, 2019c, 2020a, 2020b, 2021). Grande parte destas descrições recentes foram realizadas através de taxonomia integrativa. Uma vez que espécies em vários clados de *Adenomera* apresentam morfologia conservada, parte considerável destas descrições recentes não poderiam ter sido feitas sem integrar outras linhas de evidencia, principalmente moleculares e bioacústicas.

Adenomera é um gênero de pequenos anuros terrestres que pertence à família Leptodactylidae Werner, 1896 (1838) e que se distribui por toda a América do Sul a leste dos Andes e possui atualmente 29 espécies formalmente descritas (Frost, 2021). O histórico taxonômico do gênero é bastante complexo, tendo sido sinonimizado a *Lithodytes* e *Leptodactylus* e revalidado diversas vezes (veja Lutz, 1930; Parker, 1932, 1935; Heyer, 1973, 1974; Frost *et al*., 2006; Ponssa *et al*., 2008; Pyron e Wiens, 2011; Fouquet *et al*., 2014; da Sá *et al*., 2014). Oito clados são atualmente reconhecidos em *Adenomera* (sensu Fouquet *et al*., 2014), sete dos quais associados a espécies nominais: clados *A. andreae, A. heyeri, A. hylaedactyla, A. lutzi, A. marmorata, A. martinezi* e *A. thomei*.

O clado *Adenomera andreae* é restrito da Amazônia e compreende quatro espécies: *A. andreae* (Müller, 1923), *A. chicomendesi* Carvalho, Angulo, Kokubum, Barrera, Souza, Haddad & Giaretta, 2019c, *A. guayaro* Carvalho, Angulo, Barrera, Aguilar-Puntriano & Haddad, 2020a e *A. simonstuarti* (Angulo & Icochea, 2010), além de duas espécies candidatas identificadas como *Adenomera* sp. D e *Adenomera* sp. T (Fouquet *et al*., 2014; Carvalho *et al*., 2019c). *Adenomera andeae* foi descrita da Amazônia brasileira oriental no estado do Pará com base em caracteres morfológicos e está amplamente distribuída pelo bioma (Fouquet *et al*., 2014; Carvalho et al., 2019d). Através de caracteres morfológicos e bioacústicos, *A. simonstuarti* foi descrita de Camisea, Cusco, Peru. Duas espécies foram descritas através de taxonomia integrativa (DNA, morfologia, bioacústica): *Adenomera chicomendesi* e *A. guayaro*. A primeira foi descrita do Parque Zoobotânico da Universidade Federal do Acre, Rio Branco (Brasil) e a segunda descrita da Reserva Nacional Tambopata, distrito e província de Tambopata (Peru). Duas espécies (*Adenomera* sp. D e *Adenomera* sp.T) possuem apenas um registro cada no Peru, e são consideradas espécies candidatas não confirmadas devido à ausência de dados bioacústicos.

Carvalho *et al*. (2020a) analisaram dados moleculares, bioacústicos e morfológicos de populações topotípicas de *A. simonstuarti* e populações adicionais distribuídas em uma área total de 2 milhões de km^2 na Amazônia. Populações analisadas estão divididas em oito linhagens (*Adenomera simonstuarti* 1 à *Adenomera simonstuarti* 8, sendo a linhagem 3 relativa à espécie nominal descrita em 2010) (Carvalho. et al., 2020a). *Adenomera simonstuarti* sensu stricto parece restrita ao sudoeste da Amazônia no Peru e Brasil. O estudo ressalta que as linhagens adicionais podem representar espécies crípticas, porém a delimitação do status taxonômico das mesmas depende da análise de dados bioacústicos—linha de evidência essencial na diferenciação interespecífica em *Adenomera*.

Recentemente, Albertina P. Lima e Miquéias Ferrão coletaram espécimes e cantos de anúncio de uma espécie de *Adenomera* habitante das florestas de areia branca do interflúvio entre os rios Engro e Solimões, na Reserva de Desenvolvimento Sustentável Rio Negro, Amazônia central (Amazonas, Brasil). Dados moleculares revelaram que estes espécimes representam uma nova linhagem dentro de *A. simonstuarti* sensu lato. Além disto, dados bioacústicos desta nova linhagem são fortemente divergentes daqueles de *A. simonstuarti* sensu stricto. As duas linhas de evidências indicam congruentemente que este táxon representa uma nova espécie.

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A new species of foam-nesting frog (Anura: Leptodactylidae: *Adenomera*) from white-sand forests of central Amazonia, Brazil

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Resumo

Por meio da taxonomia integrativa nos descrevemos uma nova espécie de rãzinha-da-serrapilheira do gênero *Adenomera* das florestas de areia branca da Reserva de Desenvolvimento Sustentável Rio Negro, Amazônia central, Brasil. Dentro do clado *A. andreae,* a nova espécie se aninhou dentro do complexo *A. simonstuarti* como irmã da linhagem *A. simonstuarti* 2 do baixo Juruá. A nova espécie é atribuída ao gênero *Adenomera* por ter tamanho menor que 34,1 mm, ausência de franjas e membranas entre os dedos e ausência de espinhos nos polegares de machos adultos. Difere de outras espécies amazônicas pela combinação dos seguintes caracteres: ausência de tubérculo antebraquial; pontas dos dedos achatadas ou ligeiramente achatadas, com expansões visíveis; presença de uma faixa escura quase sólida na parte inferior do antebraço; canto de anúncio com nota única formado por 11–21 pulsos parcialmente fundidos; frequências fundamental e dominante variando entre 1.765–2.239 Hz e 3.448–4.349 Hz, respectivamente; duração do canto variando entre 100–199 ms. Embora tenhamos amostrado muitos módulos permanentes de amostragem em florestas ombrófilas na região de Manaus e no interflúvio Purus-Madeira na última década, a nova espécie foi encontrada apenas em florestas de areia branca à oeste do Interflúvio Negro-Solimões, o que indica que *Adenomera* sp. nov. pode ser endêmica ou pelo menos especialista neste tipo de ambiente no interflúvio Negro-Solimões.

Palavras-chave Anfíbios, taxonomia integrativa, campina, campinarana, Interflúvio Negro-Solimões.

Abstract

Through integrative taxonomy we describe a new species of foam-nesting frog of the genus *Adenomera* from white-sand forests of the Reserva do Desenvolvimento Sustentável Rio Negro, Central Amazonia, Brazil. Within the *A. andreae* Clade, the new species nest within *A. simonstuarti* complex as sister to the lineage *A. simonstuarti* 2 from Lower Juruá. The new species is assigned to the genus *Adenomera* by having smaller than 34.1 mm, lack of fringing and webbing between toes and the absence of spines in adult males' thumbs. It differs from other *Adenomera* Amazonian species by the combination of the following characters: absence of antebrachial tubercle; toe tips flattened or slightly flattened, with visible expansions; presence of nearly solid dark-colored stripe on underside of forearm; single-note call formed by 11–21 partly fused pulses; fundamental and dominant frequencies varying between 1,765–2,239 Hz and 3,448–4,349 Hz, respectively; call duration varying between 100–199 ms. Although we have sampled many permanent sampling modules in ombrophilous forests in Manaus region and in the Purus-Madeira interfluve in the last decade, the new species was only found in the white-sand forest from West Negro-Solimões Interfluve, which indicate that *Adenomera* sp. nov. might be an endemic or at least a specialist in this kind of environment in the Negro-Solimões interfluve.

Keywords Amphibia, integrative taxonomy, campina, campinarana, West Negro-Solimões Intefluve.

Introduction

Leptodactylid frogs of the genus *Adenomera* Steindachner, 1867 are distributed throughout South America east of the Andes and comprises 29 described species (Frost, 2023). The taxonomic history of this genus is very complex. Over the last 50 years, some systematic studies reviewed the phylogenetics position of *Adenomera* (Heyer, 1973; 1974; Frost et al., 2006; Pyron & Wiens, 2011; de Sá et al., 2014). The genus was described by Steindachner (1867) to accommodate *A. marmorata*. Later, Lutz (1930) synonymized it to *Parvulus*, a subgenus of *Leptodactylus* Fitzinger, 1826, but Parker (1932) gave priority to the genus *Adenomera* and elevated it as a subgenus of *Leptodactylus*. Then, Parker (1935) reallocated *A. marmorata* to the genus *Leptodactylus*. Four decades later, *Adenomera* was resurrected by Heyer (1974) to harbor taxa of the *Leptodactylus marmoratus* species group (sensu Heyer, 1968). To avoid the paraphyly of *Leptodactylus*, Frost et al. (2006) placed *Adenomera* as a synonym of *Lithodytes* Fitzinger, 1843, and the latter as a synonym (subgenus) of *Leptodactylus*. Based on molecular data, Pyron & Wiens (2011) recovered *Adenomera* as a sister to *Lithodytes*, and this clade as a sister to *Leptodactylus*; then, removed the two former taxa from the synonym of *Leptodactylus*. The relationship *Adenomera* + *Lithodytes* was corroborated by de Sá et al. (2014) through a total evidence analysis, which recuperated (*Adenomera* + *Lithodytes*) + (*Hydrolaetare* + *Leptodactylus*). Fouquet et al. (2014) published a comprehensive phylogenetic tree in which *Adenomera* is classified into eight major species clades: *A. lutzi* clade, *A. heyeri* clade, *A.* sp. I clade, *A. andreae* clade, *A. marmorata* clade, *A. thomei* clade, *A. martinezi* clade and *A. hylaedactyla* clade. However, Carvalho et al. (2021) recovered *Adenomera* sp. I as *Adenomera juikitam* sensu stricto and, through acoustic, morphologic and genetic data, concluded that *A. juikitam* belongs to *A. heyeri* clade instead a different clade.

The genus *Adenomera* presents a high prevalence of morphologically cryptic species (Carvalho et al., 2020a). Some species in the genus also show high levels of intraspecific polymorphism (e.g., Cassini et al., 2020). Congeneric sympatry and syntopy are common in *Adenomera*; up to three species might occur in the same region, sometimes in syntopy (e.g., Carvalho et al., 2021). These factors make the species delimitation in *Adenomera* challenging. Nevertheless, 14 of the 29 currently recognized species were described in the last 10 years (Frost, 2022) and several candidate species await formal description (Fouquet et al. 2014). The massive taxonomic advance in the taxonomy of *Adenomera* has been possible due to the use of integrative taxonomy (Carvalho et al., 2019a; c). Despite morphological crypsis the advertisement call in *Adenomera* is markedly divergent between species and represents a powerful source of diagnostic characters (Angulo & Icochea, 2010; Carvalho & Giaretta 2013a, b; Carvalho et al., 2019c, 2021).

The *Adenomera andreae* clade comprises four described species: [*A. andreae* (Müller, 1923): *A. chicomendesi* Carvalho, Angulo, Kokubum, Barrera, Souza, Haddad & Giaretta, 2019, *A. guayaro* Carvalho, Angulo, Barrera, Aguilar-Puntriano & Haddad, 2020 and *A. simonstuarti* (Angulo & Icochea, 2010), and two candidate species (*Adenomera* sp. D and T; sensu Fouquet et al., 2014]. The *Adenomera andreae* clade is restricted to Amazonia, but none of the nominal species has restricted geographic distribution. *Adenomera andreae* shows the widest range, being distributed throughout Amazonia (Carvalho et al., 2019c), while *A. chicomendesi* and *A. guayaro* are widely distributed in southwestern Amazonia (Carvalho et al., 2019a; Carvalho et al., 2020a). Finally, *A. simonstuarti* is distributed in western and southwestern Amazonia (Carvalho et al., 2020b).

The species *Adenomera simonstuarti* was described from Peruvian Amazonia through morphological and bioacoustic traits of four males and two females (Angulo & Icochea, 2010). Posteriorly, Fouquet et al. (2014) indicated that the species was more widespread than previously thought, also occurring in Venezuela, Ecuador, Peru and Brazil (states of Acre and Amazonas). Fouquet et al. (2014) also suggested the existence of more than one species hidden under the name *A. simonstuarti* (see Appendix S2a of Fouquet et al. 2014). Recently, Carvalho et al. (2020b) sequenced new specimens from Brazil referred to *A. simonstuarti* and their lineage delimitation analysis recovered eight lineages within this name (hereafter *A. simonstuarti* complex). Carvalho et al. (2020b) also redescribed the species' advertisement call based on recordings from the type locality in Peru and an additional locality in the upper Juruá River Basin (Acre, Brazil). Based on molecular, morphological, and bioacoustic data, Carvalho et al. (2020b) recognized their lineage 3 as *A. simonstuarti* sensu stricto (nominal species). They also suggested that the other lineages might represent putative new species, but confirmation is pending while additional data are acquired (*e.g*., acoustic and morphologic data).

It is well documented that poorly sampled environments in Amazonia usually harbor undocumented biodiversity of anurans (Ferrão et al., 2016; Vacher et al., 2020). Physiognomies comprising the white-sand ecosystems (hereafter WSE) are great examples of poorly sampled environments (Adeney et al., 2016). The WSE occupies an area of 5% of the Brazilian Legal Amazonia and comprises two main physiognomies: *campina* – open environments characterized as patches of grasslands or scrublands (canopy < 7 m) on a matrix of exposed sandy soil; *campinarana* – closed-canopy, forested environments characterized by thin-trunked trees of low stature (canopy < 20 m) (Anderson, 1981; Ferreira, 2009; Adeney et al., 2016). Despite the increasing interest of scientists on WSE organisms (Fine & Baraloto., 2016; Vicentini, 2016; Lamarre et al., 2016; Capurucho et al., 2013; Borges et al., 2016; Fraga et al., 2018; Ferreira et al., 2019; Gonella et al., 2020), studies involving anurans from WSE are still rare. The few recently published studies have shown that WSE represent a source of poorly known and new species of anurans, many of which seems specialists or endemics to these environments (Carvalho et al., 2019a; Ferrão et al., 2019b; Ferrão et al., 2022; Mônico et al., in press).

In the present study, we present an unreported lineage from the white-sand forests of Central Amazonia and describe it through integrative taxonomy as a new species of the *Adenomera simonstuarti* complex.

MATERIAL AND METHODS

Sampling

Fieldwork was conducted between 2019 and 2023 in three RAPELD (long-term ecological research modules) permanent sampling modules at the Rio Negro Sustainable Development Reserve (hereafter, RDS Rio Negro), municipality of Iranduba, state of Amazonas, Brazil (Fig. 1). Modules are installed near the km 18 (3°06'33.6"S, 60°40'29.0"W; 73 m above sea level [hereafter [asl]), km 26 (3°03'31.0"S, 60°45'42.0"W; 73 m asl) and km 50 (2°50'10.0"S, 60°50'20.0"W; 19 m asl) of the AM-352 highway. Adults were euthanized with 2% benzocaine topical solution, fixed in 10% formalin and preserved in 70% ethanol. Before fixation, tissue samples were collected of each specimen and stored in 100% ethanol. Specimens were deposited in the herpetological collections of the Instituto Nacional de Pesquisas da Amazônia – **INPA-H** (Manaus, Brazil), Museu Paraense Emílio Goeldi – **MPEG** (Belém, Brazil) and Museu de Zoologia da Universidade de Campinas – **ZUEC-AMP** (Campinas, Brazil).

Advertisement calls of six males of the new species (**INPA-H** 44867, **MPEG** 44649, **INPA-H** 44868–44869, **MPEG** 44652, **INPA-H** 44877 [field numbers APL 21878–81, 23721–22, respectively]) were recorded with a Sennheiser K6/ME66 unidirectional microphone (Sennheiser, Germany) coupled to a Marantz PMD660 digital recorder (Kanagawa, Japan), and with a Sony PCM-D50 digital recorder with built-in microphone. Recordings were stored in wav files with sampling rate of 44.1 kHz and sample size of 16 bits. The microphone was positioned ~50–100 cm from the focal active male. Air temperature during the recordings was 25ºC. Recordings were deposited in the Neotropical Jacques Vielliard of the University of Campinas - **FNJV**

(Campinas, Brazil) under accession number FNJV 59561–59566. We also deposited a video of the species calling, photographs and a recordinig in the Sapoteca of the Programa de Pesquisa em Biodiversidade (PPBio), INPA.

To improve comparisons, 16 individuals of *Adenomera simonstuarti* sensu stricto were collected at Unidade de Gestão Ambiental do Acurauá (hereafter UGAI Acurauá), municipality of Tarauacá, state of Acre, Brazil. An individual of this locality was included in the phylogenetic inference of Carvalho et al. (2020) and nest with samples of *A. simonstuarti* sensu stricto from Peru, and advertisement calls of Acre population males match with the ones described in the original description of Angulo and Icochea, (2010). All males of the Acre population were found in the field through their vocalization, ensuring we collected the target taxa and not a close relative (e.g., *A. andreae*). See appendix 4 for morphometric measurements of individuals of *A. simonstuarti* sensu stricto collected in the present study.

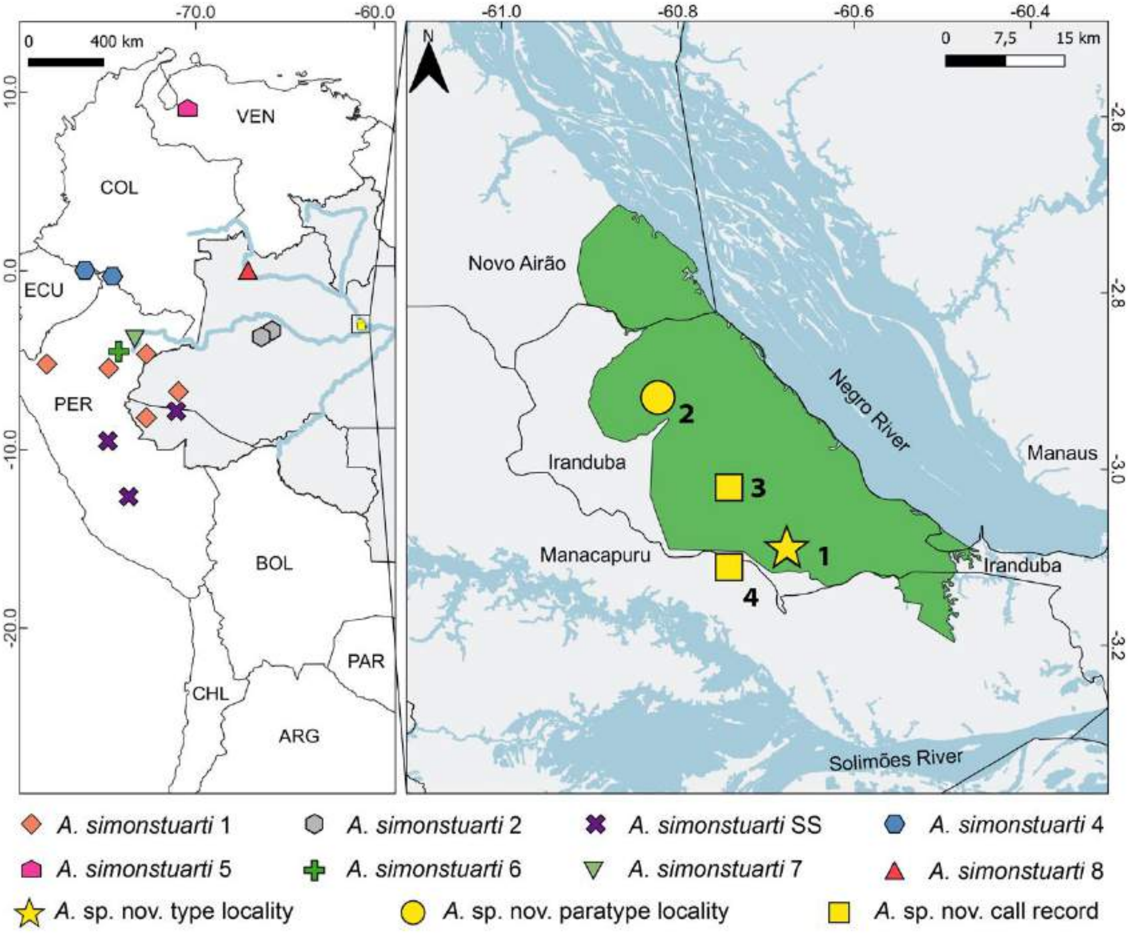

Figure 1. Geographic distribution of *Adenomera simonstuarti* species complex in central Amazonia, Amazonas, Brazil. Green area = Reserva de Desenvolvimento Sustentável Rio Negro. Numbers: permanent sampling modules at (1) km 18, (2) km 26 and (3) 50 of the AM-352 highway; (4) Vale da Benção Community, Ramal do 25, Manacapuru. South American countries: BOL, Bolivia; COL, Colombia; PAR, Paraguay; PER, Peru; VEN, Venezuela.

Morphology

External morphology of the new species is described based on 21 males and 5 females. Sex was determined through direct inspection of sexual characters, such the presence of vocal slits, vocal sac and fleshy ridge on the snout tip in males, and oviducts in females. Morphometric measurements were taken to the nearest 0.1 mm using a digital caliper and an ocular micrometer coupled to a stereomicroscope. The following 16 morphometric measurements were taken following Watters *et al*. (2016): snout-vent length (SVL), head length (HL), head width (HW), snout length (SL), eye-nostril distance (EN), eye diameter (ED), interorbital distance (IOD) internarial distance (IND), tympanum diameter (TD), upper arm length (UAL), hand length (HAL), forearm length (FLL), thigh length (THL), tibia length (TL), and foot length (FL), and tarsus length (TSL). Toe tip development (character states) follows Heyer *et al*. (1973). Snout shape terminology follows Heyer *et al.* (1990). Morphological description follows Carvalho et al. (2020a).

Vocalization

The advertisement call description and the following acoustic parameters follow Carvalho *et al*. (2019a): call duration (CD), notes per call (NpC), note duration (ND), note rise time (NrT), pulses per note (PpN), pulse duration (PD; measured for the first, central and last pulses of each note), dominant frequency (DF), fundamental frequency and frequency modulation (FM). Calls were analyzed with *Raven* 1.6.1 (Bioacoustics Research Program, 2017) configured as follows: Hamming window, filter bandwidth of 65 Hz, overlap of 90%, hop size of 2 ms, and Discrete Fourier Transform of 1,024 points. Dominant frequency was measured with the *peak frequency* function. Figures were produced in *R platform* (R Core Team, 2022) with the packages *seewave* 2.1.0 (Sueur et al., 2008) and *tuneR* 1.3.2 (Ligges et al., 2017). *Seewave* was set as follows: Hanning window, Fast Fourier Transform of 256 points, overlap of 90%.

Molecular phylogenetics

Genomic DNA was extracted from tissues of four individuals of *Adenomera* sp. nov. DNA was extracted using a Wizard genomic DNA Purification Kit (Promega Corp., Madison, WI, USA) according to the manufacturer's protocol. Fragments of Cytochrome c oxidase subunit I (COI) were amplified through polymerase chain reaction (PCR) using CHmL4 (5’-TYTCWACWAAYCAYAAAGAYATCGG-3’) and CHmR4 (5’-ACYTCRGGRTGRCCRAARAATCA-3’) (Che et al. 2012), under the following conditions: 60 s at 94°C followed by 35 cycles of 94°C (20 s), 50°C (50 s) and 72°C (90 s), and final extension of 10 minutes at 72°C. The final volume of the PCR reaction was 15 µL and contained 0.6 µL of 50 mM MgCl2, 1.2 µL of 10 mM dNTPs (2.5 mM each dNTP), 1.5 µL of tampon 10x (75 mM Tris HCl, 50 mM KCl, 20 mM (NH4)2SO4), 0,5 µL of each primer (10 µM), 9.55 µL of ddH2O and 0.15 µL of 1 U Taq DNA Polymerase and 1 µL of DNA (30–50 ng/µL).

The PCR products were purified using Exonuclease I and Thermosensitive Alkaline Phosphatase (Thermo Fisher Scientific, Waltham, MA, USA). After this, sequencing reactions were performed using standard protocols of the Big Dye^{TM}

Terminator Kit (Applied Biosystems, Waltham, USA). We use an automated sequencer ABI Prism 3130 (ThermoFisher Scientific, Waltham, USA) to sequence the amplicons. Sequences were edited with Geneious 5.3.4 (Kearse et al., 2012). Newly generated sequences were deposited in the online repository GenBank under accession numbers OQ974333–36.

To infer phylogenetic relationships, we inserted the sequences we generated into a data set containing sequences retrieved from GenBank. Our dataset contains the genes cytochrome b (Cytb), cytochrome c oxidase subunit I (COI), recombination activating gene 1 (RAG1) and pro-opiomelanocortin C. These sequences represent all species of *Adenomera andreae* Clade, including all lineages of *A. simonstuarti* and *A.* sp. D and T (see Carvalho et al., 2020), and representants of all nominal clades (*i.e., A. heyeri* Clade, *A. hylaedactyla* clade, *A. lutzi* clade, *A. marmorata* clade, *A. martinezi* clade and *A. thomei* clade) as well as *Lithodytes lineatus* used as outgroup. In total, 65 sequences of CYTB, 76 sequences of COI, 65 sequences of RAG1 and 65 sequences of POMC were retrieved from GenBank (see Appendix 1). To align sequences of each gene we used the MAFFT online server following default parameters under the G-INS-i strategy. The final matrix was concatenated in Geneious 5.3.4 (Kearse et al., 2012) and is composed of 53 terminals and 3,293 base pairs (bp) (667 pb for Cytb; 657 for COI; 1422 for RAG1; 547 for POMC).

We divided the dataset considering first, second, and third codons of each protein-coding gene, and to infer partition schemes and evolutionary models we perform PartitionFinder 2.1.1 (Lanfear et al., 2017) under the corrected Akaike information criterion (AICc). The best evolutionary models for partitions in the concatenated matrix were: TIM+G for Cytb 1st and COI 3rd codons; SYM+I+G for Cytb 2nd codon; GTR+I+G for Cytb 3rd codon; TRNEF+I+G for COI 1st codon; F81+I+G for COI 2nd codon; TRN+I+G for RAG1 1st and 2nd codons; GTR+G for RAG1 3rd and POCM 1st codons; TVM+I+G for POMC 2nd codon; GTR+I for POMC 3rd codon. Phylogenetic relationship was reconstructed through Maximum Likelihood (ML). The ML tree was inferred using IQTREE (Nguyen et al., 2015) implemented in the online server http://iqtree.cibiv.univie.ac.at/ (Trifinopoulos et al., 2016). Clade support was estimated with 10,000 ultrafast bootstrap replications (Hoang et al., 2018) using 5,000 maximum iterations, 3,000 replicates and minimum correlation coefficient of 0.99.

Using the COI alignment, we calculated pairwise genetic distances (uncorrected p-distance and Kimura-two-parameter distance; Kimura, 1980) between the new species and closely related taxa of the *A. simonstuarti* species complex using MEGA 6 (Tamura et al., 2013).

Morphometric and bioacoustic analyses

Due to the phenotypic similarity between *A. simonstuarti* sensu stricto and the new species, we performed a Principal Component Analysis (PCA) to test the existence of statistical difference between the morphometric and bioacoustic multidimensional space each species occupies. Morphological analysis was performed only for males due to the low number of collected females in *A. simonstuarti* sensu stricto. Therefore, the same 16 morphometric and 9 acoustic measurements taken from the new species were also taken from 14 (morphology) and 6 (acoustic) adult males of *A. simonstuarti*, respectively. To perform morphometric PCA we transform the raw data into 15 morphometric ratios as follows: HL/SVL, HW/HL, SL/HL, END/SL, IN/HW, ED/HW, IOD/HW, TYM/ED, FAL/THL, UAL/FAL, HAL/FAL, TL/TAL, FL/THL, THL/TL, TAL/TL. To perform acoustic PCA we use the following parameters: call duration, note duration, notes per call, pulses per note, pulse duration, note rise time, fundamental frequency, dominant frequency and frequency modulation. After performing acoustic PCA, we used the scores to perform a T-test and verify if the PC1 values for each species differs significantly.

Interspecific morphological comparisons

Phenotypic comparisons between the new species and its congeners were restricted to nominal species of *Adenomera* distributed in Amazonia: *A. amicorum* Carvalho, Moraes, Lima, Fouquet, Peloso, Pavan, Drummond, Rodrigues, Giaretta, Gordo, Neckel-Oliveira, and Haddad, 2021; *A. andreae* (Müller, 1923); *A. aurantiaca* Carvalho, Moraes, Lima, Fouquet, Peloso, Pavan, Drummond, Rodrigues, Giaretta, Gordo, Neckel-Oliveira, and Haddad, 2021; *A. chicomendesi* Carvalho, Angulo, Kokubum, Barrera, Souza, Haddad, and Giaretta, 2019a; *A. glauciae* Carvalho, Simões, Gagliardi-Urrutia, Rojas-Runjaic, Haddad and Castrovejo-Fisher, 2020b; *A, gridipappi* Carvalho, Moraes, Lima, Fouquet, Peloso, Pavan, Drummond, Rodrigues, Giaretta, Gordo, Neckel-Oliveira, and Haddad, 2021; *A. guarayo* Carvalho, Angulo, Barrera, Aguilar-Puntriano, and Haddad, 2020a; *A. heyeri* Boistel, Massary, and Angulo, 2006; *A. hylaedactyla* (Cope, 1868); *A. inopinata* Carvalho, Moraes, Lima, Fouquet, Peloso, Pavan, Drummond, Rodrigues, Giaretta, Gordo, Neckel-Oliveira, and Haddad, 2021; *A. juikitam* Carvalho and Giaretta, 2013a; *A. kayapo* Carvalho, Moraes, Lima, Fouquet, Peloso, Pavan, Drummond, Rodrigues, Giaretta, Gordo, Neckel-Oliveira, and Haddad, 2021; *A. lutzi* Heyer, 1975; *A. martinezi* (Bokermann, 1956); *A. phonotriccus* Carvalho, Giaretta, Angulo, Haddad, and Peloso, 2019b; *A. simonstuarti* (Angulo and Icochea, 2010); and *A. tapajonica* Carvalho, Moraes, Lima, Fouquet, Peloso, Pavan, Drummond, Rodrigues, Giaretta, Gordo, Neckel-Oliveira, and Haddad, 2021. Detailed comparisons are provided for closely related species indicated by phylogenetic relationships (*A. simonstuarti*).

RESULTS

Phylogenetic relationships and genetic distances

Individuals of *Adenomera* sp. nov. nest together as a completely new lineage within the *A. simonstuarti* species complex (sensu Carvalho et al., 2020b), which nest within the *A. andreae* clade (Fig. 2). The new species is sister to the lineage *A. simonstuarti* 2 collected in the lower Juruá River, in Brazil. Clades representing these lineages are the shallowest within the species complex and genetic p-distance (Table 1) between them is 2.9% for COI. Peruvian and Brazilian individuals of *A. simonstuarti* sensu stricto is recovered as sister to *A. simonstuarti* 1, *A. simonstuarti* 2 and *A.* sp. nov. The lineage *A. simonstuarti* 8 is placed as the most basal within the species complex. Genetic p-distance between the new species and *A. simonstuarti* sensu stricto is 5.1%.

Table 1. Pairwise genetic distances (%) between taxa of the *Adenomera simonstuarti* species complex and related species of the *A. andreae* clade and outgorup. Interspecific uncorrected p-distances (lower diagonal) and Kimura-2 parameters (upper diagonal) were based on a fragment of COI.

	Species	1	2	3	4	5	6	7	8	9	10	12	14	15
1	*A.* sp. nov.	**0.0**	3.9	2.9	5.4	5.6	5.0	5.4	5.5	7.0	13.7	17.4	13.8	25.2
2	*A. simonstuarti* 1	3.8	**0.02**	3.7	5.7	6.1	5.2	6.2	4.6	7.1	14.0	16.7	14.4	26.2
3	*A. simonstuarti* 2	2.8	3.6	**0.0**	5.5	7.0	5.6	6.1	5.5	7.5	14.6	17.1	14.4	25.2
4	*A. simonstuarti* SS	5.1	5.4	5.2	**0.0**	5.4	5.0	5.4	5.3	7.4	13.6	16.7	14.8	24.2
5	*A. simonstuarti* 4	5.3	5.8	6.5	5.1	**0.0**	4.1	7.5	5.7	7.7	14.5	15.7	14.0	23.7
6	*A. simonstuarti* 5	4.8	4.9	5.3	4.8	3.9	**n/c**	6.6	3.9	7.3	13.8	16.6	15.1	24.8
7	*A. simonstuarti 6*	5.1	5.8	5.8	5.2	7.0	6.2	**n/c**	6.1	7.0	14.1	17.6	14.4	24.8
8	*A. simonstuarti 7*	5.3	4.4	5.2	5.0	5.4	3.8	5.8	**n/c**	6.7	15.5	16.6	14.7	25.8
9	*A. simonstuarti 8*	6.5	6.7	7.0	6.9	7.2	6.9	6.5	6.4	**n/c**	13.0	16.5	12.6	23.5
10	*A. andreae*	12.3	12.5	12.9	12.2	12.9	12.3	12.6	13.7	11.7	**0.6**	17.0	15.3	24.0
12	*A. guarayo*	15.1	14.6	14.9	14.6	13.9	14.5	15.2	14.5	14.5	14.9	**0.3**	19.2	25.1
14	*A. chicomendesi*	12.3	12.8	12.8	13.1	12.5	13.3	12.8	13.0	11.4	13.4	16.6	**0.5**	26.2
15	*L. lineatus*	21.1	21.8	21.1	20.4	20.1	20.8	20.9	21.5	19.9	20.3	21.0	21.8	**n/c**

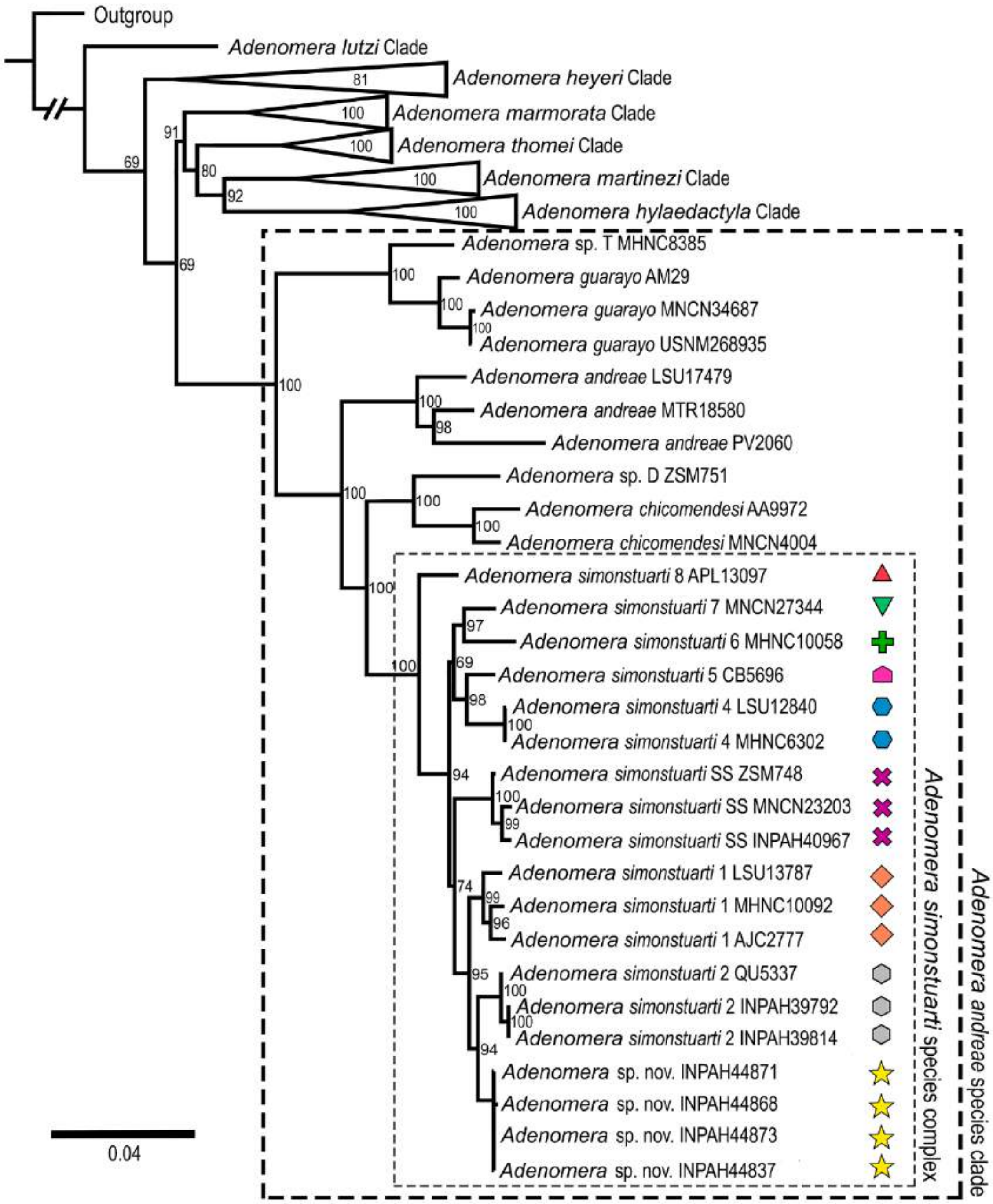

Figure 2. Phylogenetic tree of the *Adenomera andreae* species clade with a focus on the *A. simonstuarti* species complex. Maximum likelihood inferred based on Cytb, COI, RAG1 and POMC. Lineage numbering within *A. simonstuarti* species complex follows Carvalho et al. 2020. Species names are preceded by respective museum voucher numbers. Symbols are the same as in Figure 1.

Morphometric and bioacoustic analyses

The first two Principal Components (PCs) of morphometric and bioacoustic PCA explained ~ 44% and ~ 92% of data variance, respectively. Neither the centroids of morphometric (Fig. 3A) nor bioacoustic (3B) spaces occupied by *Adenomera* sp. nov. overlap with those of *Adenomera simonstuarti* sensu stricto. The three morphometric ratio which most contributed to the variation of PC1 are ED/HW, TL/TAL and TYM/ED. The three bioacoustic parameters which most contributed to the variation of PC1 are pulses per note, dominant frequency, and fundamental frequency. For data of other variables in PC1 and PC2 of morphometric and bioacoustic analyses, see table 2.

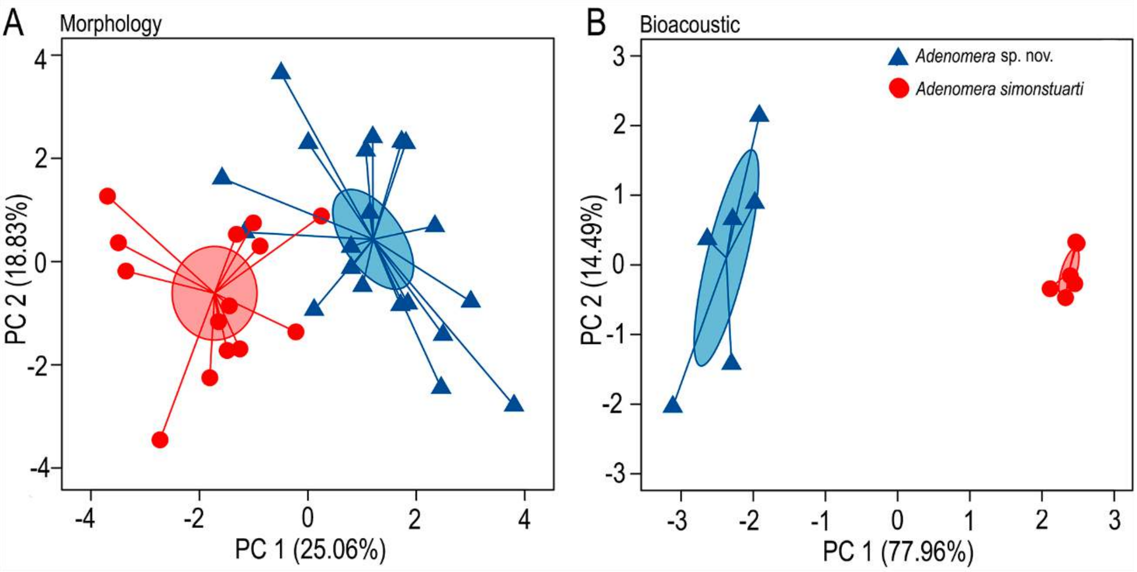

Figure 3. Morphometric and bioacoustic Principal Component Analyses. Analyses were based on 15 morphometric ratios of males and 9 parameters of calls of *Adenomera* sp. nov. (blue triangles) and *A. simonstuarti* sensu stricto (red circles). Ellipses means standard error with confidence interval = 95%. Recordings used in the analysis: FNJV 59562–59571.

For bioacoustic, we perform T-test using the PC1 scores of acoustic PCA analysis and the result corroborates those of this previous analysis (T-test: t = 28.1993; df = 10,P = 0.0001).

Table 2: Loadings of 15 morphometric and 9 acoustic ratios on the first principal components. For morphometric PCA, values were generated by a principal component analysis on 20 males of *Adenomera* sp nov. and 14 males of *Adenomera simonstuarti* sensu stricto. For acoustic PCA, values were generated by a principal component analysis on 6 males of *Adenomera* sp nov. and 6 males of *Adenomera simonstuarti* sensu stricto.

Morphometric			Acoustic		
Variables	PC 1	PC 2	Variables	PC 1	PC 2
HL/SVL	0.295	-0.205	CD	0.969	-0.101
HW/HL	-0.571	0.456	ND	-0.969	-0.001
SL/HL	0.338	0.363	NpC	0.964	-0.106
END/SL	0.473	0.159	NrT	0.696	0.474
IN/HW	0.425	-0.124	PpN	-0.993	-0.003
ED/HW	0.826	0.161	PD	0.952	-0.032
IOD/HW	0.672	-0.044	FF	0.987	-0.099

TYM/ED	-0.705	-0.260	DF	-0.995	0.048
FAL/THL	0.243	0.812	FM	0.045	0.955
UAL/FAL	-0.131	-0.697			
HAL/FAL	0.121	-0.376			
TL/TAL	-0.739	0.278			
FL/THL	0.222	0.314			
THL/TL	-0.093	-0.814			
TAL/TL	0.703	-0.419			

Table 3. Spectral and temporal parameters of the advertisement call of *Adenomera* sp. nov. and *A. simonstuarti* sensu stricto. Values depict average, standard deviation and range. Symbols: *, same values of call duration because the call is composed of only one note; **, measured by Carvalho et al. (2020b) from calls of the type locality in Cusco and Tarauacá. Abbreviation: SS, sensu stricto. Traits acronyms are described in the main text.

Call traits	*Adenomera* sp. nov. (n = 6)	*A. simonstuarti* SS (n = 6)	***A. simonstuarti* SS (n = 2)
CD (ms)	142 ± 19.0 (100–199), n = 148	4,700 ± 1,400 (1,800–7,000), n = 93	800–6,500
ND	*	64 ± 10 (40–93), n = 93	57–79
NpC	1 ± 0 (1–1), n = 148	22.4 ± 6.5 (9–33), n = 93	4–30
NrT (%)	28.4 ± 19.9 (2–73), n = 90	50 ± 14 (16–76), n = 93	13–73
PpN	14.8 ± 1.9 (11–21), n = 148	3.4 ± 0.7 (2–6), n = 93	2–3
PD (ms)	10 ± 3.3 (4–23), n = 444	26.4 ± 6.4 (10–53), n = 192	10–53
FF (Hz)	1,986 ± 0.1 (1,765–2,239), n = 148	3,987 ± 0.16 (3,617–4,263), n = 93	-
DF (Hz)	3,899 ± 1.3 (3,448–4,349), n = 148	1,991 ± 0.05 (1,851–2,224), n = 93	1,873–2,046
FM (Hz)	273.8 ± 238.1 (-173–861), n = 90	261.7 ± 119.7 (-173–517), n = 93	43–301

TAXONOMIC ACCOUNT

Adenomera sp. nov.
Adenomera gr. *heyeri* (Lima et al. 2021)
(Tables 3–4; Figs 4–7; 9B, C, D)

Holotype

INPA-H 44867 (Field number APL 21878), an adult male collected at km 26 of the AM-352 highway, Reserva do Desenvolvimento Sustentável do Rio Negro (03º05'35" S, 60º40'36" W; 76 m asl), municipality of Iranduba, state of Amazonas, Brazil on 11 December 2020 by M. Ferrão, A. P. Lima and W. E. Magnusson.

Paratopotypes

Twenty-four adult individuals from the same locality as the holotype. Eight males MPEG 44649, INPA-H 44868–44871, ZUEC AMP 25694, INPA-H 44872–44873 (field numbers 21879– 21886, respectively) collected on 11 December 2020 by M. Ferrão, A. P. Lima and W. E. Magnusson; four females INPA-H 44874, ZUEC AMP

25695, MPEG 44650, INPA-H 44875 (field numbers APL 23715– 23718, respectively) collected on 10 December 2021 by M. Ferrão, A. P. Lima and B. Martins; four males INPA-H 44876, MPEG 44651–44652, INPA-H 44877 (field numbers APL 23719–23722, respectively) collected on 11 December 2021 by M. Ferrão, A. Lima and B. Martins; four males INPA-H 44878–44880, ZUEC AMP 25696 (field numbers APL 23723– 23726, respectively) collected on 12 December 2021 by M. Ferrão, A. P. Lima and B. Martins; a male INPA-H 44881 (field number APL 23736) collected on 19 January 2022 by B. Martins; a male INPA-H 44882 (field number APL 23739) collected on 3 February 2022 by B. Martins; a male ZUEC AMP 25697 (field number APL 23944) and a female INPA-H 44883 (field number APL 23947) collected on 14 May 2022 by B. Martins.

Paratype

One adult male INPA-H 44885 (field number BCM1), collected at km 50 of the AM-352 Highway, Reserva do Desenvolvimento Sustentável do Rio Negro (2°50'10.0"S 60°50'20.0"W), municipality of Iranduba, state of Amazonas, Brazil on 12 January 2023 by B. Martins.

Generic placement

The species *Adenomera* sp. nov can be assigned to the genus *Adenomera* based on its small SVL in comparison with other leptodactylids (smaller than 34.1 mm), lack of fringing and webbing between the toes (Fig. 5) and absence of spines in adult males' thumb (Fig. 5A).

Diagnosis

The species *Adenomera* sp. nov. can be recognized by the following combination of characters. (1) Medium size (adult male SVL = 21.2–23.0 mm, n = 21; adult female SVL 22.1–24.3, n = 5); (2) snout rounded in dorsal view and acuminated in lateral view; (3) absence of antebrachial tubercle; (4) toe tips moderately to fully expanded (character states C–D); (5) throat in males with condensed melanophores near the jaw and scattered melanophores on the central portion, (6) advertisement call composed of a single pulsed note; (7) notes formed by 11–21 partly fused pulses; (8) dominant frequency of 3,448–4,349 Hz, (9) dominant frequency contained within the second harmonic.

Table 4. Morphometric measurements of the type series of *Adenomera* sp. nov. (RDS Rio Negro, Iranduba, Amazonas, Brazil) and *A. simonstuarti* sensu stricto (Tarauacá, Acre, Brazil). Values depict average, standard deviation and range. Trait acronyms are explained in the text. * Holotype included.

Adenomera sp. nov.				*Adenomera simonstuarti* sensu stricto	
Trait	Holotype	Males (n = 21)*	Females (n = 5)	Males (n = 14)	Females (n = 2)
SVL	22.9	21.9 ± 0.5 (21.2–23.0)	23.7 ± 0.9 (22.1–24.3)	24.9 ± 0.7 (23.9–26.4)	24.4 ± 2.0 (23.0–25.8)
HL	8.4	7.9 ± 0.3 (7.4–8.4)	8.1 ± 0.4 (7.4–8.5)	8.9 ± 0.3 (8.2–9.4)	8.5 ± 0.5 (8.1–8.8)
HW	8.5	8.1 ± 0.3 (7.6–8.7)	8.4 ± 0.6 (7.6–9)	9.2 ± 0.3 (8.7–9.7)	9.2 ± 0.6 (8.7–9.6)

SL	3.8	3.5 ± 0.2 (3.3–3.9)	3.4 ± 0.3 (2.9–3.8)	3.8 ± 0.2 (3.5–4.0)	3.4 ± 0.5 (3.1–3.8)
EN	2.0	2.0 ± 0.1 (1.9–2.2)	2.3 ± 0.2 (2.0–2.5)	2.0 ± 0.1 (2.0–2.2)	2.0 ± 0.4 (1.8–2.3)
IND	2.4	2.3 ± 0.1 (2.0–2.4)	2.3 ± 0.1 (2.1–2.5)	2.6 ± 0.1 (2.5–2.7)	2.5 ± 0.1 (2.4–2.5)
ED	2.4	2.5 ± 0.1 (2.2–2.7)	2.6 ± 0.2 (2.3–2.8)	2.3 ± 0.2 (2.1–2.6)	2.3 ± 0.1 (2.2–2.4)
IOD	5.4	5.4 ± 0.2 (5.0–5.8)	5.6 ± 0.4 (4.9–5.9)	5.8 ± 0.2 (5.6–6.3)	5.6 ± 0.2 (5.4–5.7)
TD	1.4	1.4 ± 0.1 (1.2–1.5)	1.4 ± 0.1 (1.2–1.5)	1.5 ± 0.1 (1.3–1.8)	1.5 ± 0.1 (1.5–1.6)
FAL	4.7	4.5 ± 0.3 (4.0–5.0)	5.1 ± 0.5 (4.7–6)	5.0 ± 0.3 (4.7–5.8)	5.1 ± 0.3 (5.1–5.6)
UAL	4.4	4.1 ± 0.4 (3.1–4.8)	4.6 ± 0.5 (4.1–5.4)	5.0 ± 0.3 (4.5–5.5)	5.3 ± 0.3 (5.1–5.6)
HAL	4.9	4.5 ± 0.2 (4.0–4.9)	4.7 ± 0.2 (4.5–4.9)	5.2 ± 0.2 (4.8–5.6)	5.1 ± 0.4 (4.8–5.3)
TL	9.5	9.7 ± 0.5 (8.9–10.8)	10.9 ± 0.2 (10.5–11.1)	11.1 ± 0.6 (10.0–12.1)	11.4 ± 0.4 (11.1–11.6)
FL	10.5	10.0 ± 0.4 (9.5–10.7)	10.7 ± 0.3 (10.5–11.2)	11.5 ± 0.5 (10.5–12.2)	11.6 ± 0.6 (11.2–12.0)
THL	9.4	9.2 ± 0.3 (8.9–10)	9.7 ± 0.8 (8.4–10.2)	10.8 ± 0.7 (9.8–11.8)	11.1 ± 0.6 (10.6–11.5)
TSL	5.5	5.6 ± 0.3 (5.0–6.2)	6.1 ± 0.2 (5.9–6.5)	6.2 ± 0.4 (5.5–6.8)	6.2 ± 0.5 (5.8–6.5)

Morphological and acoustic interspecific comparisons

Adult males of *Adenomera* sp. nov. have SVL of 21.2–23.0 mm, being smaller than those of *A. glauciae* (SVL 27.6–30.4; Carvalho et al., 2020b), *A. gridipappi* (SVL 25.4–27.7 mm; Carvalho et al., 2021) and *A. lutzi* (SVL 25.7–33.5 mm; Kok et al., 2007) and larger than *A. juikitam* (SVL 19.1–19.5mm; Carvalho and Giaretta, 2013a) and *A. kayapo* (SVL 17.5–21.0 mm; Carvalho et al., 2021). *Adenomera* sp. nov. has a snout rounded in dorsal view and differs from *A. martinezi* (snout pointed in dorsal view; Carvalho & Giaretta, 2013b). The absence of antebrachial tubercle differs *Adenomera* sp. nov. from *A. amicorum*, *A. aurantiaca*, *A. glauciae, A. gridipappi*, *A. inopinata*, *A. kayapo*, *A. lutzi*, *A. tapajonica* and *A. phonotriccus* (antebrachial tubercle present in all mentioned species; Kok et al., 2007; Carvalho et al., 2019b; Carvalho et al., 2020c; Carvalho et al., 2021). *Adenomera* sp. nov. has toe tips moderately to fully expanded, states C–D (sensu Heyer, 1973) and differs from *A. cotuba*, *A. hylaedactyla*, *A. juikitam* and *A. martinezi* (states A–B; Carvalho et al., 2019c; Carvalho & Giaretta, 2013a, b). *Adenomera* sp. nov. differs from *A. andreae*, *A. chicomendesi*, *A. guarayo*, *A. heyeri* and *A. hylaedactyla* by the presence of nearly solid dark-colored stripe on underside of forearm (absence; Carvalho et al., 2019a–c; Carvalho et al., 2021). *Adenomera* sp. nov. differs from *A. simonstuarti* by having smaller SVL, absence of a longitudinal dark blotch on each side of the throat demarking the lateral vocal sac in males and for the absence of prominent stretch marks on the dorsum (SVL 23.4–26.2 mm, presence of longitudinal dark blotches and presence of prominent stretch marks; Angulo and Icochea, 2010; Carvalho et al., 2020b; present study; see appendix 5).

The advertisement call of *Adenomera* sp. nov. is composed of a single pulsed-note, which differs from *A. amicorum*, *A. glauciae*, *A. gridipappi*, *A. inopinata* and *A. simonstuarti* sensu stricto (multi-note calls in all cited species; Angulo and Icochea, 2010; Carvalho et al., 2020b; Carvalho et al., 2021). *Adenomera* sp. nov. has calls composed of 11–21 pulses, which differs it from *A. amicorum* (4–10 pulses; Carvalho et al., 2021c), *A. andreae* (3–10 pulses; Carvalho et al., 2019c), *A. aurantiaca* (5–7 pulses;

Carvalho et al., 2021), *A. chicomendesi* (22–35 pulses; Carvalho et al., 2019a), *A. gridipappi* (2–4; Carvalho et al., 2021), *A. heyeri* (4–12 pulses; Carvalho et al., 2021), *A. hylaedactyla* (4–10; Carvalho et al., 2019c), *A. inopinata* (4–5 pulses; Carvalho et al., 2021) and *A. tapajonica* (3–5 pulses; Carvalho et al., 2021) and *A. glauciae* (unpulsed; Carvalho et al., 2020b) and *A. simonstuarti* sensu stricto (2–6 pulses; this study). *Adenomera* sp. nov. has incomplete pulses and differs from *A. aurantiaca*, *A. guarayo*, *A. inopinata* and *A. phonotriccus* (complete pulses in all mentioned species; Carvalho et al., 2019b; Carvalho et al., 2020a; Carvalho et al., 2021). *Adenomera* sp. nov. has dominant frequency of 3,448–4,349 Hz and differs from *A. kayapo* (4,570–4,990 Hz; Carvalho et al., 2021) and *A. simonstuarti* sensu stricto (1,851–2,224 Hz; this study). The dominant frequency of *Adenomera* sp. nov. is placed in the second harmonic and differs from *A. simonstuarti* sensu stricto (dominant frequency in the fundamental harmonic).

Description of holotype

Adult male (Fig. 4A, B, C; 5A, C) Snout subovoid in dorsal view and acuminate in profile. Dorsal skin smooth and glandular, warty on flank. Dorsolateral folds indistinct. Sacral region, dorsal surface of tibia and posterior surface of tarsus with white-tipped tubercles. Vertebral stripe on sacral region. Tibia long than thigh. Throat, belly and ventral surface of limbs smooth. Pair of lumbar glands. Posterior surface of thigh with a pair of paracloacal glands. Nostrils are closer to the snout tip than the eyes and oriented dorsolaterally; fleshy ridge on the snout tip. Eye nostril distance = 83% eye diameter, eye diameter = inter narial distance. Head wider than long. Internarial distance > 25% of head width. Canthus rostralis defined; loreal region slightly concave. Triangle-shaped mark on the head. Tympanum distinct, nearly 60% of the eye diameter; black-coloured supratympanic fold well developed, extending from the posterior corner of the eye to base of arm. Postcommissural gland ovoid. Subgular vocal sac; vocal slits present. Vomerine teeth in two straight rows posterior to choanae and arranged in transverse series parallel to choanae. Tongue lanceolate (sensu Duellman, 1970) and free behind. Relative fingers lengths IV < I ≈ II < III; absence of fringes or webbing in fingers; finger tips rounded, slight expanded but without disc; inner metacarpal tubercle elliptical; outer metacarpal tubercle rounded; distinct rounded grayish-coloured subarticular tubercles of the underside of fingers; supernumerary tubercles rounded; antebrachial tubercle absent; prepollical spine absent. Elliptical axilar grand. Toe lengths IV > III > V > II > I; toe tips flattened or slightly flattened, with visible expansions (character states C–D according to Heyer [1973]); fringes or webbing absent; Inner metatarsal tubercle elliptical; outer metatarsal tubercle rounded. Tarsal fold from the inner metatarsal tubercle extending 2/3 of tarsus length. Subarticular tubercles among elliptical and rounded and supernumerary tubercles rounded.

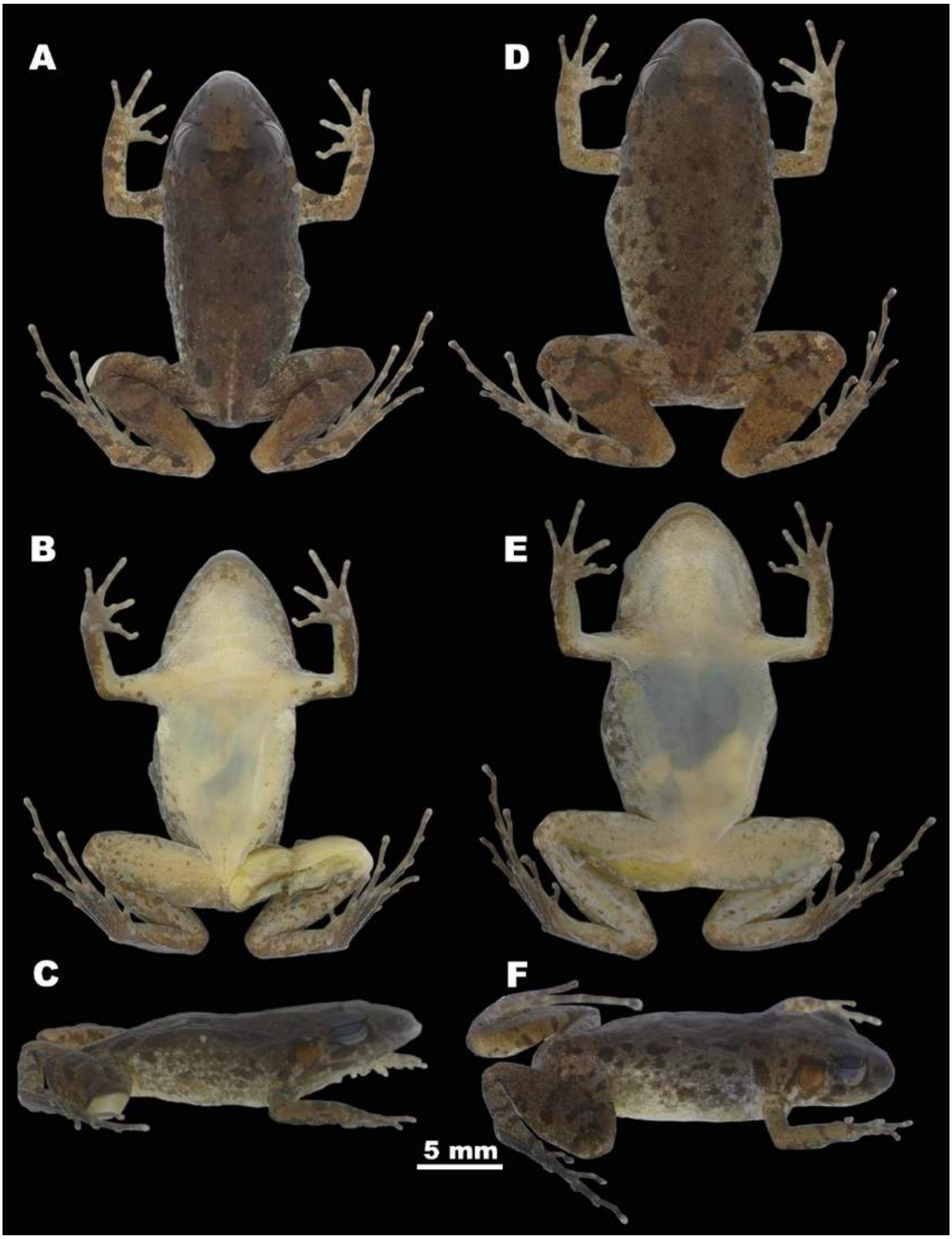

Figure 4. Male holotype and female paratype of *Adenomera* sp. nov. (A–C) Male holotype, INPA-H 44868 and (D–F) female paratopotype INPA-H 44875. Photographs: B. C. Martins.

Figure 5. Ventral views of the hand and foot of the *Adenomera* sp. nov. (A, C) Male holotype INPA-H 44867 and (B, D) female paratopotype, INPA-H 44875 of *Adenomera* sp. nov. Scale bars: 3 mm. Photographs: B. C. Martins.

Color holotype in life

We followed Köhler (2012) as color catalogue. Name of the color in *italic.* Color code = cc. Repeated colors will not show color code. Snout tip with a *cinnamon-drab* (cc 50), fleshy ridge *pale neutral grey* (cc 296). *Light sky blue* (cc 191) coloration of the blotches on the upper and lower lips. Postcommissural gland with melanophores.

Tympanum *dark carmine* (cc 61) in the edge and *Buff* (cc 5) in the center. *Vandyke brown* (cc 281) supratympanic fold. Thoracic dorsal surface of body *Prout's brown* (cc 47); lumbar region *cinnamon-drab* (cc 50) with white-tipped tubercles. Interorbital region *sepia* (cc 286). Flank *Dark spectrum yellow* (cc 78). *Sepia* (cc 286) triangle-shaped blotch. Dorsal surface of forelimbs *Tawny* (cc 60) with *Raw umber* (cc 280) blotches. Dorsal surface of hindlimbs *True cinnamon* (cc 260) with transverse *Raw umber* bars. *Medium chrome orange* (cc 75) vertebral stripe in sacral region. Paracloacal region and lumbar glands *sepia* coloration. Throat *Pale mauve* (cc 204) with low density of melanophores around the jaw; belly *light buff* (cc 2) and chest and underside of limbs as the same color as throat. Underside of forearm with *dark grayish olive* (cc 275) nearly solid stripe. Palm of hand, sole of foot, digits and subarticular tubercles almost completely covered with melanophores. Metatarsal, proximal and medial phalanx have a *Fuscuous* (cc 283) stripe in the ventral view. This character is not present in distal phalanx and toe tip.

Color holotype in preservative

See figures 4A, B, C and 5A, C to see the holotype's color in preservative. Snout tip with a *pale neutral grey* coloration (cc 296), as well as the fleshy ridge. *Pale neutral grey* coloration of the blotches on the upper and lower lips. Postcommissural gland with melanophores. Tympanum *dark grab* (cc 45). *Vandyke brown* (cc 281) supratympanic fold. Thoracic dorsal surface of body *hair brown* (cc 277); lumbar region *cinnamon-drab* (cc 50) with white-tipped tubercles. Interorbital region *sepia* (cc 279). Flank *pale buff* (cc 1). *Dark grayish brown* (cc 284) triangle-shaped blotch. Dorsal surface of forelimbs *pale buff* (cc 1) with *drab* (cc 19) blotches. Dorsal surface of hindlimbs *tawny olive* (cc 17) with transverse bars *sepia* (cc 279) with white-tipped tubercles. *Pale buff* vertebral stripe in sacral region. Paracloacal region and lumbar glands *sepia* coloration. Throat *light buff* (cc 2) with melanophores and a greater density around the jaw; belly *light buff* and chest and underside of limbs pale *pinkish buff* (cc 3). Underside of forearm with *brownish olive* (cc 276) nearly solid stripe. Palm of hand, sole of foot, digits and subarticular tubercles almost completely covered with melanophores. All individuals have *fuscuous* stripe in metatarsal and proximal and medial phalanx (see Fig. 5C, D).

Intraspecific variation

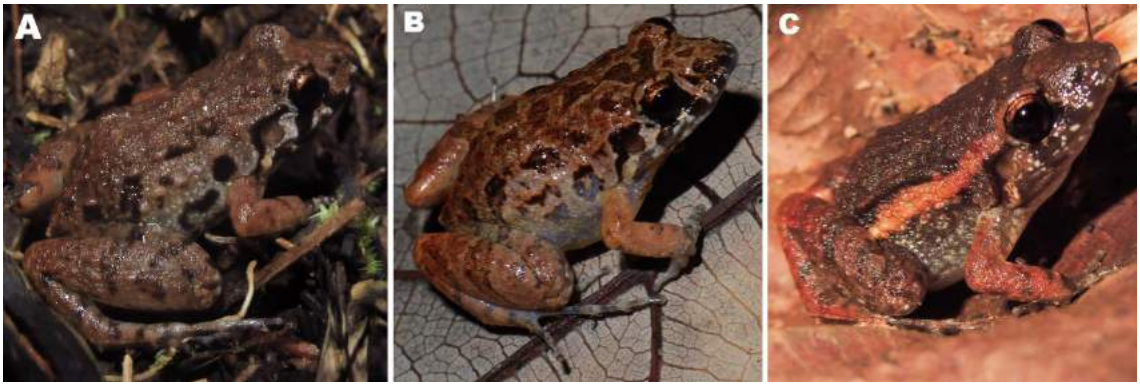

Figure 6. Three dorsal coloration patterns of *Adenomera* sp. nov. in life. (A)Absence of dark blotches or only a few, (B) presence of many dark blotches and (C) presence of a dorsolateral stripe. Photographs: S. P. Dantas (A, C); A. T. Mônico (B). Unvouchered specimens.

Morphometric variation of the new species is summarized in Table 4. The type series shows three coloration patterns on dorsum: absence of dark blotches or only a few (Fig. 6A; 7A); presence of many dark blotches (Fig 6B; 7D); and presence of a dorsolateral stripe (Fig. 6C; 8G). Sixty-eight percent of the type series (including the holotype) fits the first mentioned pattern; 20% have many dark spots; and only 12% have a dorsolateral stripe. A sacral stripe is present in 64% of the series. About 52% of paratypes have toe tip shape in the stage D (sensu Heyer 1972), while 48% have toe tip shape in an intermediary form of stages C–D. All individuals have a triangle-shaped mark on the head (Fig. 6), which is less visible in individuals without blotches on the dorsum. The texture of dorsum varies between rough and smooth, with few to many glandules. The iris is always *chrome orange* (color code 74 from Kohler., 2012). Throat and belly have a small variation in melanophore density (see Fig. 7B, E, H).

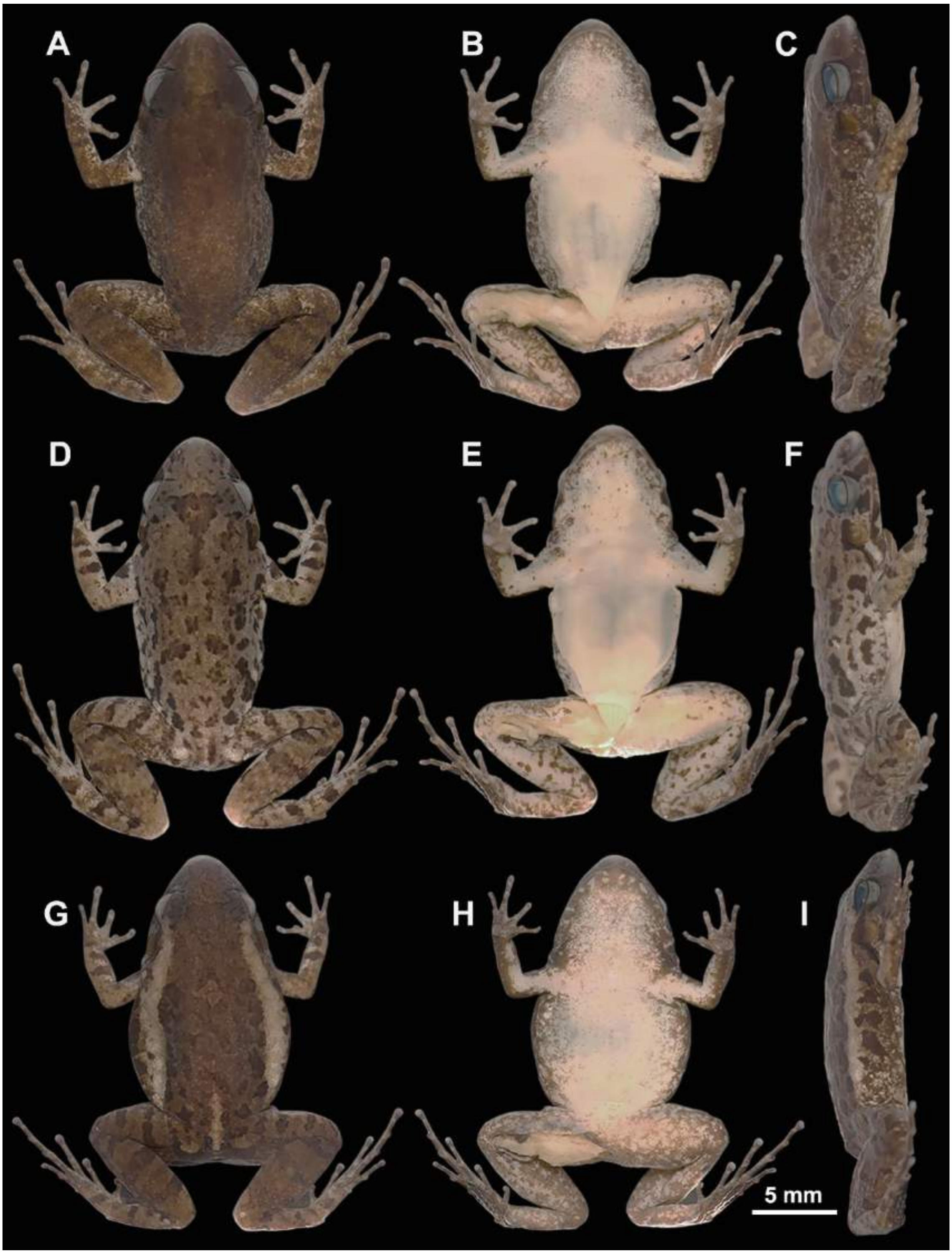

Figure 7. Dorsal, ventral and lateral views of the three coloration patterns of *Adenomera* sp. nov. in preservative. Paratypes: A–C (INPA-H 44869); D–F (INPA-H 44870); and G–I (INPA-H 44877). Photographs: L. R. Mendonça.

Advertisement call

The advertisement call of *Adenomera* sp. nov. consists of a single note with partially fused pulses. Pulse number varies from 11 to 21; pulse duration from 4 to 23 ms. The note duration varies from 100 to 199 ms. The fundamental frequency of the note varies from 1,765 to 2,239 Hz, the dominant frequency varies from 3,746 to 4,349 Hz and corresponds to the second harmonic (Table 3; Fig. 8).

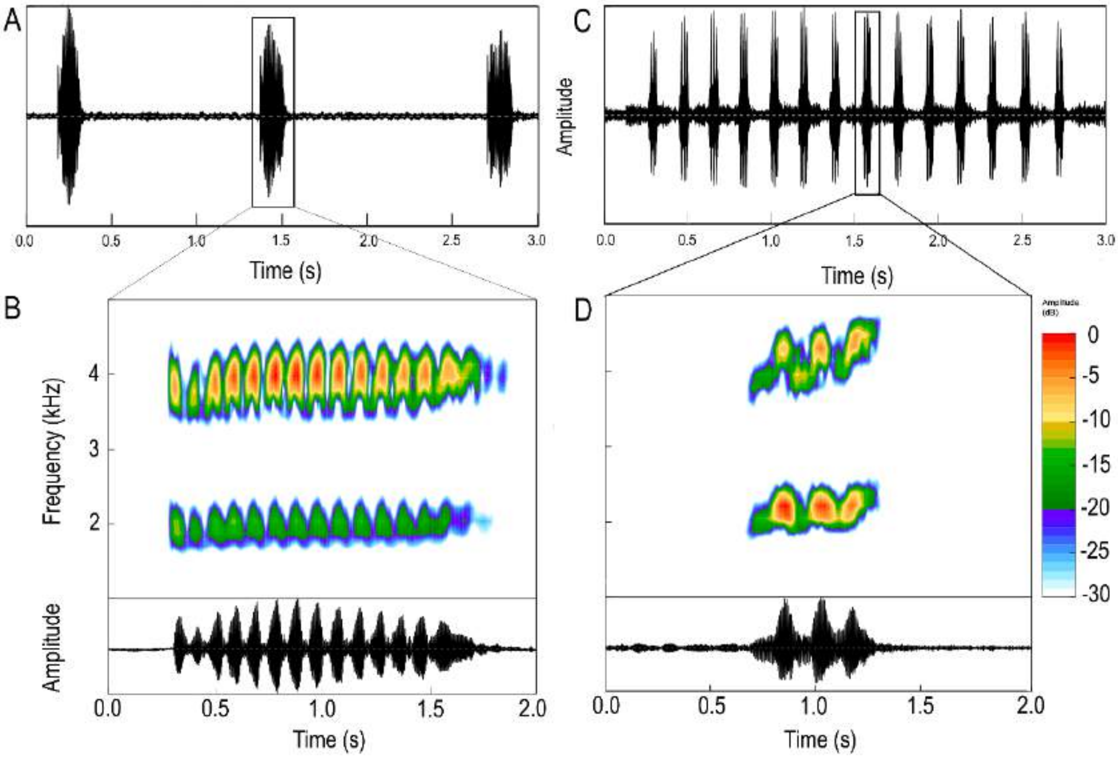

Figure 8. Advertisement call of *Adenomera* sp. nov. (A, B) and *A. simonstuarti* sensu stricto (C, D). (A, B) INPA-H 44876 (FNJV 59564), RDS Rio Negro, Iranduba, Amazonas, Brazil. (C, D) INPA-H 44904 (FNJV 59568), Taracuá, Acre, Brazil.

Distribution, habitat and natural history

The species *Adenomera* sp. nov. is only known from the White-sand ecosystems between West Negro and Solimões River, specifically in Reserva de Desenvolvimento Sustentável Rio Negro and nearby localities, municipalities of Iranduba and Manacapuru, Amazonas, Brazil, where these ecosystems are predominant (Fig. 1; Fig. 9A). Two other species of *Adenomera* occur in sympatry with the new species: *A. hylaedactyla* and *A. andreae.* Although these species occur in the same region, the habitat is not the same; *A. andreae* inhabit mainly unflooded forests, while *A. hylaedactyla* occurs in open areas. On the other hand, *Adenomera* sp. nov. inhabits white-sand forests under flooding regimes close to streams. At the type locality, *A. andreae* and the new species occur in syntopy in the border between forests under flooding regimes and those unflooded.

Males call on the ground, above or hidden in the leaf litter (Fig. 9B). They start calling at dusk (~17:00h) and remain calling until ~18:00h. Sometimes, it is possible to listen isolated individuals singing after this time, but it is not usual.

Adult males are easily found, and juveniles are also not difficult to observe. However, females (Fig. 9B) are very secretive. The new species build foam nests for depositing their eggs (Fig. 9D). The foam nests are very difficult to find once its localization is under the leaf litter among the roots of palm trees and ferns. Aspects of breeding biology of the new species is the subject of another study conducted by third parts.

Figure 9. Natural aspects of *Adenomera* sp. nov. (A) Example of species habitat; (B) male during vocalization on the leaf litter; (C) female hide into leaf litter; (D) foam nest. Photographs: A. T. Mônico (A), S. P. Dantas (B–D).

Conservation

The species *Adenomera* sp. nov. is distributed into an area of approximately 150km^2 belonging to RDS Rio Negro and close locality. Although the species being known only from a small area, it is very common in this place and expected to occur in other parts of the RDS Rio Negro and in the nearby Jaú National Park. The new species occurs mainly in riparian white-sand forests; these environments are very sensible to anthropization once streams can be easily polluted. However, the fact that the known distribution is mostly in a protected area and its local abundance likely favor the species preservation.

Discussion

Over the years, many undescribed species were erroneously related to nominal species which were supposedly widespread (*e.g.*, *Allobates caldwellae*, *Atelopus manauensis*, *Pristimantis guianensis*; Lima et al., 2020; Jorge et al. 2020; Mônico et al., 2022). However, the integration of molecular, acoustic and morphological data in recent years can help taxonomists to delimit and describe new species (Fouquet et al., 2014; Moraes et al., 2022; Carvalho et al., 2021). The crypsis of the genus *Adenomera* linked to syntopy and sympatry has challenged taxonomists. A great example of crypsis is that of the *A. simonstuarti* species complex. Fouquet et al. (2014) recovered six lineages belonging to this complex (*A. simonstuarti* sensu stricto and other five lineages). Then, Carvalho et al. (2020) published additional data and delimited eight lineages. However, none of them represented the new species from RDS Rio Negro described in the present study. This new species is the easternmost taxon belonging to the *A. simonstuarti* complex, which is an indication that this complex might be more widespread and diverse than previously thought.

The species *Adenomera* sp. nov. is the first in the *A. simonstuarti* complex to be described since the original description of *A. simonstuarti* (Angulo and Icochea, 2010). It is sister to *A.* aff. *simonstuarti* 2 (sensu Carvalho et al., 2020) from the Cumaru community at Reserva Extrativista Baixo Juruá, lower Juruá River, Brazil. Despite the low genetic divergence between these taxa suggests a conspecific status, the low number of individuals and the absence of acoustic data of *A.* aff. *simonstuarti* Lineage 2 hamper its identification. Although we find plausible that *Adenomera* sp. nov. occurs in other areas within the Negro-Solimões interfluve, the species is only known from RDS Rio Negro and a nearby locality. Additional sampling between known distribution of these taxa is necessary to identify the contact zone between them and properly test whether they are or not conspecific taxa.

The first species of the genus to be described from a white-sand environment (WSE) is *Adenomera* sp. nov. Although this species is likely a specialist in WSE, it seems limited to WSE in the Negro-Solimões interfluve. Other frogs have been suggested as specialists or endemics to WSE in this interfluve, such as *Scinax albertinae* (Ferrão et al. 2022), *Pristimantis* aff. *ockendeni* (Mônico, personal communication) and *Osteocephalus vilarsi* (Ferrão et al. 2019). Moreover, two candidate species (*Rhinella* aff. *probocidea* and *Pristimantis* aff. *orcus*) apparently has their geographic distribution associated with WSE and limited within the West Negro-Solimões interfluve (M. Ferrão and A. T. Mônico, unpublished data). The congruence of frog species sharing this habitat specialization and distribution pattern endemic reinforce the Jaú region as an area of endemism in Amazonia (see Borges & Da Silva, 2012).

Conclusion

The species *Adenomera* sp. nov. is assigned to *Adenomera simonstuarti* species complex belonging to the *Adenomera andreae* clade. The integration of morphological, acoustic and molecular data helps us to delimit this lineage. Although genetic and morphology being distinct between the new species and *Adenomera simonstuarti* sensu stricto as we show, the advertisement call is highly divergent. The new species has a single-note call while the *Adenomera simonstuarti* has a multi-note call. Temporal and spectral parameters are also very distinct between then. *Adenomera* sp. nov. is the easternmost lineage into *Adenomera simonstuarti* species complex and the first species of the genus to be described from white-sand forests.

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Appendix 1

Species of *Adenomera* and *Lithodytes* used in phylogenetic analyses, with respective voucher, Genbank acession number and references.

Taxon	Molecular voucher	Clade	GenBank accession numbers				References
			CYTB	COI	RAG1	POMC	
Adenomera sp. nov.	INPA-H44867	*andreae*	---	OQ974333	---	---	This study
Adenomera sp. nov.	INPA-H 44868	*andreae*	---	OQ974334	---	---	This study
Adenomera sp. nov.	INPA-H 44871	*andreae*	---	OQ974335	---	---	This study
Adenomera sp. nov.	INPA-H44873	*andreae*	---	OQ974336	---	---	This study
Adenomera andreae 1	MTR18580	*andreae*	KF674837	KF674525	KF674205	KF673893	Fouquet et al., 2014
Adenomera andreae 2	LSU17479	*andreae*	KF674839	KF674527	KF674207	KF673895	Fouquet et al., 2014
Adenomera andreae 3	DT1416	*andreae*	KF674841	KF674529	KF674209	KF673897	Fouquet et al., 2014
Adenomera chicomendesi 1	AA9972	*andreae*	JQ321831	KF674586	KF674267	KF673954	Carvalho et al., 2019a
Adenomera chicomendesi 2	MNCN4004	*andreae*	KF674903	KF674594	KF674275	KF673962	Carvalho et al., 2019a
Adenomera simonstuarti 1	LSU13787	*andreae*	KF674885	KF674575	KF674256	KF673943	Carvalho et al., 2019a
Adenomera simonstuarti 1	AJC2777	*andreae*	KF674884	KF674574	KF674255	KF673942	Carvalho et al., 2019a
Adenomera simonstuarti 1	MHNC10092	*andreae*	KF674883	KF674573	KF674254	KF673941	Carvalho et al., 2019a
Adenomera simonstuarti 2	QU5337	*andreae*	KF674887	KF674577	KF674258	KF673945	Carvalho et al., 2019a
Adenomera simonstuarti 2	INPAH39792	*andreae*	---	MT472181	---	---	Carvalho et al., 2019a
Adenomera simonstuarti 2	INPAH39814	*andreae*	---	MT472182	---	---	Carvalho et al., 2019a
Adenomera simonstuarti 3	MNCN23203	*andreae*	KF674889	KF674579	KF674260	KF673947	Carvalho et al., 2019a
Adenomera simonstuarti 3	ZSM748	*andreae*	KF674888	KF674578	KF674259	KF673946	Carvalho et al., 2019a
Adenomera simonstuarti 3	INPAH40967	*andreae*	---	MT472180	---	---	Carvalho et al., 2019a
Adenomera simonstuarti 4	LSU12840	*andreae*	KF674891	KF674581	KF674262	KF673949	Carvalho et al., 2019a
Adenomera simonstuarti 4	MHNC6302	*andreae*	KF674892	KF674582	KF674263	KF673950	Carvalho et al., 2019a
Adenomera simonstuarti 5	CB5696	*andreae*	KF674890	KF674580	KF674261	KF673948	Carvalho et al., 2019a
Adenomera simonstuarti 6	MHNC10058	*andreae*	KF674893	KF674583	KF674264	KF673951	Carvalho et al., 2019a
Adenomera simonstuarti 7	MNCN27344	*andreae*	KF674894	KF674584	KF674265	KF673952	Carvalho et al., 2019a
Adenomera simonstuarti 8	APL13097	*andreae*	KF674895	KF674585	KF674266	KF673953	Carvalho et al., 2019a
Adenomera guarayo	MNCN34687	*andreae*	KF674878	KF674568	KF674249	KF673936	Fouquet et al., 2014
Adenomera guarayo	AM29	*andreae*	KF674880	KF674570	KF674251	KF673938	Fouquet et al., 2014
Adenomera guarayo	USNM268935	*andreae*	KF674879	KF674569	KF674250	KF673937	Fouquet et al., 2014
Adenomera sp. T	MHNC8385	*andreae*	KF674877	KF674567	KF674248	KF673935	Fouquet et al., 2014
Adenomera sp. D	ZSM751	*andreae*	KF674881	KF674571	KF674252	KF673939	Fouquet et al., 2014

Adenomera heyeri 1	AF269	*heyeri*	KF675009	KF674700	KF674383	KF674068	Fouquet et al., 2014
Adenomera heyeri 2	AF127	*heyeri*	KC603972	KC604000	KF674387	KC604068	Carvalho et al., 2021
Adenomera inopinata	INPAH40517	*heyeri*	---	MN866438	---	---	Carvalho et al., 2021
Adenomera amicorum	MTR11092	*heyeri*	KF675023	KF674714	KF674398	KF674082	Carvalho et al., 2021
Adenomera amicorum	INPAH40506	*heyeri*	---	MT162498	---	---	Carvalho et al., 2021
Adenomera aurantiaca	INPAH40518	*heyeri*	---	MN866439	---	---	Carvalho et al., 2021
Adenomera cotuba	AAGUFU2463	*heyeri*	---	MT162505	---	---	Carvalho et al., 2021
Adenomera cotuba	AAGUFU1400	*heyeri*	---	MT162504	---	---	Carvalho et al., 2021
Adenomera gridipappi	PMJ154	*heyeri*	KF675028	KF674719	KF674403	KF674087	Carvalho et al., 2021
Adenomera gridipappi	INPAH40512	*heyeri*	---	MT162510	---	---	Carvalho et al., 2021
Adenomera kayapo	CFBH43885	*heyeri*	---	MT162528	---	---	Carvalho et al., 2021
Adenomera kayapo	DT1798	*heyeri*	KF675018	KF674709	KF674393	KF674077	Carvalho et al., 2021
Adenomera tapajonica	INPAH40515	*heyeri*	---	MN866436	---	---	Carvalho et al., 2021
Adenomera tapajonica	INPAH40516	*heyeri*	---	MN866437	---	---	Carvalho et al., 2021
Adenomera phonotriccus	DT2123	*heyeri*	KF675021	KF674712	KF674396	KF674080	Carvalho et al., 2021
Adenomera phonotriccus	DT2016	*heyeri*	KF675020	KF674711	KF674395	KF674079	Carvalho et al., 2021
Adenomera hylaedactyla 1	APL15864	*hylaedactyla*	KF674917	KF674608	KF674289	KF673976	Fouquet et al., 2014
Adenomera hylaedactyla 2	CFBH8289	*hylaedactyla*	KF674929	KF674620	KF674301	KF673988	Fouquet et al., 2014
Adenomera diptyx 1	IIBPH905	*hylaedactyla*	KC603966	KC603994	KF674278	KC604062	Fouquet et al., 2014
Adenomera diptyx 2	RGA5254	*hylaedactyla*	KF674910	KF674601	KF674282	KF673969	Fouquet et al., 2014
Adenomera coca	AMG1	*hylaedactyla*	KF674969	KF674660	KF674342	KF674028	Carvalho et al., 2020
Adenomera lutzi	ROM40167	*lutzi*	KC603974	KC604002	KF674366	KC604070	Fouquet et al., 2014
Adenomera sp. P	AJC2390	*lutzi*	KF675036	KF674727	KF674411	KF674095	Fouquet et al., 2014
Adenomera glauciae	MCP13890	*lutzi*	---	MT956671	---	---	Carvalho et al., 2020b
Adenomera glauciae		*lutzi*	---	MT956673	---	---	Carvalho et al., 2020b
Adenomera ajurauna 1	CTMZ02393	*marmorata*	KF675079	KF674770	KF674457	KF674138	Fouquet et al., 2014
Adenomera ajurauna 2	H182	*marmorata*	KF675080	KF674771	KF674458	KF674139	Fouquet et al., 2014
Adenomera araucaria	MCP10769	*marmorata*	KC603969	KC603997	KF674440	KC604065	Fouquet et al., 2014
Adenomera bokermanni 1	K1730	*marmorata*	KF675115	KF674806	KF674494	KF674174	Fouquet et al., 2014
Adenomera bokermanni 2	MTR18508	*marmorata*	KF675116	KF674807	KF674495	KF674175	Fouquet et al., 2014
Adenomera engelsi 1	MTR18497	*marmorata*	KC603970	KC603998	KF674450	KC604066	Fouquet et al., 2014
Adenomera engelsi 2	MNRJ72224	*marmorata*	KF675073	KF674764	KF674451	KF674132	Fouquet et al., 2014
Adenomera kweti 1	MTR18496	*marmorata*	KF675065	KF674756	KF674442	KF674124	Carvalho et al., 2020

Adenomera kweti 2	TRPD9	*marmorata*	KF675066	KF674757	KF674443	KF674125	Carvalho et al., 2020
Adenomera marmorata 1	AF467	*marmorata*	KF675092	KF674783	KF674470	KF674151	Fouquet et al., 2014
Adenomera marmorata 3	MTR15511	*marmorata*	KF675096	KF674787	KF674474	KF674155	Fouquet et al., 2014
Adenomera nana 1	MTR18505	*marmorata*	KF675076	KF674767	KF674454	KF674135	Fouquet et al., 2014
Adenomera nana 2	CFBH3251	*marmorata*	KF675077	KF674768	KF674455	KF674136	Fouquet et al., 2014
Adenomera sp. A	MNRJ47474	*marmorata*	KF675112	KF674803	KF674491	KF674171	Fouquet et al., 2014
Adenomera sp. N	CFBH10241	*marmorata*	KF675097	KF674788	KF674475	KF674156	Fouquet et al., 2014
Adenomera sp. O	CFBH7321	*marmorata*	KF675098	KF674789	KF674476	KF674157	Fouquet et al., 2014
Adenomera sp. S	ITH0585	*marmorata*	KF675067	KF674758	KF674444	KF674126	Fouquet et al., 2014
Adenomera martinezi	CHUNB40218	*martinezi*	KF675006	KF674697	KF674380	KF674065	Fouquet et al., 2014
Adenomera saci 1	MTR14648	*martinezi*	KF675001	KF674692	KF674375	KF674060	Fouquet et al., 2014
Adenomera saci 2	CHUNB49509	*martinezi*	KF675004	KF674695	KF674378	KF674063	Fouquet et al., 2014
Adenomera sp. B	ESTR307	*martinezi*	KF675007	KF674698	KF674381	KF674066	Fouquet et al., 2014
Adenomera thomei 1	MNRJ60463	*thomei*	KF675104	KF674795	KF674483	KF674163	Fouquet et al., 2014
Adenomera thomei 2	CFBH10573	*thomei*	KC603971	KC603999	KF674481	KC604067	Fouquet et al., 2014
Adenomera sp. L 1	MTR20994	*thomei*	KF675113	KF674804	KF674492	KF674172	Fouquet et al., 2014
Adenomera sp. L 2	MTR21951	*thomei*	KF675114	KF674805	KF674493	KF674173	Fouquet et al., 2014
Adenomera sp. M	MD2568	*thomei*	KF675099	KF674790	KF674477	KF674158	Fouquet et al., 2014
Adenomera juikitan	MRT2811	*thomei*	KF675053	KF674744	KF674429	KF674112	Carvalho et al., 2021
Adenomera juikitan	MRT11878	*thomei*	kf675050	KF674741	KF674426	KF674109	Carvalho et al., 2021
Lithodytes lineatus	MC55	outgroup	JQ321833	KC604003	KC604025	KC604060	Fouquet et al., 2013

Appendix 2

Call parameters of *Adenomera* sp. nov. and *A. simonstuarti* used to perform Principal Component Analysis. CD = Call duration (ms); ND = Note duration (ms); PpN = Pulses per note; PD = Pulse duration (ms); FF = Fundamental frequency; DF = Dominant frequency; NrT = Note rise time; FM = Frequency modulation.

Voucher	Species	CD	NpC	ND	PpN	PD	FF	DF	NrT	FM
INPA-H 44867	*A.* sp. nov	0.152	0.152	0.152	16.313	0.01	1936	3946	2.6	23.07
MPEG 44649	*A.* sp. nov	0.136	0.136	0.136	14.559	0.009	2178	3925	31.6	-0.07
INPA-H 44868	*A.* sp. nov	0.132	0.132	0.132	13.842	0.01	1909	3796	42.07	562.73
INPA-H 44869	*A.* sp. nov	0.130	0.130	0.130	13.692	0.009	1843	3897	46.87	321.6
INPA-H 44876	*A.* sp. nov	0.126	0.126	0.126	13.52	0.01	2052	3857	17.13	425.07
MPEG 44651	*A.* sp. nov	0.169	0.169	0.169	16.507	0.011	1958	3912	30.33	310.4
INPA-H 44903	*A. simonsatuarti*	4.53	0.06	0.06	3.33	0.02	3698	1957	33.56	287.67
INPA-H 44904	*A. simonsatuarti*	3.68	0.06	0.06	2.68	0.02	4036	2024	53.56	306.78
INPA-H 44905	*A. simonsatuarti*	5.60	0.058	0.058	3.13	0.016	4019	2008	45.2	218.87
INPA-H 44911	*A. simonsatuarti*	5.1	0.077	0.077	4.28	0.02	4101	2002	52.39	325.89
Unvouchered	*A. simonsatuarti*	4.94	0.066	0.066	3.22	0.02	3895	3895	47.6	241.67
Unvouchered	*A. simonsatuarti*	3.27	0.065	0.065	3	0.021	4029	2014	52.33	230.33

Appendix 3. Morphometric traits of *Adenomera* sp. nov.

Voucher	Sex	SVL	HL	HW	SL	END	IND	ED	IOD	TD	FAL	UAL	HAL	TL	FL	THL	TAL
INPA-H44881	M	21.4	7.4	8.0	3.6	2.0	2.4	2.3	5.4	1.5	4.8	3.1	4.3	10.3	9.7	9.5	5.8
INPA-H 44871	M	22.0	7.6	8.0	3.9	2.3	2.3	2.5	5.4	1.5	4.6	4.0	4.4	10.0	9.5	8.9	6.0
INPA-H 44879	M	23.0	8.0	8.6	3.4	2.0	2.5	2.5	5.7	1.5	4.8	3.6	4.7	10.8	10.2	9.1	5.2
INPA-H 44873	M	22.1	7.8	7.9	3.5	2.0	2.3	2.5	5.5	1.4	4.0	3.9	4.7	9.2	9.6	8.9	5.9
MPEG 44651	M	22.4	7.9	8.0	3.5	2.0	2.3	2.6	5.1	1.3	4.6	4.0	4.9	10.3	10.4	9.0	5.7
INPA-H 44882	M	22.5	8.4	8.7	3.3	2.0	2.5	2.7	5.5	1.4	4.7	3.5	4.7	9.4	9.9	9.6	5.5
INPA-H 44876	M	21.3	8.0	8.2	3.7	2.0	2.2	2.5	5.5	1.3	4.9	3.9	4.3	10.0	9.8	9.0	5.8
INPA-H 44877	M	21.9	8.0	8.6	3.3	2.0	2.4	2.2	5.3	1.3	4.5	3.8	4.5	9.6	10.3	9.5	5.4
MPEG 44652	M	21.6	7.8	8.0	3.7	2.3	2.2	2.4	5.5	1.3	5.0	4.0	4.4	9.9	9.6	9.2	5.7
INPA-H 44868	M	21.7	7.9	8.1	3.5	2.0	2.3	2.5	5.3	1.5	4.7	4.3	4.8	9.6	10.0	10.0	5.4
INPA-H 44869	M	22.4	8.3	8.1	3.6	2.0	2.4	2.4	5.6	1.3	4.1	3.9	4.6	9.3	9.8	9.4	5.7
INPA-H 44870	M	21.9	7.5	7.6	3.5	2.0	2.3	2.6	5.2	1.3	4.1	4.1	4.7	9.7	10.4	9.3	6.0
MPEG 44649	M	21.6	8.0	7.8	3.5	2.1	2.3	2.5	5.4	1.4	4.5	4.7	4.4	9.8	10.5	9.7	5.8
INPA-H 44880	M	21.2	8.2	8.2	3.7	2.1	2.3	2.3	5.3	1.3	4.4	4.2	4.8	9.3	9.7	8.9	5.8
ZUEC 25694	M	21.3	8.1	8.0	3.7	2.0	2.2	2.4	5.4	1.3	4.7	4.7	4.7	9.9	10.7	8.9	6.0
INPA-H44872	M	21.5	7.8	7.8	3.6	2.1	2.3	2.4	5.5	1.5	4.4	4.9	4.6	8.9	9.7	9.1	6.2
INPA-H 44867	M	22.9	8.5	8.3	3.8	2.0	2.4	2.4	5.5	1.4	4.7	4.4	5.0	9.5	10.5	9.4	5.5
INPA-H 44878	M	22.1	7.8	8.2	3.4	2.0	2.2	2.2	5.4	1.4	4.5	4.4	4.0	9.8	10.1	8.9	5.2
ZUEC 25697	M	21.7	7.5	7.7	3.5	2.2	2.1	2.4	5.3	1.3	4.5	4.6	4.5	9.1	10.4	9.4	5.0
ZUEC 25696	M	22.0	8.2	8.5	3.3	2.0	2.0	2.6	5.8	1.3	4.1	4.2	4.6	9.8	10.1	8.9	5.3
INPA-H 44885	M	21.9	8.1	8.5	3.3	2.0	2.3	2.2	5.6	1.4	4.2	4.8	4.2	9.5	10.5	9.7	5.5
INPA-H 44874	F	24.1	8.2	9	3.4	2.1	2.3	2.4	5.6	1.5	4.8	4.1	4.5	11	10.9	10	6.1
INPA-H 44875	F	24.2	8.5	8.8	3.8	2.4	2.5	2.8	5.8	1.5	6	4.4	4.9	11	10.5	9.8	6.5
MPEG 44650	F	24.3	8.2	8.7	3.4	2.5	2.4	2.7	5.7	1.4	5.1	5.4	4.8	11	11.2	10.2	6
ZUEC 25695	F	23.7	8	8	3.7	2.4	2.4	2.7	5.9	1.3	4.7	4.2	4.6	10.5	10.5	10.2	5.9
INPA-H 44883	F	22.1	7.4	7.6	2.9	2	2.1	2.3	4.9	1.2	4.9	4.7	4.8	11.1	10.6	8.4	6.1

Appendix 4

Morphometric traits of *Adenomera simonstuarti*.

Voucher	Sex	SVL	HL	HW	SL	END	IND	ED	IOD	TD	FAL	UAL	HAL	TL	FL	THL	TAL
ZUEC 25700	M	24.3	9.1	9.4	3.9	2	2.6	2.4	5.8	1.43	4.8	4.5	5.1	10.6	11.3	10.5	6.0
MPEG 44653	M	24.7	8.7	9.1	3.5	2.1	2.6	2.4	6.3	1.5	4.75	5.1	5.25	11.1	12.0	11.2	6.0
ZUEC 25698	M	25.0	8.7	9.0	3.9	2.2	2.6	2.3	5.7	1.53	5	5.5	5.3	11.6	12.0	11.1	6.8
INPA-H 44903	M	25.3	9.1	9.3	3.8	2.1	2.6	2.6	5.9	1.33	5.3	5.1	5.35	11.8	11.9	10.5	6.8
INPA-H 44905	M	25.2	9.4	9.1	4	2.1	2.5	2.5	5.6	1.55	4.9	5.05	5.4	11.6	12.0	11.3	6.3
INPA-H 44912	M	24.5	9.0	9.1	3.9	2.1	2.7	2.3	5.8	1.45	4.8	4.95	4.8	11.3	11.8	10.0	6.4
INPA-H 44910	M	26.4	9.4	9.7	4	2.1	2.6	2.1	5.8	1.75	5.8	5.05	5.6	12.1	12.2	11.3	6.8
INPA-H 44907	M	25.0	8.9	9.2	3.6	2	2.5	2.3	5.6	1.7	4.9	5.15	5.1	10.8	11.5	11.8	6.2
INPA-H 44908	M	24.5	8.8	9.0	3.8	2	2.6	2.3	5.7	1.5	4.9	4.55	4.9	10.5	10.5	9.8	5.8
MPEG 44655	M	23.9	8.2	8.7	3.6	2	2.5	2.1	5.8	1.45	4.7	4.95	4.8	10.6	10.9	10.6	6.0
INPA-H 44904	M	24.0	8.6	8.8	3.5	2	2.5	2.1	5.9	1.5	4.7	5	5.05	10.0	11.5	9.8	6.0
MPEG 44654	M	24.3	8.5	9.1	3.9	2	2.5	2.2	5.6	1.53	4.75	5.3	5.05	10.6	10.9	9.9	5.5
ZUEC 25699	M	25.3	8.9	9.1	3.9	2.1	2.5	2.1	6	1.63	4.9	4.75	5.25	11.5	11.6	11.6	6.7

INPA-H 44911	M	26.0	9.0	9.5	4	2.1	2.5	2.6	6.1	1.53	5.15	5.25	5.35	11.5	11.5	11.6	5.7
INPA-H 44909	F	23.0	8.1	8.7	3.1	1.8	2.4	2.4	5.4	1.45	4.8	5.1	4.8	11.1	11.2	10.6	5.8
INPA-H 44906	F	25.8	8.8	9.6	3.8	2.3	2.5	2.2	5.7	1.58	5.35	5.55	5.3	11.6	12.0	11.5	6.5

Appendix 5

Dorsal and ventral views of males (A–D) and females (E–F) of *Adenomera simonstuarti* from the municipality of Tarauacá, state of Acre, Brazil. Photographs: L. R. Mendonça.