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

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Abstract

By using integrative taxonomy, we describe a new species of terrestrial foam-nesting frog of the genus *Adenomera* from white-sand forests of the Rio Negro Sustainable Development Reserve, Central Amazonia, Brazil. Within the *A. andreae* clade, the new species belongs to the *A. simonstuarti* complex where it is sister to the lineage from the lower Juruá River. The new species is assigned to the genus *Adenomera* by having adult SVL smaller than 34.1 mm, by its lack of fringing and webbing between toes and by the absence of spines on the thumb of adult males. It differs from other *Adenomera* by the following combination of characters: antebrachial tubercle absent; toe tips flattened or slightly flattened, with visible expansions; nearly solid, dark-coloured stripe on underside of forearm; single-note advertisement call; notes formed by 11–21 incomplete pulses; call duration varying between 100 and 199 ms; fundamental frequency 1,765–2,239 Hz; dominant frequency 3,448–4,349 Hz; and endotrophic tadpoles with spiracle present and labial teeth absent. Over the last decade, we have inventoried many permanent sampling modules in ombrophilous forests in the Manaus Region and in the Purus-Madeira interfluve, but the new species was found only in the white-sand forest from West Negro-Solimões Interfluve. *Adenomera* **sp. nov.** may be endemic to, or at least a specialist in, this environment.

Key Words

campina, *campinarana*, integrative taxonomy, tadpoles, West Negro-Solimões Interfluve

Introduction

Leptodactylid frogs of the genus *Adenomera* Steindachner, 1867 comprise 30 described species distributed throughout South America east of the Andes (Frost 2024). The taxonomic history of this genus is very complex and, over the last 50 years, numerous systematic studies have reviewed its taxonomic validity, phylogenetic position and species diversity (Heyer 1973, 1974; Frost et al. 2006; Pyron and Wiens 2011; de Sá et al. 2014). The genus was originally described by Steindachner (1867) to accommodate a single species, *A. marmorata*. Later, Lutz (1930) synonymised the genus with *Parvulus*, a subgenus of *Leptodactylus* Fitzinger, 1826, but Parker (1932) soon gave priority to the name *Adenomera* and elevated it as a subgenus of *Leptodactylus*. Four decades later, *Adenomera* was resurrected by Heyer (1974) to accommodate taxa of the *Leptodactylus marmoratus* species group.

To avoid paraphyly of *Leptodactylus* rendered by *Vanzolinius* Heyer, 1974, Frost et al. (2006), supported by evidence from Heyer (1998) and Kokubum and Giaretta (2005), declared *Adenomera* a synonym of *Lithodytes* Fitzinger, 1843 and the latter taxon a synonym (subgenus) of *Leptodactylus*. Based on molecular data, Pyron and Wiens (2011) recovered *Adenomera* as sister to *Lithodytes* and this clade as sister to *Leptodactylus*. The authors also removed the two former taxa from the synonymy with *Leptodactylus*. The sister relationship between *Adenomera* and *Lithodytes* was corroborated by de Sá et al. (2014) through a total evidence analysis, which recovered the clade comprising the two genera as sister to the one grouping *Hydrolaetare* and *Leptodactylus*. Fouquet et al. (2014) performed a comprehensive phylogenetic analysis and recovered eight major clades within *Adenomera*: *A. lutzi* clade, *A. heyeri* clade, *Adenomera* 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 *A. juikitam* and, based on acoustic, morphologic and genetic data, concluded that *A. juikitam* instead belongs to the *A. heyeri* clade.

The genus *Adenomera* displays a high prevalence of morphologically cryptic species (e.g. Angulo and Icochea (2010); Carvalho and Giaretta (2013a); Carvalho et al. (2020a); Zaracho et al. (2023)). Some species also show high levels of intraspecific polymorphism (e.g. Cassini et al. (2020)) and congeneric sympatry and syntopy are common; up to three species may occur in the same region (e.g. Carvalho et al. (2021)). These factors make species delimitation in *Adenomera* challenging. Nevertheless, 15 of the 30 currently recognised species were described in the last 10 years (Frost 2024) and several candidate species still await formal description (Fouquet et al. 2014). The massive advance in the taxonomy of *Adenomera* has been made possible by the use of integrative taxonomy (Carvalho et al. 2019a, 2019c). In particular, despite morphological crypsis, advertisement calls are markedly divergent amongst species and represent a powerful source of reliable diagnostic characters (Angulo and Icochea 2010; Carvalho and 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. guarayo* Carvalho, Angulo, Barrera, Aguilar-Puntriano & Haddad, 2020; and *A. simonstuarti* (Angulo & Icochea, 2010)—and two candidate species, *Adenomera* sp. D and *Adenomera* sp. T (Fouquet et al. 2014). While the *A. andreae* clade is restricted to Amazonia, none of the nominal species has a restricted geographic distribution. *Adenomera andreae* shows the widest range, being distributed throughout Amazonia (Carvalho et al. 2019c), while *A. chicomendesi* and *A. guarayo* are widely distributed in south-western Amazonia (Carvalho et al. 2019a, 2020a). *Adenomera simonstuarti* is distributed in western and south-western Amazonia (Carvalho et al. 2020b).

Adenomera simonstuarti was described from Peruvian Amazonia, based on morphological and acoustic data of four males and two females (Angulo and Icochea 2010). Subsequently, Fouquet et al. (2014) reported that the species was more widespread than previously thought, also occurring in Venezuela, Ecuador and Brazil (States of Acre and Amazonas). They also suggested the existence of more than one species hidden under the name *A. simonstuarti* (Fouquet et al. 2014, appendix S2a). Recently, Carvalho et al. (2020b) sequenced additional specimens from Brazil referred to as *A. simonstuarti* and their delimitation analysis recovered eight lineages within this name (hereafter, the *A. simonstuarti* complex). They also re-described 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) recognised their lineage 3 as *A. simonstuarti* sensu stricto. They also identified the other lineages as putative new species, pending confirmation with additional data (e.g. acoustic and morphologic data).

Poorly sampled environments in Amazonia usually harbour undocumented biodiversity of anurans (Ferrão et al. 2016; Vacher et al. 2020). Physiognomies comprising the white-sand ecosystems (hereafter, WSE) exemplify such environments (Adeney et al. 2016). The WSE occupies an area of 5% of the Amazonia and comprises two main physiognomies in Brazil: *campina*—open environments characterised as patches of grasslands or scrublands (canopy < 7 m) on a matrix of exposed sandy soil; and *campinarana*—closed-canopy, forested environments characterised by thin-trunked trees of low stature (canopy < 20 m) (Anderson 1981; Ferreira 2009; Adeney et al. 2016). Despite the increasing interest in WSE organisms (Capurucho et al. 2013; Fine and Baraloto 2016; Vicentini 2016; Lamarre et al. 2016; Borges et al. 2016; Fraga et al. 2018; Gonella et al. 2020), studies of anurans from WSE are rare. The few such studies recently published show that WSE represents a source of poorly known and new species of anurans, many of which appear to be specialists in or endemic to these environments (Carvalho et al. 2019a; Ferrão et al. 2019, 2022; Mônico et al. 2023).

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

Methods

Sampling

Fieldwork was conducted between 2019 and 2023 in three long-term ecological research sites (RAPELD) in the Rio Negro Sustainable Development Reserve (hereafter, RDS Rio Negro), Municipality of Iranduba, State of Amazonas, Brazil (Fig. 1). Modules are located near km 18 (3°06'33.6"S, 60°40'29.0"W; 73 m above sea level

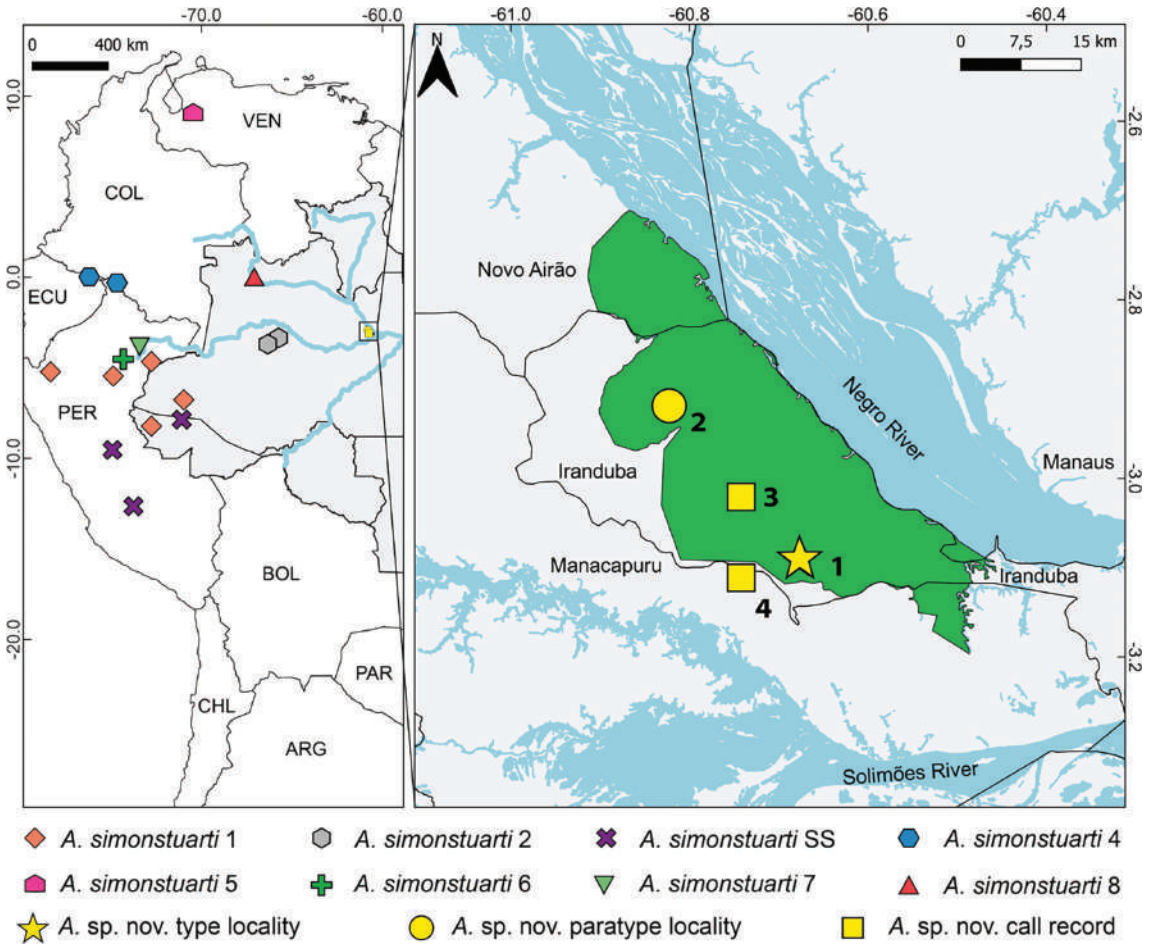

Figure 1. Geographic distribution of the *Adenomera simonstuarti* species complex (left) and a detailed view of the geographic distribution of the new species in central Amazonia, Amazonas, Brazil (right). Green area: Rio Negro Sustainable Development Reserve. Numbers: permanent sampling modules at (1) km 18, (2) km 26 and (3) km 50 along the AM-352 highway; (4) Vale da Benção Community, Ramal do 25, Manacapuru. South American countries: ARG, Argentina; BOL, Bolivia; CHL, Chile; COL, Colombia; ECU, Ecuador; PAR, Paraguay; PER, Peru; VEN, Venezuela.

[hereafter [a.s.l.]), km 26 (3°03'31.0"S, 60°45'42.0"W; 73 m a.s.l.) and km 50 (2°50'10.0"S, 60°50'20.0"W; 19 m a.s.l.) of the AM-352 highway. Adults were euthanised with 2% aqueous benzocaine topical solution, fixed in 10% neutral-buffered formalin and preserved in 70% ethanol. Before fixation, tissue samples of each specimen were collected and stored in 100% ethanol. Tadpoles were collected from two foam nests in the calling site of two uncollected males near the sampling module at km 18. They were euthanised as described above, fixed and preserved in 5% neutral-buffered formalin. Adults 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); tadpoles were deposited at **INPA-H**.

Advertisement calls of six males of the new species (INPA-H 44867 [holotype], MPEG 44649, INPA-H 44868–69, MPEG 44652 and INPA-H 44877) 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 a sampling rate of 44.1 kHz and sample size of 16 bits. The microphone was positioned 50–100 cm from the calling male. Air temperature during all recordings was 25 °C. Recordings were deposited in the Neotropical Jacques Vielliard sound repository of the University of Campinas (**FNJV;** Campinas, Brazil) under accession numbers FNJV 59561–66.

To facilitate interspecific comparisons, 16 specimens and the advertisement calls of six males of *Adenomera simonstuarti* sensu stricto were collected and recorded, respectively, at Unidade de Gestão Ambiental Acurauá, Municipality of Tarauacá, State of Acre, Brazil. A specimen from this locality (INPA-H 40967) was included in the phylogenetic inference of Carvalho et al. (2020b) and nests with samples of *A. simonstuarti* sensu stricto from Peru and male advertisement calls of the Acre population match with those in the original description by Angulo and Icochea (2010). All males of the Acre population were found in the field by their vocalisation, ensuring that we collected the target species.

Morphology

The description of external morphology of adults of the new species is based on 21 males and five females. Sex was determined through direct assessment of sexual characters: the presence of vocal slits, vocal sac and a fleshy ridge on the snout tip in males and absence in females. Maturity was determined, based on breeding behaviour in males (calling activity) and examination of secondary sexual characters in females (mature oocytes visible through the belly skin). The following 16 morphometric measurements (Watters et al. 2016) were taken to the nearest 0.1 mm using digital calipers and an ocular micrometer coupled to a stereomicroscope: 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**), foot length (**FL**) and tarsus length (**TSL**). Toe tip development (character states) follows Heyer (1973). Snout shape follows Heyer et al. (1990). Terminology for other morphological characters follows Carvalho et al. (2020a). We follow the colour catalogue of Köhler (2012): colour names are italicised; cc, colour code. Repeated colours do not repeat codes. See Suppl. material 1: table S1 for morphometric raw data.

The larval developmental stage was determined according to Gosner (1960). The following morphometric measurements were taken with a micrometer coupled to a stereomicroscope from 10 tadpoles at stages 35 (n = 7) and 41 (n = 3): total length (**TL**), body length (**BL**), tail length (**TAL**), maximum tail height (**MTH**), tail muscle height (**TMH**), tail muscle width (**TMW**), inter-nostril distance (**IND**) and interorbital distance (**IOD**) (Altig and McDiarmid 1999); body height (**BH**), body width at spiracle level (**BW**), eye-nostril distance (**END**), eye diameter (**ED**) and oral-disc width (**ODW**) (Lavilla and Scrocchi 1986); body width at eye level (**HW**) (Lima et al. 2015); and vent-tube length (**VTL**) (Lins et al. 2018). Morphological description is based on seven tadpoles at stage 35. Terminology and diagnostic characters follow Altig and McDiarmid (1999) and Schulze et al. (2015).

Vocalisation

Description of the advertisement call and the following acoustic parameters follow Carvalho et al. (2019a) and Köhler et al. (2017): call duration (**CD**), notes per call (**NpC**), note duration (**ND**), note repetition rate (**NrR**) note rise time (**NrT**), pulses per note (**PpN**), pulse duration (**PD**; measured for the first, central and last pulses of each note), pulse repetition rate (**PrR**), dominant frequency (**DF**), fundamental frequency (**FF**) and frequency modulation (**FM**). See Suppl. material 1: table S2 for bioacoustic raw data.

Calls were analysed with Raven 1.5.1 (Bioacoustics Research Program 2014) configured as follows: Hamming window (size = 20 ms), filter bandwidth 65 Hz, overlap 90%, hop size 2 ms and Discrete Fourier Transform 1,024 samples. The dominant frequency and rise time were measured with the peak frequency and peak time relative functions. Figures were produced in R platform (R Core Team 2021) 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: Hamming window, Fast Fourier Transform 256 points, overlap 90%.

Molecular phylogenetics

Genomic DNA was extracted from tissues of four specimens of the new species 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 the primers CHmL4 (5'-TYTCWACWAAYCAYAAAGAYATCGG-3') and CHmR4 (5'-ACYTCRGGRTGRCCRAARAATCA-3') (Che et al. 2012). Reaction conditions were: 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 min at 72 °C. The final volume of the PCR reaction was 15 µl and contained 0.6 µl of 50 mM $MgCl_2$, 1.2 µl of 10 mM dNTPs (2.5 mM each dNTP), 1.5 µl of tampon 10× (75 mM Tris HCl, 50 mM KCl, 20 mM $(NH_4)_2SO_4$), 0.5 µl of each primer (10 µM), 9.55 µl of ddH_2O, 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). Subsequent sequencing reactions were performed using standard protocols of the Big Dye™ Terminator Kit (Applied Biosystems, Waltham, USA). We used 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 are deposited in the online repository GenBank under accession numbers OQ974333–36.

To infer phylogenetic relationships, we inserted the generated sequences into a dataset containing sequences retrieved from GenBank (Suppl. material 1: table S3). 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 the *Adenomera andreae* clade, including all known lineages of *A. simonstuarti*, as well as *Adenomera* sp. D and *Adenomera* sp. T (Fouquet et al. 2014; Carvalho et al. 2020b) and species belonging to the other six clades (Suppl. material 1: table S3). *Lithodytes lineatus* was used to root the tree. 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 and comprises 53 terminals and 3,293 base pairs (bp) (667 for Cytb, 657 for COI, 1,422 for RAG1 and 547 for POMC).

We divided the dataset considering first, second and third codon positions for each protein-coding gene and we used PartitionFinder 2.1.1 (Lanfear et al. 2017) under

the corrected Akaike Information Criterion (AICc) to infer partition schemes and evolutionary models. The best evolutionary models for partitions in the concatenated matrix were TIM+G for Cytb 1st and COI 3rd positions; SYM+I+G for Cytb 2nd position; GTR+I+G for Cytb 3rd position; TRNEF+I+G for COI 1st position; F81+I+G for COI 2nd position; TRN+I+G for RAG1 1st and 2nd positions; GTR+G for RAG1 3rd and POCM 1st positions; TVM+I+G for POMC 2nd position; and GTR+I for POMC 3rd position. Phylogenetic relationships were reconstructed through Maximum Likelihood (ML) 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 replicates (Hoang et al. 2018) using 5,000 maximum iterations, 3,000 replicates and a minimum correlation coefficient of 0.99. Lineage numbering within the *A. simonstuarti* species complex follows Carvalho et al. (2020b), except by *A. simonstuarti* 3, which is referred to as *A. simonstuarti* sensu stricto (SS) in the present study.

Based on 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). Mean distances are presented as in the main text, minimum–maximum values in the Suppl. material 1: table S4.

Morphometric analysis

Due to the phenotypic similarity between *A. simonstuarti* sensu stricto and the new species, we performed a Principal Component Analysis (PCA) associated with a Multivariate Analysis of Variance (MANOVA) to test for a statistical difference between the morphometric multidimensional spaces of each species. Analysis was performed only for males due to the low number of females collected for *A. simonstuarti* sensu stricto. The same 16 morphometric measurements taken from the new species were also taken from 14 adult males of *A. simonstuarti*. To perform morphometric PCA, we transformed the raw data into 15 morphometric ratios: HL/SVL, HW/SVL, SL/SVL, END/SVL, IND/SVL, ED/SVL, IOD/SVL, TD/SVL, FAL/SVL, UAL/SVL, HAL/SVLL, TL/SVL, FL/SVL, THL/SVL and TAL/SVL. The PCA and MANOVA were run using the functions prcomp and manova of the package stats 4.1 (R Core Team 2021); an ellipse representing the standard errors of points in the graphic representation of PCA was drawn using the function ordiellipse of the package vegan 2.5-7 (Oksanen et al. 2020) with parameter kind set as *se*. See Suppl. material 1: table S1 for morphometric measurements of *Adenomera* sp. nov. and *A. simonstuarti* sensu stricto.

Interspecific morphological comparisons

Succinct morphological comparisons of adults were made with all 30 nominal congeners but detailed morphological and acoustic comparisons were restricted to species of the *Adenomera andreae* clade (*A. andreae* [Müller, 1923]; *A. chicomendesi* Carvalho, Angulo, Kokubum, Barrera, Souza, Haddad & Giaretta, 2019; *A. guarayo* Carvalho, Angulo, Barrera, Aguilar-Puntriano & Haddad, 2020; and *A. simonstuarti*) and species distributed in Amazonia (*A. amicorum* Carvalho, Moraes, Lima, Fouquet, Peloso, Pavan, Drummond, Rodrigues, Giaretta, Gordo, Neckel-Oliveira & Haddad, 2021; *A. aurantiaca* Carvalho, Moraes, Lima, Fouquet, Peloso, Pavan, Drummond, Rodrigues, Giaretta, Gordo, Neckel-Oliveira & Haddad, 2021; *A. glauciae* Carvalho, Simões, Gagliardi-Urrutia, Rojas-Runjaic, Haddad and Castrovejo-Fisher, 2020; *A. gridipappi* Carvalho, Moraes, Lima, Fouquet, Peloso, Pavan, Drummond, Rodrigues, Giaretta, Gordo, Neckel-Oliveira & Haddad, 2021; *A. heyeri* Boistel, Massary & Angulo, 2006; *A. hylaedactyla* (Cope, 1868); *A. inopinata* Carvalho, Moraes, Lima, Fouquet, Peloso, Pavan, Drummond, Rodrigues, Giaretta, Gordo, Neckel-Oliveira & Haddad, 2021; *A. kayapo* Carvalho, Moraes, Lima, Fouquet, Peloso, Pavan, Drummond, Rodrigues, Giaretta, Gordo, Neckel-Oliveira & Haddad, 2021; *A. lutzi* Heyer, 1975; *A. martinezi* (Bokermann, 1956); *A. phonotriccus* Carvalho, Giaretta, Angulo, Haddad & Peloso, 2019; and *A. tapajonica* Carvalho, Moraes, Lima, Fouquet, Peloso, Pavan, Drummond, Rodrigues, Giaretta, Gordo, Neckel-Oliveira & Haddad, 2021). Larval comparisons were made with all nominal species for which tadpoles are described (*A. andreae*, *A. guarani*, *A. hylaedactyla*, *A. marmorata*, *A. saci* and *A. thomei*), except for *A. bokermanni* because the tadpole described for it might correspond to another species (Carvalho and Giaretta 2013b). Comparisons were made based on published data (i.e. taxonomic descriptions, re-descriptions and revisions), except the one for *Adenomera simonstuarti*, which is based on direct analysis of specimens (Appendices 1, 2).

Results

Phylogenetic relationships and genetic distances

Individuals of *Adenomera* sp. nov. nest together as a new monophyletic lineage (bootstrap support = 100) within the *A. simonstuarti* species complex (sensu Carvalho et al. (2020b)), which nests within the *A. andreae* clade (Fig. 2). The new species is sister to the lineage *A. simonstuarti* 2 from the lower Juruá River in Brazil. Clades representing *A. simonstuarti* 2 and *Adenomera* sp. nov. are the shallowest within the species complex; the average p-distance for COI between them equals 2.9% (2.5–3.3%) (Table 1; Suppl. material 1: table S4). Peruvian and Brazilian individuals of *A. simonstuarti* sensu stricto are recovered as sister to the clade comprising *A. simonstuarti* 1, *A. simonstuarti* 2 and *Adenomera* sp. nov. Genetic p-distance between the new species and *A. simonstuarti* sensu stricto averages 5.2% (4.3–5.9%).

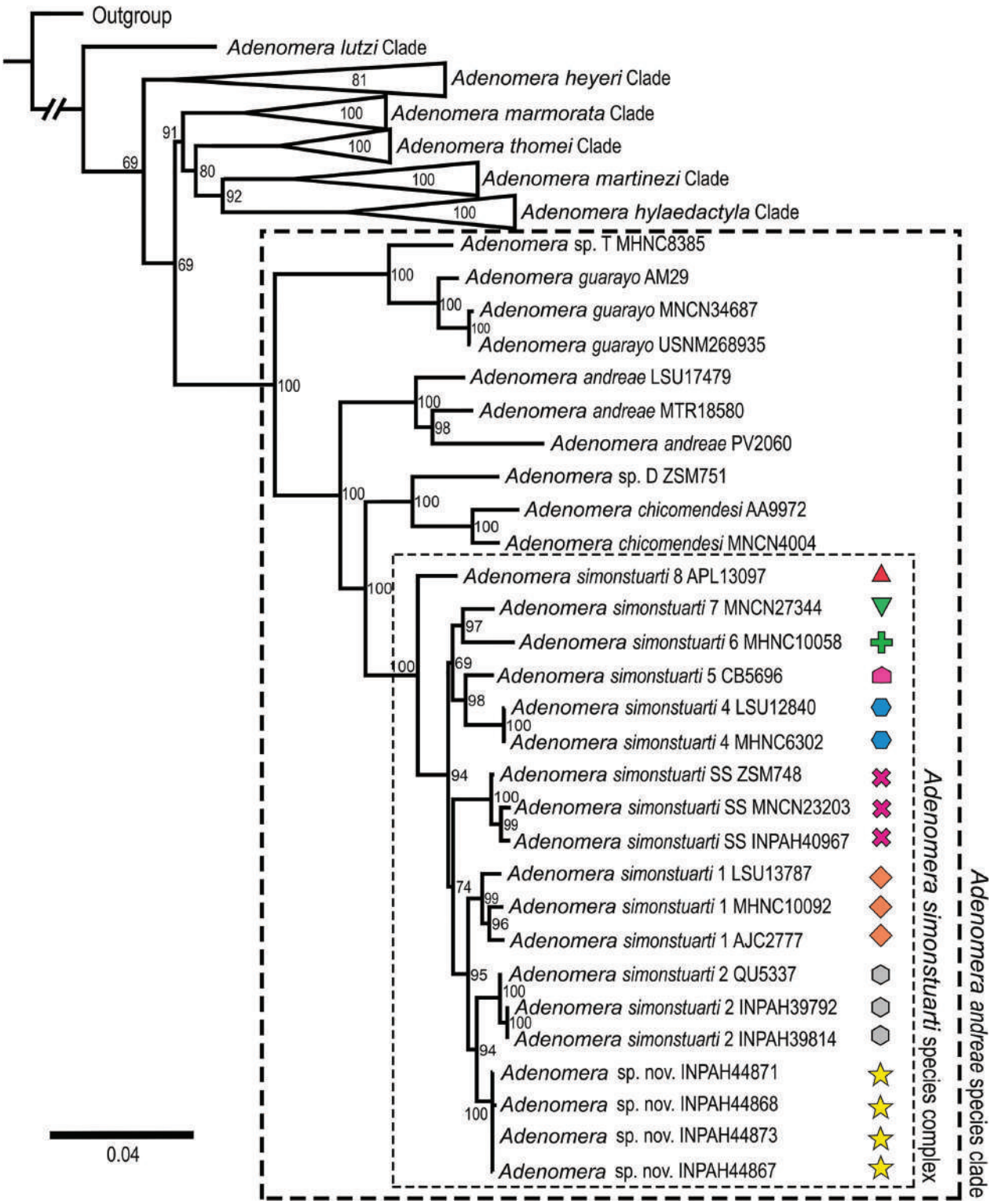

Figure 2. Phylogenetic relationships of the *Adenomera andreae* species clade with a focus on the *A. simonstuarti* species complex. Maximum Likelihood values are inferred from sequence data for Cytb, COI, RAG1 and POMC genes. Lineage numbering within *A. simonstuarti* species complex follows Carvalho et al. (2020b), except for *A. simonstuarti* sensu stricto (SS). Species names are followed by the corresponding museum voucher numbers. Symbols are as in Fig. 1.

Morphometric analysis

The first two principal components (PCs) of morphometric PCA explained ~ 53% of data variance. Spaces occupied by *Adenomera* sp. nov. and *A. simonstuarti* SS are significantly different (Pillai = 0.286, df = 32, $p = 0.004$) and do not overlap (Fig. 3). The three morphometric ratios that contribute most of the variation along PC1 are END/SVL, ED/SVL and DSL/SVL. See Table 2 for data regarding other PCA variables.

Table 1. Average pairwise genetic distances (%) between lineages of the *Adenomera simonstuarti* species complex and related species of the *A. andreae* clade. Interspecific uncorrected p-distances (lower diagonal) and Kimura 2-parameter distances (upper diagonal) are based on a fragment of the COI gene. Intraspecific p-distances are shown along the diagonal in bold. See Supplementary file 4 for minimum and maximum values.

	Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*Adenomera* sp. nov. (n = 4)	0.3	4.0	3.0	5.5	5.7	5.1	5.5	5.6	7.1	13.8	13.9	17.5	14.0	16.9
2	*A. simonstuarti* 1 (n = 3)	3.9	2.1	3.7	5.7	6.1	5.2	6.7	4.6	7.1	14.0	14.4	16.7	14.2	18.1
3	*A. simonstuarti* 2 (n = 3)	2.9	3.6	0.4	5.5	7.0	5.6	6.1	5.5	7.5	14.6	14.4	17.1	14.1	17.3
4	*A. simonstuarti* SS (n = 3)	5.2	5.4	5.2	0.8	5.4	5.0	5.4	5.3	7.4	13.6	14.8	16.7	14.0	18.0
5	*A. simonstuarti* 4 (n = 2)	5.4	5.8	6.5	5.1	0.0	4.1	7.5	5.7	7.7	14.5	14.0	15.7	14.5	17.1
6	*A. simonstuarti* 5 (n = 1)	4.9	4.9	5.3	4.8	3.9	NA	6.2	3.9	7.3	13.8	15.1	16.6	13.5	17.3
7	*A. simonstuarti* 6 (n = 1)	5.2	5.8	5.8	5.2	7.0	6.2	NA	6.1	6.9	14.1	14.4	17.6	14.7	17.6
8	*A. simonstuarti* 7 (n = 1)	5.4	4.4	5.2	5.0	5.4	3.8	5.8	NA	6.7	15.5	14.7	16.6	15.0	19.5
9	*A. simonstuarti* 8 (n = 1)	6.6	6.7	7.0	6.9	7.2	6.9	6.5	6.4	NA	13.0	12.6	16.5	14.1	17.3
10	*A. andreae* (n = 3)	12.3	12.5	12.9	12.2	12.9	12.3	12.6	13.7	11.7	6.6	15.3	17.0	12.8	16.1
11	*A. chicomendesi* (n = 2)	12.4	12.8	12.8	13.1	12.5	13.3	12.8	13.0	11.4	13.4	4.9	19.2	10.3	19.4
12	*A. guarayo* (n = 3)	15.2	14.6	14.9	14.6	13.9	14.5	15.2	14.5	14.5	14.9	16.6	2.7	17.9	8.8
13	*Adenomera* sp. D (n = 1)	12.4	12.6	12.5	12.4	12.8	12.0	12.9	13.1	12.5	11.5	9.4	15.6	NA	17.9
14	*Adenomera* sp. T (n = 1)	14.0	15.7	15.1	15.6	14.9	15.1	15.3	16.8	15.1	14.2	16.7	8.2	15.6	NA

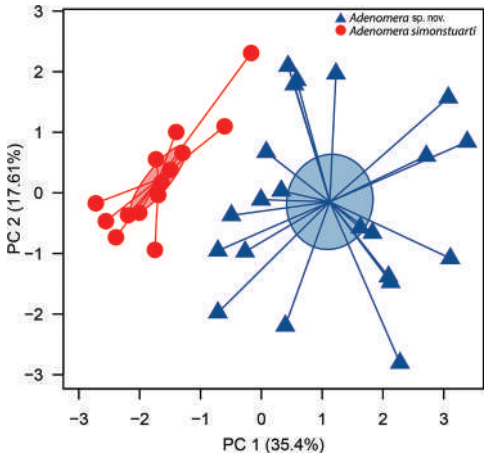

Figure 3. Morphometric Principal Component Analysis. Analyses were based on 15 morphometric ratios of 21 males of *Adenomera* sp. nov. and 14 males of *A. simonstuarti* sensu stricto. Ellipse represents the standard error with 95% confidence interval.

Table 2. Loadings of 15 morphometric and 9 bioacoustic ratios on the respective first principal components. Values were generated from data for 21 males of *Adenomera* sp. nov. and 14 males of *A. simonstuarti* sensu stricto.

Variables	PC 1	PC 2
HL/SVL	0.480	0.697
HW/SVL	0.378	0.777
SL/SVL	0.732	-0.325
END/SVL	0.845	-0.355
IND/SVL	0.348	0.168
ED/SVL	0.743	-0.112
IOD/SVL	0.720	0.145
TD/SVL	0.199	-0.420
FAL/SVL	0.571	-0,152
UAL/SVL	-0.174	-0.046
HAL/SVL	0.097	0.009
TL/SVL	0.099	-0.118
FL/SVL	-0.041	0.172
THL/SVL	-0.165	-0.051
TSL/SVL	0.448	-0.215

Taxonomic account

Order Anura Fischer von Waldhein, 1813
Family Leptodactylidae Werner, 1896
Subfamily Leptodactylinae Werner, 1896
Genus *Adenomera* Steindachner, 1867

***Adenomera albarena* sp. nov.**

https://zoobank.org/0FAB2CE9-3291-453D-8C58-E092BD288DCA

Tables 3, 4, Figs 4–7, 9B–D

Chresonymy. *Adenomera* gr. *heyeri* (Lima et al. 2021).

Type material. *Holotype*. INPA-H 44867, an adult male collected at km 26 of the AM-352 highway, Rio Negro Sustainable Development Reserve (03°05'35"S, 60°40'36"W; 76 m a.s.l.), Municipality of Iranduba, State of Amazonas, Brazil, on 11 December 2020 by M. Ferrão, A. P. Lima and W. E. Magnusson.

***Paratypes*.** Twenty-four adults collected at the same locality as the holotype; eight males MPEG 44649, INPA-H 44868–73 and ZUEC-AMP 25694 collected on 11 December 2020 by M. Ferrão, A. P. Lima and W. E. Magnusson; four females INPA-H 44874–75, ZUEC-AMP 25695 and MPEG 44650 collected on 10 December 2021 by M. Ferrão, A. P. Lima and B. Martins; four males INPA-H 44876–77 and MPEG 44651–52 collected on 11 December 2021 by M. Ferrão, A. P. Lima and B. Martins; four males INPA-H 44878–80 and ZUEC-AMP 25696 collected on 12 December 2021 by M. Ferrão, A. P. Lima and B. Martins; a male INPA-H 44881 collected on 19 January 2022 by B. Martins; a male INPA-H 44882 collected on 3 February 2022 by B. Martins; a male ZUEC-AMP 25697 and a female INPA-H 44883 collected on 14 May 2022 by B. Martins. One adult male INPA-H 44885, collected at km 50 of the AM-352 Highway, Rio Negro Sustainable Development Reserve (2°50'10.0"S, 60°50'20.0"W), Municipality of Iranduba, State of Amazonas, Brazil, on 12 January 2023 by B. Martins.

Table 3. Morphometric measurements of the type series of *Adenomera albarena* (Rio Negro Sustainable Development Reserve, Iranduba, Amazonas, Brazil) and *A. simonstuarti* sensu stricto (Tarauacá, Acre, Brazil). Values depict average, standard deviation and range. Abbreviation: SS, sensu stricto. Trait acronyms are explained in the text. * Holotype included.

	Adenomera albarena			*Adenomera simonstuarti* SS	
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)

Etymology. The specific epithet *albarena* is formed by the combination of two Latin words: "alba" (white) and "arena" (sand). This is a reference to the white-sand forests of central Amazonia, the distinctive environment inhabited by this species.

Vernacular names. White-sand terrestrial foam-nesting frog (English), rana terrestre de arena blanca (Spanish) and rãzinha da areia branca (Portuguese).

Diagnosis. The species *Adenomera albarena* is recognised 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 of males subovoid in dorsal view and acuminate in lateral view; (3) absence of antebrachial tubercle; (4) toe tips moderately to fully expanded (character states C, D sensu Heyer (1973)); (5) throat in males with condensed melanophores near the jaw and scattered melanophores on the central portion; 6) nearly solid dark-coloured stripe present on the underside of the forearm; (7) Advertisement call composed of a single pulsed note; (8) notes formed by 11–21 pulses; (9) pulses incomplete; (10) dominant frequency 3,448–4,349 Hz; (11) dominant frequency coinciding with the second harmonic; (12) Endotrophic tadpoles; (13) with labial teeth absent; (13) spiracle present; and (14) internarial distance 44–52% of IOD.

Interspecific comparisons. *Adenomera albarena* differs from all congeners, except *A. simonstuarti* by having a nearly solid dark-coloured stripe on the underside of the forearm (Heyer 1973, 1975; Kwet and Angulo 2002; Almeida and Angulo 2006; Kok et al. 2007; Kwet 2007; Angulo and Reichle 2008; Berneck et al. 2008; Kwet et al. 2009; Angulo and Icochea 2010; Carvalho and Giaretta 2013a, 2013b; Carvalho et al. 2019a, 2019b, 2019c, 2019d, 2020a, 2020c, 2021; Cassini et al. 2020; Zaracho et al. 2023).

Amongst Amazonian congeners, adult male *Adenomera albarena* have SVL 21.2–23.0 mm, which is smaller than *A. glauciae* (SVL 27.6–30.4; Carvalho et al. (2020b)), *A. gridipappi* (SVL 25.4–27.7 mm; Carvalho et

Table 4. Spectral and temporal parameters of the advertisement calls of *Adenomera albarena* 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 at the type locality in Cusco, Peru and Tarauacá, Brazil; ***, dominant frequency correspond to the first harmonic in *A. simonstuarti* and to the second harmonic in the new species. Abbreviation: SS, sensu stricto. Trait acronyms are described in the main text.

Call traits	***Adenomera albarena* (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 (ms)	142 ± 19.0 (100–199), n = 148*	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
NrR	0.8 ± 0.15 (0.6–1.2), n = 30	4.8 ± 0.3 (4.3–5.4), n = 30	4.6 ± 0.1 (4.5–4.9)
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
PrR	107 ± 9.7 (94–138), n = 60	53 ± 8.7 (37–78), n = 30	–
FF (Hz)	1,986 ± 0.1 (1,765–2,239), n = 148***	3,987 ± 0.16 (3,617–4,263), n = 93***	3596–4156***
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

al. (2021)), *A. lutzi* (SVL 25.7–33.5 mm; Kok et al. (2007)) and *A. simonstuarti* (SVL 23.4–26.2 mm; Angulo and Icochea (2010); Carvalho et al. (2020b); present study), but larger than *A. kayapo* (SVL 17.5–21.0 mm; Carvalho et al. (2021)). *Adenomera albarena* has a snout rounded in dorsal view, which differs from *A. martinezi* (snout pointed in dorsal view; Carvalho & Giaretta (2013b)). The absence of an antebrachial tubercle distinguishes *A. albarena* from *A. amicorum*, *A. aurantiaca*, *A. cotuba*, *A. glauciae*, *A. gridipappi*, *A. inopinata*, *A. kayapo*, *A. lutzi*, *A. tapajonica* and *A. phonotriccus* (antebrachial tubercle present; Kok et al. (2007); Carvalho and Giaretta (2013b), Carvalho et al. (2019b, 2020c, 2021)). *Adenomera albarena* has toe tips moderately to fully expanded (states C and D, sensu Heyer (1973)), which differs from *A. cotuba*, *A. hylaedactyla* and *A. martinezi* (toe tips pointed to poorly expanded, states A and B, respectively; Carvalho et al. (2019c); Carvalho and Giaretta (2013a, 2013b)). *Adenomera albarena* differs from *A. heyeri* by having white ventral colouration (yellow colouration; Carvalho et al. (2021)). Although *A. albarena* differs morphologically from *A. andreae*, *A. chicomendesi* and *A. guarayo* only by having a nearly solid dark-coloured stripe on the underside of the forearm (absence in *A. andreae*, *A. chicomendesi* and *A. guarayo*; Carvalho et al. (2019a, 2019b, 2019c)), it also differs acoustically from these species (see below).

The advertisement call of *Adenomera albarena* is composed of incomplete pulses, which differs from *A. aurantiaca*, *A. guarayo*, *A. inopinata* and *A. phonotriccus* (complete pulses; Carvalho et al. (2019b, 2020a, 2021)). Amongst those with incomplete pulses, calls of *A. albarena* sp. nov. differ from *A. amicorum*, *A. glauciae*, *A. gridipappi* and *A. simonstuarti* sensu stricto by having a single note (multi-note calls; Angulo and Icochea (2010); Carvalho et al. (2020b, 2021)). *Adenomera albarena* has calls composed of 11–21 pulses, which differ from *A. amicorum* (4–10 pulses; Carvalho et al. (2021)), *A. andreae* (3–10 pulses; Carvalho et al. (2019c)), *A. chicomendesi* (22–35 pulses; Carvalho et al. (2019a)), *A. gridipappi* (2–4 pulses; Carvalho et al. (2021)), *A. heyeri* (4–12 pulses; Carvalho et al. (2021)), *A. hylaedactyla* (4–10 pulses; Carvalho et al. (2019c)), *A. tapajonica* (3–5 pulses; Carvalho et al. (2021)), *A. glauciae* (unpulsed; Carvalho et al. (2020b)) and *A. simonstuarti* sensu stricto (2–6 pulses; this study). *Adenomera albarena* has a dominant frequency of 3,448–4,349 Hz, which 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 albarena* sp. nov. is placed in the second harmonic, which differs from *A. simonstuarti* sensu stricto (dominant frequency in the fundamental harmonic).

Lack of labial teeth distinguishes tadpoles of *Adenomera albarena* from exotrophic tadpoles of *A. guarani*, *A. saci* and *A. thomei* (present in all mentioned species; De la Riva (1995); Almeida and Angulo (2006); Carvalho and Giaretta (2013a)). Endotrophic tadpoles of *A. albarena* differ from those of *A. hylaedactyla* and *A. andreae* by the presence of a spiracle (absent in all mentioned species; Menin et al. (2009); Menin and Rodrigues (2013)); from *A. marmorata* by an internarial distance 44–52% of IOD (IND/IOD = 74%; Heyer et al. (1990)).

Description of the holotype. Adult male (Figs 4A, B, C, 5A, C). Dorsal skin glandular, warty on flank. Dorsolateral fold indistinct. Sacral region, dorsal surface of tibia and posterior surface of tarsus with white-tipped tubercles. Vertebral stripe in sacral region. Throat, belly and ventral surface of limbs smooth. Pair of lumbar glands. Posterior surface of thigh with a pair of paracloacal glands. Snout subovoid in dorsal view and acuminate in profile. Nostril closer to the snout tip than to the eye and orientated dorsolaterally; fleshy ridge on the snout tip. Eye nostril distance 83% of eye diameter, eye diameter equals internarial distance. Head wider than long. Internarial distance > 25% of head width. Canthus rostralis defined; loreal region slightly concave. Triangular interorbital blotch. Tympanum distinct, nearly 60% of eye diameter; black-coloured supratympanic fold well developed, extending from posterior corner of 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 finger lengths IV < I ≈ II < III; fringes or webbing on fingers absent; finger tips rounded, slightly expanded, but without disc; inner metacarpal tubercle elliptical; outer metacarpal tubercle rounded; distinct rounded greyish subarticular tubercles on the underside of fingers; supernumerary tubercles rounded; antebrachial tubercle absent. Elliptical axillary gland. Tibia slightly longer than thigh (TL/THL = 1.01). Relative toe lengths I < II < V < III < IV; toe tips flattened or slightly flattened, with visible expansions (character states C and D, sensu Heyer (1973)); fringes or webbing absent; inner metatarsal tubercle elliptical; outer metatarsal tubercle rounded. Tarsal fold from the inner metatarsal tubercle extends 2/3 of tarsus length. Subarticular tubercles elliptical or rounded, supernumerary tubercles rounded.

Colour of the holotype in life. Snout tip ***Cinnamon-drab*** (cc 50), with a fleshy ridge ***Pale neutral grey*** (cc 296). Blotches on upper and lower lips ***Light sky blue*** (cc 191). Postcommissural gland with melanophores. Tympanum ***Dark carmine*** (cc 61) at its edge and ***Buff*** (cc 5) in the centre. Supratympanic fold ***Vandyke brown*** (cc 281). Thoracic dorsal surface of body ***Prout's brown*** (cc 47); lumbar region ***Cinnamon-drab*** with white-tipped tubercles. Interorbital region ***Sepia*** (cc 286). Flank ***Dark spectrum yellow*** (cc 78). Triangular blotch ***Sepia***. Dorsal surface of forelimbs ***Tawny*** (cc 60) with ***Raw umber*** (cc 280) blotches. Dorsal surface of hind-limbs ***True cinnamon*** (cc 260) with transverse ***Raw umber*** bars. Vertebral stripe in sacral region ***Medium chrome orange*** (cc 75).

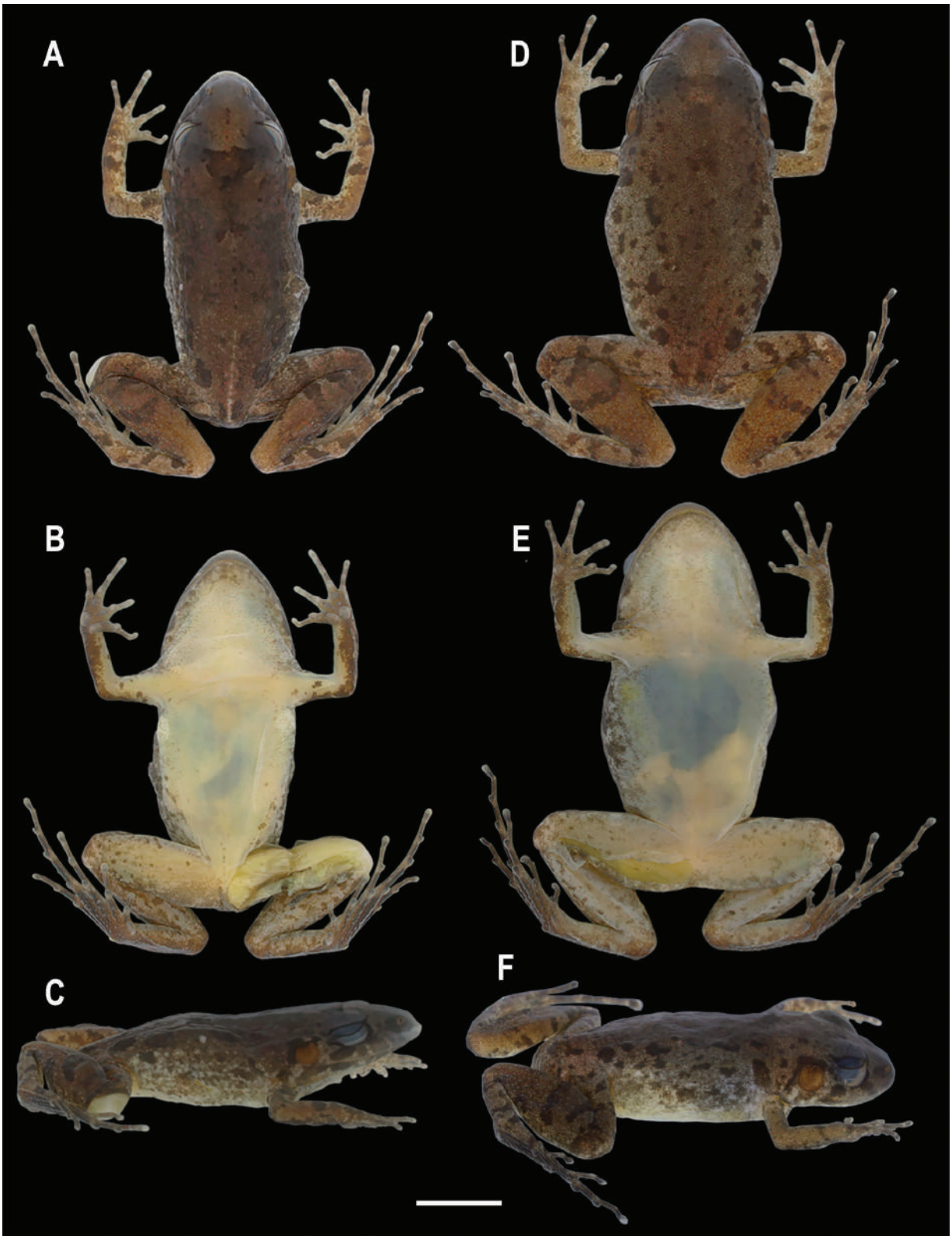

Figure 4. Male holotype and female paratype of *Adenomera albarena*. **A–C.** Male holotype, INPA-H 44867; **D–F.** Female paratype INPA-H 44875. Scale bar: 5 mm.

Paracloacal region and lumbar glands ***Sepia***. Throat ***Pale mauve*** (cc 204) with low density of melanophores around the jaw; belly ***Light buff*** (cc 2) and chest and underside of limbs the same colour as throat. Underside of forearm with ***Dark greyish-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 ***Fuscous*** (cc 283) ventral stripe, which is not present in distal phalanx and toe tip.

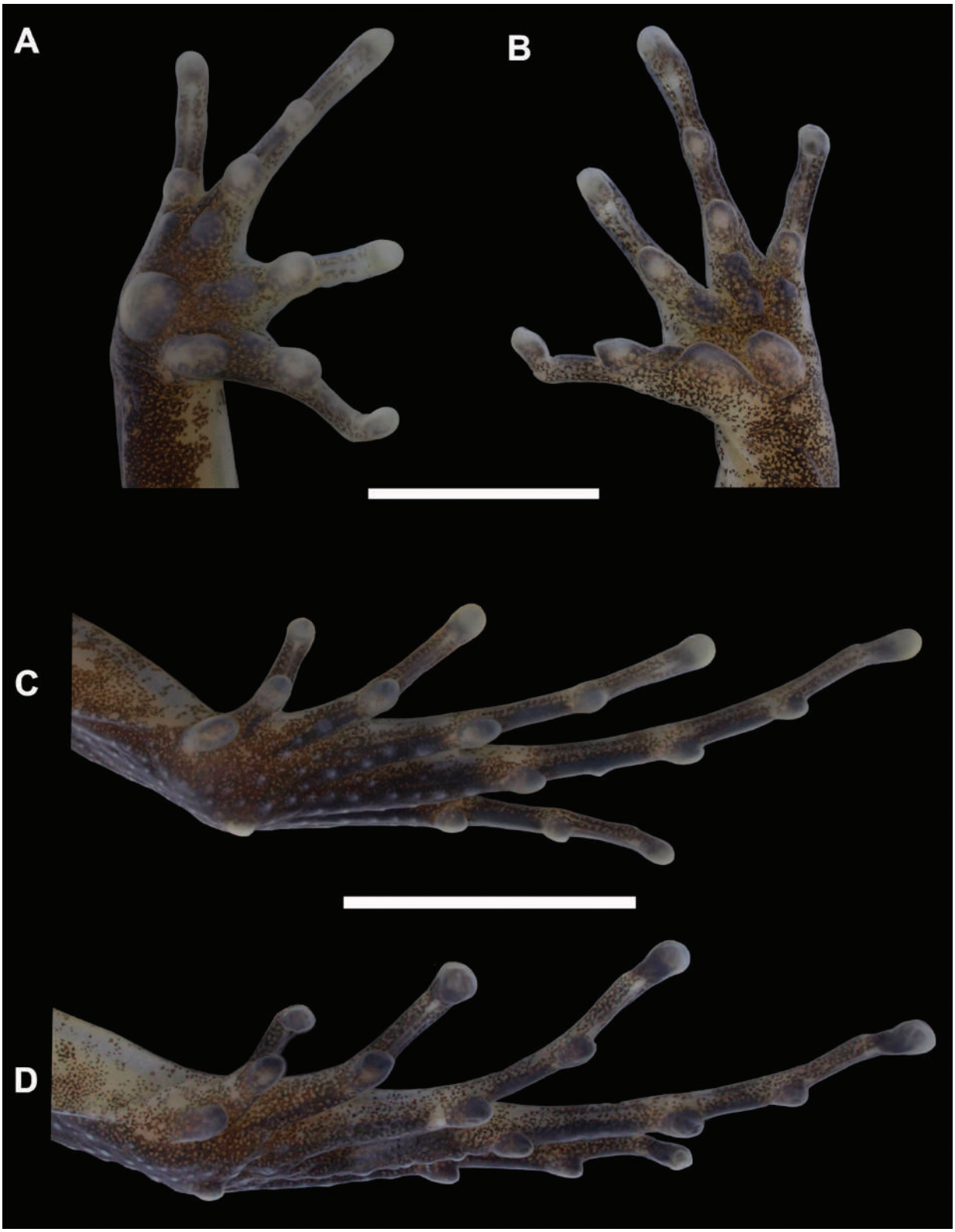

Figure 5. Ventral views of the hands and feet of *Adenomera albarena*. **A, C.** Male holotype INPA-H 44867; **B, D.** Female paratype, INPA-H 44875. Scale bars: 5 mm.

Colour of the holotype in preservative*.** See Figs 4A–C, 5A, C. Iris ***chrome orange (colour code [cc] 74). Snout tip ***Pale neutral grey*** (cc 296), as well as the fleshy ridge. Blotches on upper and lower lips ***Pale neutral grey***. Postcommissural gland with melanophores. Tympanum ***Dark grey*** (cc 45). Supratympanic fold ***Vandyke brown*** (cc 281). 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). Triangular

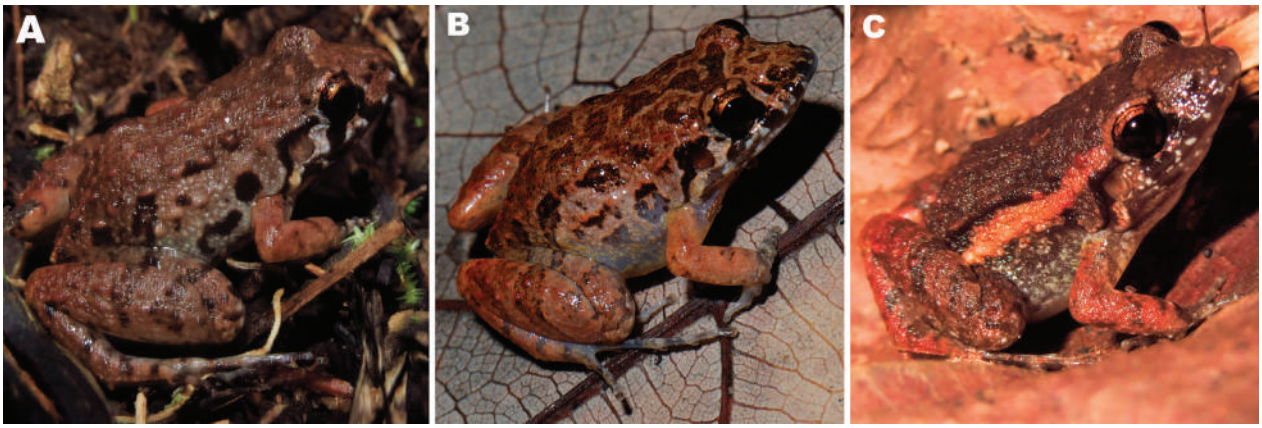

Figure 6. Three dorsal colour patterns of *Adenomera albarena* in life. **A.** Dark blotches few or absent; **B.** Many dark blotches; and C. Dorsolateral stripe. Unvouchered specimens.

interorbital blotch ***Dark greyish-brown*** (cc 284). Dorsal surface of forelimbs ***Pale buff*** (cc 1) with ***Drab*** (cc 19) blotches. Dorsal surface of hind-limbs ***Tawny olive*** (cc 17); transverse bars ***Sepia*** with white-tipped tubercles. Vertebral stripe ***Pale buff*** in sacral region. Paracloacal region and lumbar glands ***Sepia***. 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 nearly solid ***Brownish-olive*** (cc 276) stripe. Palm of hand, sole of foot, digits and subarticular tubercles almost completely covered with melanophores. Stripe in metatarsal and proximal and medial phalanx ***Fuscous*** (cc 283) (Figs 5C, D).

Intraspecific variation. Morphometric variation of the new species is summarised in Table 3. The type series shows three dorsal colour patterns: dark blotches few or absent (Figs 6A, 7A); many dark blotches (Figs 6B, 7D); and a dorsolateral stripe (Figs 6C, 7G). Sixty-eight percent of the type series (including the holotype) has the first pattern, 20% have many dark blotches and only 12% have a dorsolateral stripe. A sacral stripe is present in 64% of specimens. About 52% of paratypes have a toe tip shape in stage D (sensu Heyer (1973)), while 48% have an intermediate shape, stages C or D. All individuals have a triangular mark on the head (Fig. 6), which is less visible in individuals that lack dorsal blotches. Texture of the dorsum varies from rough to smooth, with few to many glandules. Throat and belly show slight variation in melanophore density (Fig. 7B, E, H).

Advertisement call. The advertisement call of *Adenomera albarena* consists of a single note with partially fused pulses. Pulse number varies from 11 to 21; pulse duration from 4 to 23 ms; and pulse repetition rate from 94 to 138 pulses per second. Note duration varies from 100 to 199 ms and note repetition rate from 0.6 to1.2 notes per minute. The fundamental frequency of the note coincides with the first harmonic and 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 4; Fig. 8).

Table 5. Morphometric measurements (mm) of 10 tadpoles of *Adenomera albarena* from Rio Negro Sustainable Development Reserve, Iranduba, Amazonas, Brazil. Trait acronyms are defined in the text; n, sample size.

Traits	Stage 35 (n = 7)	Stage 41 (n = 3)
BH	3.08 ± 0.15 (2.94–3.34)	2.50 ± 0.00 (2.50–2.50)
BL	5.63 ± 0.21 (5.40–6.00)	5.13 ± 0.15 (5.00–5.30)
BW	3.40 ± 0.13 (3.19–3.56)	2.79 ± 0.12 (2.66–2.88)
ED	0.60 ± 0.04 (0.56–0.67)	0.67 ± 0.04 (0.63–0.71)
END	0.38 ± 0.02 (0.35–0.40)	0.40 ± 0.03 (0.38–0.43)
HW	2.77 ± 0.12 (2.58–2.88)	2.58 ± 0.09 (2.50–2.68)
IND	0.76 ± 0.03 (0.70–0.79)	0.75 ± 0.09 (0.67–0.84)
IOD	1.63 ± 0.08 (1.54–1.73)	1.65 ± 0.11 (1.54–1.76)
MTH	2.67 ± 0.12 (2.48–2.80)	2.12 ± 0.04 (2.08–2.16)
ODW	1.03 ± 0.04 (0.96–1.09)	0.89 ± 0.05 (0.85–0.95)
TAL	9.23 ± 0.78 (7.80–10.10)	9.17 ± 0.35 (8.80–9.50)
TL	14.86 ± 0.91 (13.30–16.10)	14.30 ± 0.35 (13.90–14.50)
TMH	1.66 ± 0.07 (1.55–1.75)	1.04 ± 0.07 (1.00–1.13)
TMW	1.25 ± 0.08 (1.14–1.35)	1.18 ± 0.04 (1.14–1.22)
VTL	0.76 ± 0.11 (0.63–0.95)	0.74 ± 0.11 (0.63–0.86)

Tadpole. Body elliptical in dorsal and ventral views, globular in lateral view (Fig. 9A–C); BH 86–96% and 51–58% of BW and BL, respectively (Table 5). Body length 36–41% of TL, with a well-marked constriction in the postorbital region (Fig. 9A) that is more pronounced in dorsal than ventral view and less marked in some tadpoles. Snout rounded in dorsal and lateral views; eye-nostril distance 6–7% of BL. Internarial region convex; internarial distance 44–52% of IOD. Nostrils small, rounded, located and directed anterolaterally; visible in lateral view, poorly visible in dorsal view; closer to snout than to eyes. Border of external nares without fleshy marginal rim; border smooth, slightly below the level of marginal region. Eyes large, diameter 34–39% and 139–190% of IOD and END, respectively; located laterally, but directed anterolaterally. Interorbital region slightly concave; interorbital distance 46–50% of BW. Spiracle very small, single, sinistral; directed posterodorsally, located immediately above the edge of the lateral body surface at the level

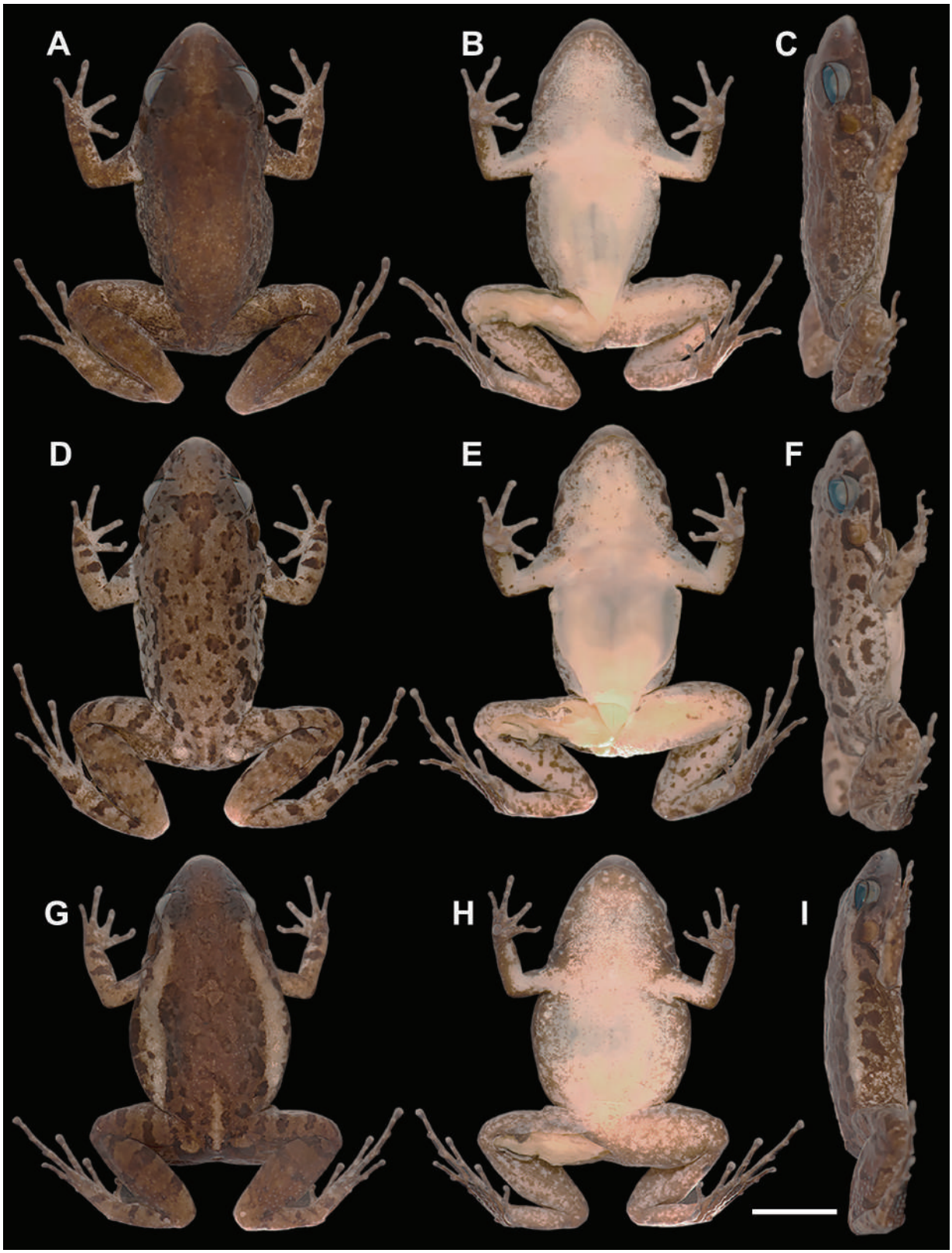

Figure 7. Dorsal, ventral and lateral views of the three colour patterns of *Adenomera albarena* in preservative. Paratypes: **A–C.** (INPA-H 44869, male); **D–F.** (INPA-H 44870, male) and **G–I** (INPA-H 44877, male). Photographs: L. R. Mendonça. Scale bar: 5 mm.

of the hind-limb insertion; poorly visible in dorsal and lateral views. Spiracular opening elliptical; aperture small, narrower than spiracle width, inner wall fused to the body wall. Widely coiled intestines positioned ventrally, perpendicular to main body axis, concealing other internal organs. Vent tube medial with sinistral displacement; comma-shaped, directed upwards; opening very small, rounded, directed dorsally; mostly free

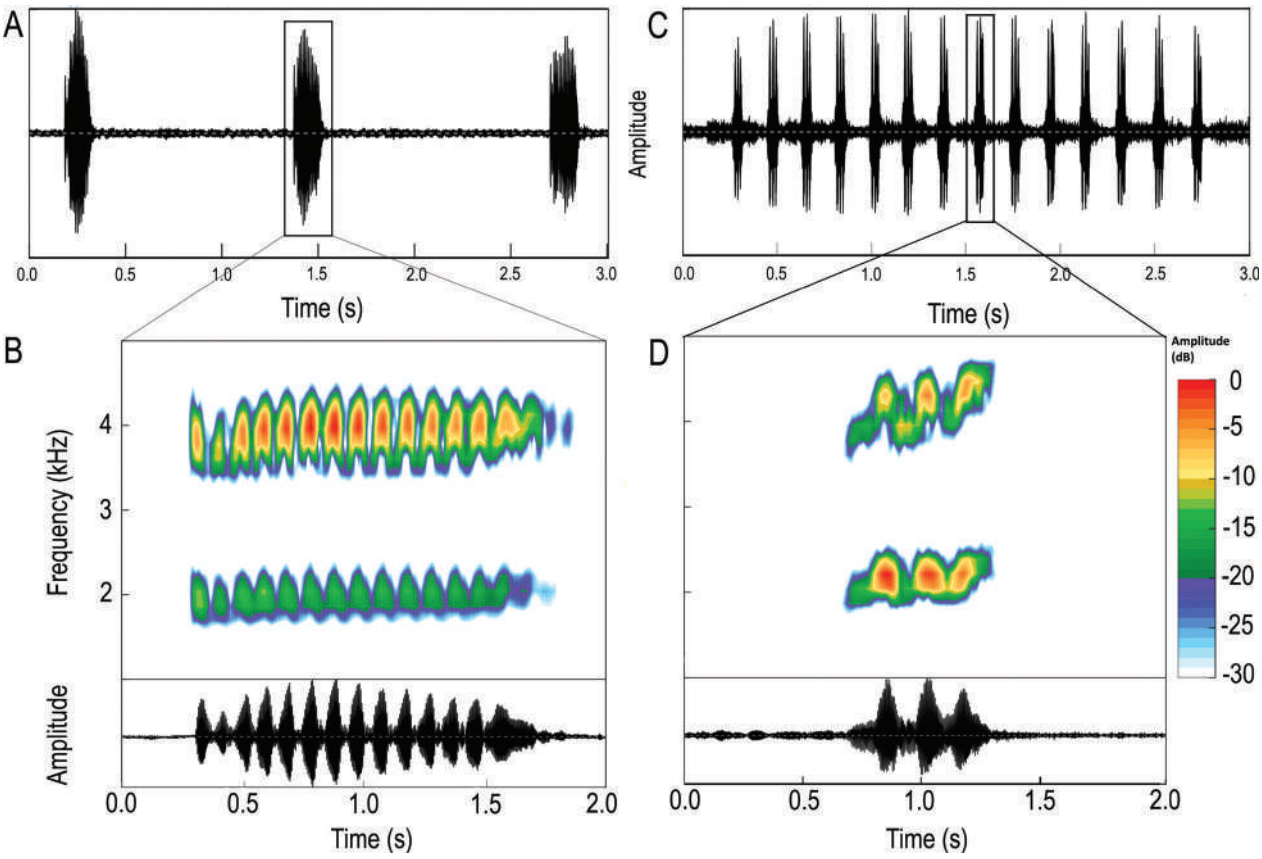

Figure 8. Advertisement call of *Adenomera albarena* (**A, B**) and *A. simonstuarti* sensu stricto (**C, D**). **A, B.** INPA-H 44876 (FNJV 59564), Rio Negro Sustainable Development Reserve, Iranduba, Amazonas, Brazil. **C, D.** INPA-H 44904 (FNJV 59568), Tarauacá, Acre, Brazil.

from the ventral fin, dorsal wall attached only near the body junction. Tail moderately long, length 142–178% and 59–64% of BL and TL, respectively; low, maximum tail height 83–94% of BH; tip rounded. Musculature moderately robust with strongly acuminate tip, which reaches the tail tip; higher than wide near the tail-body junction, tail muscle width 70–79% of TMH; tail muscle height 43–49% of MTH. Dorsal fin external margin slightly convex, originating from posterior third of body; higher than body, slightly higher than ventral fin, with maximum height at its central portion. Ventral fin external margin poorly arched; maximum height at mid-tail; same height as or shorter than body. Oral disc small, ODW 29–33% of BW; unemarginated; located and directed anteroventrally (Fig. 9D). Upper and lower labium present; upper labium with 4–6 short, rounded papillae on lateral region, interleaved by a large medial gap, but arranged in a straight line; posterior labium projected posteriorly, with 3–6 short rounded papillae laterally, interleaved by a medial gap, but arranged in a straight line; anterior gap larger than posterior. Submarginal papillae absent. Jaw sheath keratinised only at the external borders; upper jaw sheath arch-shaped, lower jaw sheath V-shaped. Serrations on each sheath extend its entire length. Labial teeth absent; two labial ridges on the upper labium and three on the lower, formulae 2(2)/3(1).

In preservative, dorsal surface of body brown; anterior half of body darker and with more melanophores than posterior half, with very fine translucent vermiculation on posterior half. Dorsal hind-limbs translucent with numerous melanophores. Tail mostly translucent grey, brown at the tail/body junction; caudal musculature whitish-grey; fins translucent grey; small melanophores on the caudal musculature. Vent tube translucent grey. Ventral surface of body translucent grey with numerous melanophores anteriorly; posterior portion translucent, except for lateral regions, which are light brown.

Distribution, habitat and natural history. *Adenomera albarena* is known only from the white-sand ecosystems between West Negro and the Solimões Rivers, specifically in the RDS Rio Negro and nearby localities, Municipalities of Iranduba and Manacapuru, Amazonas, Brazil, where these ecosystems are dominant (Figs 1, 10A). Two other species of *Adenomera* occur in sympatry: *A. hylaedactyla* and *A. andreae.* Although these species occur in the same region, their habitat is not the same; *A. andreae* mainly inhabits non-flooded forests, while *A. hylaedactyla* occurs in open areas. On the other hand, *Adenomera albarena* inhabits white-sand forests subject to flooding regimes close to streams. At the type locality, *A. andreae* and the new species occur in syntopy at the border between forests subject to flooding regimes and those that do not flood.

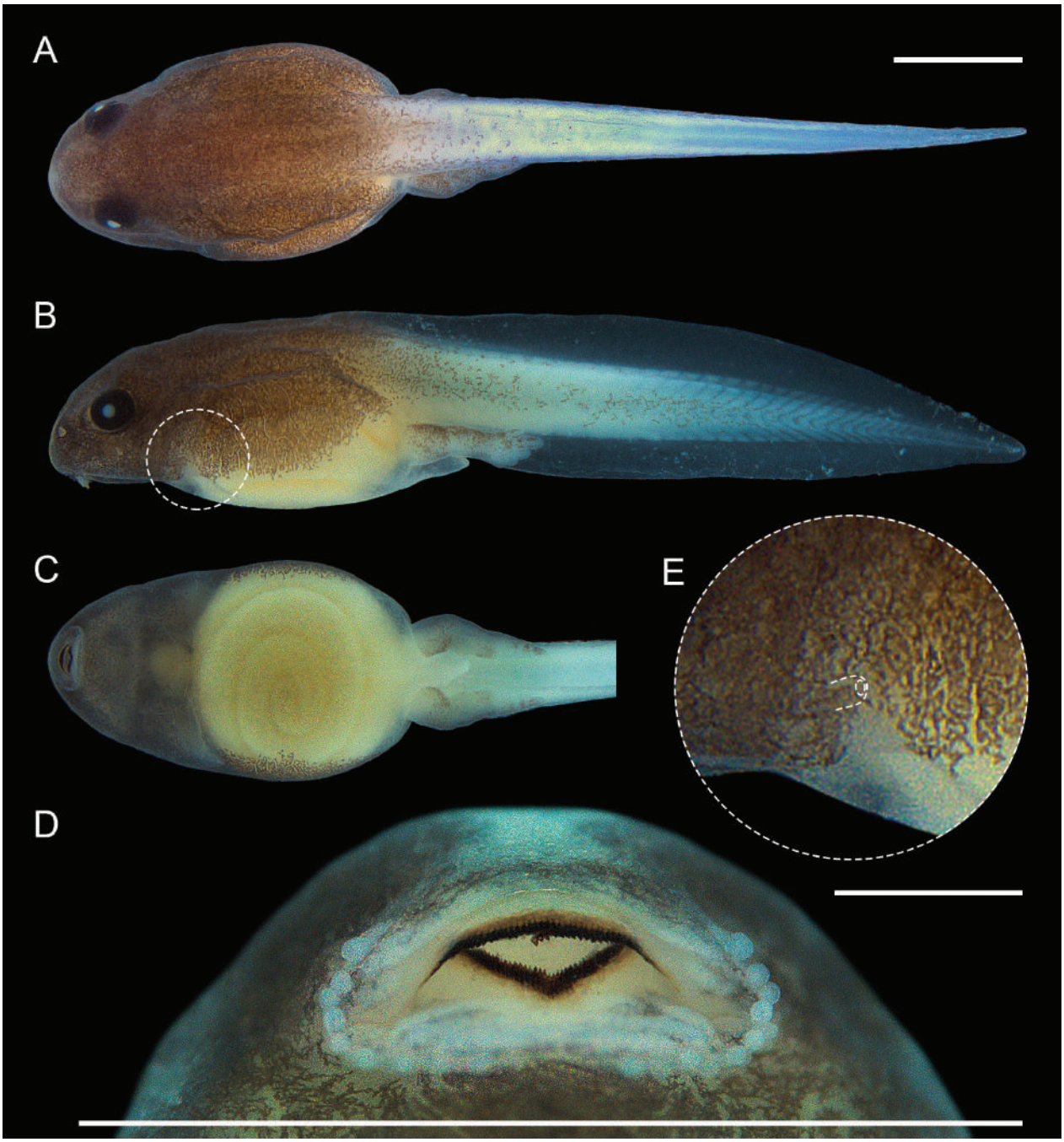

Figure 9. Preserved tadpole of *Adenomera albarena*, Gosner stage 35. Dorsal (**A**), lateral (**B**) and ventral views of the body (**C**) and ventral view of the oral disc (**D**). **E.** Detailed view of the spiracle, with its shape and aperture highlighted by dotted lines. Scale bar: 2 mm (**A–D**); 1 mm (**E**).

Males call from the ground, above or hidden in the leaf litter (Fig. 10B). They start calling at ~ 16:00 h and continue calling until ~ 19:00 h. Isolated individuals sometimes call later, but it is unusual.

Adult males are found easily and juveniles are also not difficult to observe, but females are very secretive (Fig. 10C). Males excavate underground chambers in which foam nests are built and females deposit their eggs (Fig. 10D). The chambers are very difficult to find when they are located under leaf litter amongst the roots of palm trees and ferns.

Conservation. The known geographic distribution of *Adenomera albarena* comprises an area of approximately 150 km^2 within the RDS Rio Negro and nearby localities. Although the species is known only from a small area, it is very common there and likely occurs in other parts of the RDS Rio Negro and, potentially, in the nearby Jaú National Park. Despite its abundance, the new species occurs exclusively in white-sand forests subject to flooding regimes, which are highly vulnerable to anthropogenisation (e.g. from pollution, deforestation, mining, free-ranging livestock, irregular occupation and recreational use

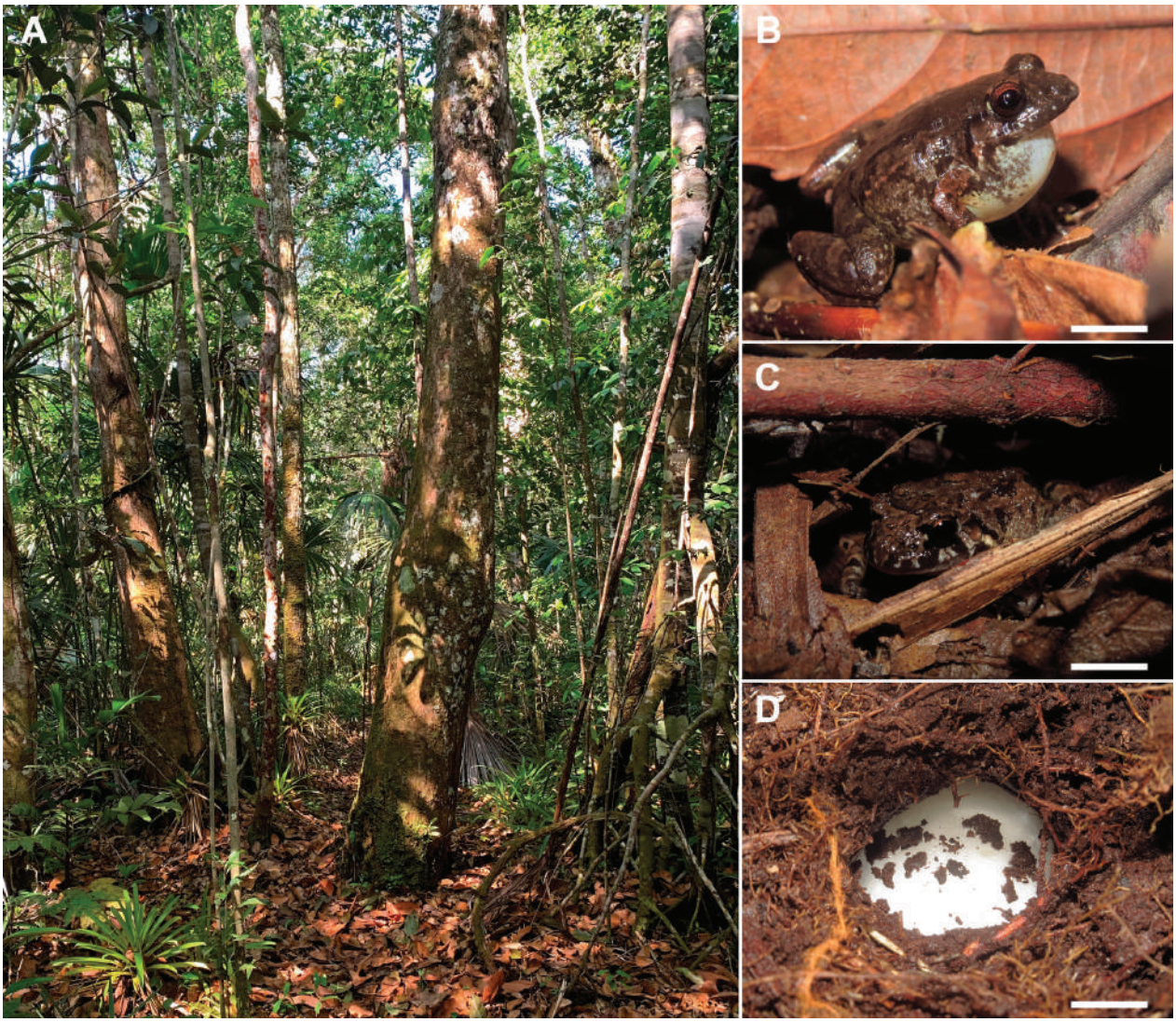

Figure 10. Natural history of *Adenomera albarena*. **A.** Example of the species' habitat; **B.** Unvouchered male vocalising on leaf litter; **C.** Unvouchered female hiding in the leaf litter; **D.** Foam nest, artificially exposed for illustration purpose. Scale bar: ~ 5 mm.

of water). Indeed, several riverine areas in this environment at RDS Rio Negro, including the type locality, have already been impacted by some of these anthropogenic drivers. Long-term monitoring that compares populations between pristine and anthropogenised areas is essential to evaluate whether and how these drivers impact the conservation status of *A. albarena.*

Discussion

For many years, several undescribed species of Amazonian anurans have been erroneously assigned to nominal species that were believed to be geographically widespread (e.g. *Allobates caldwellae* Lima et al. 2020, *Atelopus manauensis* Jorge et al. 2020; *Pristimantis guianensis* Mônico et al. 2022). However, the integration of molecular, acoustic and morphological data has allowed contemporary taxonomists to more accurately delimit and describe such cryptic species (Fouquet et al. 2014; Carvalho et al. 2021; Moraes et al. 2022). Crypsis amongst species of the genus *Adenomera*, combined with their syntopy and sympatry, has been especially challenging, as exemplified by the *A. simonstuarti* species complex. Fouquet et al. (2014) recovered six lineages within this complex (including *A. simonstuarti* sensu stricto). Carvalho et al. (2020b) subsequently considered additional data and delimited eight candidate species. However, none of those candidate species corresponds to the one from RDS Rio Negro that we describe here as a new species. As it is the easternmost taxon within the *A. simonstuarti* complex, this species complex may be more widespread and diverse than previously thought.

Considering the current taxonomic uncertainty of lineages *Adenomera simonstuarti* 1–2 and 4–8, the description of *A. albarena* may introduce taxonomic instability by rendering *A. simonstuarti* as paraphyletic in a few scenarios of species delimitation (e.g. conspecificity of *A. simonstuarti* SS + lineage 1 + lineage 2). In addition to a previous molecular analysis that supports eight candidate species (Carvalho et al. 2020b) and the phenotypic divergence between *A. albarena* and *A. simonstuarti*

SS (present study), Fouquet et al. (2014) document phenotypic divergence between *A. simonstuarti* SS and *A. simonstuarti* 4 (DF 2,830 Hz in *A. simonstuarti* 4 vs. 1,851–2,224 Hz in *A. simonstuarti* SS), which are also distinct from *A. albarena* (DF 3,448–4,349 Hz). Moreover, the slight genetic divergence between *A. albarena* and *A. simonstuarti* 1–2 and the large divergence between them and *A. simonstuarti* SS support the heterospecificity of the two nominal species. A comprehensive taxonomic revision is needed to resolve these taxonomic issues, but it may take decades to complete due to the difficulties in obtaining necessary field samples. On the other hand, solving lineage-by-lineage issues is much less time-consuming and can support conservation planning at the species level across threatened landscapes, such as the white-sand environment (WSE) inhabited by *A. albarena*.

Adenomera albarena is the first species of its genus to be described from a WSE. Although this species is likely a WSE specialist, its known distribution is limited to WSE in the Negro-Solimões interfluve. Other frogs may be specialists in or endemic to WSE in this interfluve, including *Scinax albertinae* (Ferrão et al. 2022), *Pristimantis campinarana* (Mônico et al. 2023) and *Osteocephalus vilarsi* (Ferrão et al. 2019). Moreover, the geographic distributions of two additional candidate species, *Rhinella* aff. *proboscidea* and *Pristimantis* aff. *orcus*, apparently are associated with WSE and limited to the West Negro-Solimões interfluve (unpublished data). The congruence of frog species sharing the same habitat specialisation and pattern of restricted distribution highlights the Jaú Region as an area of endemism within Amazonia (Borges and da Silva 2012).

Two reproductive modes are reported for *Adenomera*: endotrophic tadpoles that complete development entirely in a subterranean foam nest without an exotrophic feeding phase (mode 32, sensu Haddad and Prado (2005)); and exotrophic tadpoles with early developmental stages completed in a subterranean foam nest and later free-living aquatic stages (mode 30, sensu Haddad and Prado (2005)). A spiracle is present in all *Adenomera* with known exotrophic tadpoles (*A. guarani*, *A. saci* and *A. thomei*; Heyer (1973); Almeida and Angulo (2006); Carvalho and Giaretta (2013a); Zaracho and Kokubum (2017); Zaracho et al. (2023)). Amongst species with endotrophic tadpoles, the spiracle is absent in *A. andreae* and *A. hylaedactyla* (Heyer and Silverstone 1969; Kokubum and Sousa 2008; Menin et al. 2009; Menin and Rodrigues 2013) and present in *A. marmorata* (Heyer et al. 1990), *A.* aff. *hylaedactyla* from south-eastern Brazil (Kokubum and Giaretta 2005) and *A. albarena*. The condition of the spiracle differs interspecifically, ranging from a tube free from the body wall in some species to an attached tube in other species. The larval life history and morphological diversity make *Adenomera* an interesting model to investigate the evolution of reproductive features in anurans at small phylogenetic scale. Morphological and behavioural studies of *Adenomera* are urgently required for such evolutionary studies, since tadpoles are known for only a few species.

Amongst the three sympatric species of *Adenomera* living in white-sand environments at RDS Rio Negro, all of them with endotrophic tadpoles, only *A. albarena* inhabits forests subject to flooding and has subterranean foam nests that submerged for at least a few days; flooding habitats are not occupied by *A. andreae* and *A. hylaedactyla*. Hence, the spiracle in *A. albarena* may increase larval survivorship during short flooding periods, enabling the occupancy of flooding habitats by this species. Detailed ecophysiological studies evaluating survival rates in larvae of these three species under distinct flooding conditions are needed to test this hypothesis.

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References

Adeney JM, Christensen NL, Vicentini A, Cohn-Haft M (2016) White-sand ecosystems in Amazonia. Biotropica 48(1): 7–23. https://doi.org/10.1111/btp.12293

Almeida AP, Angulo A (2006) A new species of *Leptodactylus* (Anura: Leptodactylidae) from the state of Espírito Santo, Brazil, with remarks on the systematics of associated populations. Zootaxa 1334(1): 1–25. https://doi.org/10.11646/zootaxa.1334.1.1

Altig R, McDiarmid RW (1999) Body plan: Development and morphology. In: McDiarmid RW, Altig R (Eds) Tadpoles: The Biology of Anuran Larvae. University of Chicago Press, Chicago, IL, 24–51.

Anderson AB (1981) White-sand vegetation of Brazilian Amazonia. Biotropica 13(3): 199–210. https://doi.org/10.2307/2388125

Angulo A, Icochea J (2010) Cryptic species complexes, widespread species and conservation: lessons from Amazonian frogs of the *Leptodactylus marmoratus* group (Anura: Leptodactylidae). Systematics and Biodiversity 8(3): 357–370. https://doi.org/10.1080/14772000.2010.507264

Angulo A, Reichle S (2008) Acoustic signal, species diagnosis, and species concepts: The case of a new cryptic species of *Leptodactylus* (Amphibia, Anura, Leptodactylidae) from the Chapare region, Bolivia. Zoological Journal of the Linnean Society 152(1): 59–77. https://doi.org/10.1111/j.1096-3642.2007.00338.x

Berneck BVM, Costa COR, Garcia PCA (2008) A new species of *Leptodactylus* (Anura: Leptodactylidae) from the Atlantic Forest of São Paulo State, Brazil. Zootaxa 1795(1): 46–56. https://doi.org/10.11646/zootaxa.1795.1.3

Bioacoustics Research Program (2014) Raven Pro: interactive sound analysis software. Version 1.5. Ithaca, New York: The Cornell Lab of Ornithology. https://ravensoundsoftware.com/software/raven-pro [Acessed 05 Jan 2023]

Boistel R, Massary JC, Angulo A (2006) Description of a new species of the genus *Adenomera* (Amphibia, Anura, Leptodactylidae) from French Guiana. Acta Herpetologica 1: 1–14. https://doi.org/10.13128/Acta_Herpetol-1249

Borges SH, da Silva JMC (2012) A new area of endemism for Amazonian birds in the Rio Negro Basin. The Wilson Journal of Ornithology 124(1): 15–23. https://doi.org/10.1676/07-103.1

Borges SH, Cornelius C, Moreira M, Ribas CC, Cohn-Haft M, Capurucho JM, Vargas C, Almeida R (2016) Bird communities in Amazonian white-sand vegetation patches: Effects of landscape configuration and biogeographic context. Biotropica 48(1): 121–131. https://doi.org/10.1111/btp.12296

Capurucho JMG, Cornelius C, Borges SH, Cohn-Haft M, Aleixo A, Metzger JP, Ribas CC (2013) Combining phylogeography and landscape genetics of *Xenopipo atronitens* (Aves: Pipridae), a white sand campina specialist, to understand Pleistocene landscape evolution in Amazonia. Biological Journal of the Linnean Society. Linnean Society of London 110(1): 60–76. https://doi.org/10.1111/bij.12102

Carvalho TR, Giaretta AA (2013a) Bioacoustics reveals two new syntopic species of *Adenomera* Steindachner (Anura: Leptodactylidae: Leptodactylinae) in the Cerrado of central Brazil. Zootaxa 3731(3): 533–551. https://doi.org/10.11646/zootaxa.3731.4.6

Carvalho TR, Giaretta AA (2013b) Taxonomic circumscription of *Adenomera martinezi* (Bokermann, 1956) (Anura: Leptodactylidae: Leptodactylinae) with the recognition of a new cryptic taxon though a bioacoustic approach. Zootaxa 3701(2): 207–237. https://doi.org/10.11646/zootaxa.3701.2.5

Carvalho TR, Angulo A, Kokubum MNC, Barrera DA, de Souza MB, Haddad CFB, Giaretta AA (2019a) A new cryptic species of the *Adenomera andreae* clade from southwestern Amazonia (Anura, Leptodactylidae). Herpetologica 75(3): 233–246. https://doi.org/10.1655/D-18-00049

Carvalho TR, Giaretta AA, Maciel NM, Barrera DA, Aguilar-Puntriano C, Haddad CFB, Kokubum MNC, Menin M (2019b) On the uncertain taxonomic identity of *Adenomera hylaedactyla* (Cope, 1868) and the composite type series of *A. andreae* (Müller, 1923) (Anura, Leptodactylidae). Copeia 107(4): 708–723. https://doi.org/10.1643/CH-19-237

Carvalho TR, Giaretta AA, Angulo A, Haddad CFB, Peloso PLV (2019c) A new Amazonian species of *Adenomera* (Anura: Leptodactylidae) from the Brazilian state of Pará: a tody-tyrant voice in a frog. American Museum Novitates 3919(1): 1–21. https://doi.org/10.1206/3919.1

Carvalho TR, Cassini CS, Taucce PPG, Haddad CFB (2019d) A new, morphologically cryptic species of *Adenomera* closely related to *A. araucaria* from the Atlantic Forest of southern Brazil (Anura, Leptodactylidae). Journal of Herpetology 53(2): 131–143. https://doi.org/10.1670/18-172

Carvalho TR, Angulo A, Barrera DA, Aguilar-Puntriano AC, Haddad CFB (2020a) Hiding in plain sight: A fourth new cryptic species of the *Adenomera andreae* clade (Anura: Leptodactylidae) from southwestern Amazonia. Herpetologica 76(3): 304–314. https://doi.org/10.1655/Herpetologica-D-19-00068.1

Carvalho TR, Moraes LJCL, Angulo A, Werneck FP, Icochea J, Lima AP (2020b) New acoustic and molecular data shed light on the poorly known Amazonian frog *Adenomera simonstuarti* (Leptodactylidae): Implications for distribution and conservation. European Journal of Taxonomy 682(682): 1–18. https://doi.org/10.5852/ejt.2020.682

Carvalho TR, Simões PI, Gagliardi-Urrutia LAG, Rojas-Runjaic FJM, Haddad CFB, Castroviejo-Fisher S (2020c) A new forest-dwelling frog species of the genus *Adenomera* (Leptodactylidae) from northwestern Brazilian Amazonia. Copeia 108(4): 924–937. https://doi.org/10.1643/CH-19-329

Carvalho TR, Moraes LJCL, Lima AP, Fouquet A, Peloso PLV, Pavan D, Drummond LO, Rodrigues MT, Giaretta AA, Gordo M, Neckel-Oliveira S, Haddad CFB (2021) Systematics and historical biogeography of neotropical foam-nesting frogs of the *Adenomera heyeri* clade (Leptodactylidae), with the description of six new Amazonian species. Zoological Journal of the Linnean Society 192(2): 395–433. https://doi.org/10.1093/zoolinnean/zlaa051

Cassini CS, Taucce PPG, Carvalho TR, Fouquet A, Solé M, Haddad CFB, Garcia PCA (2020) One step beyond a broad molecular phylogenetic analysis: Species delimitation of *Adenomera marmorata* Steindachner, 1867 (Anura: Leptodactylidae). PLOS ONE 15(2): e0229324. https://doi.org/10.1371/journal.pone.0229324

Che J, Chen HM, Yang JX, Jin JQ, Jiang K, Yuan ZY, Murphy RW, Zhang YP (2012) Universal COI primers for DNA barcoding amphibians. Molecular Ecology Resources 12(2): 247–258. https://doi.org/10.1111/j.1755-0998.2011.03090.x

De la Riva I (1995) A new reproductive mode for the genus *Adenomera* (Amphibia: Anura: Leptodactylidae): Taxonomic implications for certain Bolivian and Paraguayan populations. Studies on Neotropical Fauna and Environment 30(1): 15–29. https://doi.org/10.1080/01650529509360937

de Sá RO, Grant T, Camargo A, Heyer WR, Ponssa ML, Stanley E (2014) Systematics of the Neotropical genus *Leptodactylus* Fitzinger, 1826 (Anura: Leptodactylidae): phylogeny, the relevance of non-molecular evidence, and species accounts. South American

Journal of Herpetology 9(s1): S1–S128. https://doi.org/10.2994/SAJH-D-13-00022.1

Duellman WE (1970) The hylid frogs of Middle America. Monograph of the Museum of Natural History, University of Kansas 1: 1–428. https://doi.org/10.5962/bhl.title.2835

Ferrão M, Colatreli O, Fraga R, Kaefer IL, Moravec J, Lima AP (2016) High species richness of *Scinax* treefrogs (Hylidae) in a threatened Amazonian landscape revealed by an integrative approach. PLOS ONE 11(11): e0165679. https://doi.org/10.1371/journal.pone.0165679

Ferrão M, Moravec J, Moraes LJCL, Carvalho VT, Gordo M, Lima AP (2019) Rediscovery of *Osteocephalus vilarsi* (Anura: Hylidae): an overlooked but widespread Amazonian spiny-backed treefrog. PeerJ 7: e8160. https://doi.org/10.7717/peerj.8160

Ferrão M, Moravec J, Ferreira AS, Moraes LJCL, Hanken J (2022) A new snouted treefrog of the genus *Scinax* (Anura, Hylidae) from the white-sand forests of central Amazonia. Breviora 573(1): 1–36. https://doi.org/10.3099/0006-9698-573.1.1

Ferreira CAC (2009) Análise comparativa de vegetação lenhosa do ecossistema de campina na Amazônia brasileira. 2009. 277 f. Tese (Doutorado em Biologia Tropical e Recursos Naturais) - Convênio INPA e UFAM, Manaus.

Fine PVA, Baraloto C (2016) Habitat endemism in white-sand forests: Insights into the mechanisms of lineage diversification and community assembly of the Neotropical flora. Biotropica 48(1): 24–33. https://doi.org/10.1111/btp.12301

Fouquet A, Cassini CS, Haddad CFB, Pech N, Rodrigues MT (2014) Species delimitation, patterns of diversification and historical biogeography of the Neotropical frog genus *Adenomera* (Anura, Leptodactylidae). Journal of Biogeography 41(5): 855–870. https://doi.org/10.1111/jbi.12250

Fraga R, Souza E, Santos-Jr AP, Kawashita-Ribeiro RA (2018) Notes on the rare *Mastigodryas moratoi* (Serpentes: Colubridae) in the Brazilian Amazon white-sand forests. Phyllomedusa 17(2): 299–302. https://doi.org/10.11606/issn.2316-9079.v17i2p299-302

Frost DR (2024) Amphibian species of the world: an online reference. Version 6.2 (Accessed on 12 Jan. 2024). Electronic database accessible at https://amphibiansoftheworld.amnh.org/index.php. American Museum of Natural History, New York, USA. https://doi.org/10.5531/db.vz.0001

Frost DR, Grant T, Faivovich J, Bain RH, Haas A, Haddad CFB, de Sá RO, Channing A, Wilkinson M, Donnellan SC, Raxworthy CJ, Campbell JA, Blotto BL, Moler P, Drewes RC, Nussbaum RA, Lynch JD, Green DM, Wheeler WC (2006) The amphibian tree of life. Bulletin of the American Museum of Natural History 297: 1–291. https://doi.org/10.1206/0003-0090(2006)297[0001:TATOL]2.0.CO;2

Gonella PM, Barbosa-Silva RG, Fleischmann AS, Zappi DC, Baleeiro PC, Andrino CO (2020) Hidden biodiversity of Amazonian white-sand ecosystems: Two distinctive new species of *Utricularia* (Lentibulariaceae) from Pará, Brazil. PhytoKeys 169: 75–98. https://doi.org/10.3897/phytokeys.169.57626

Gosner KL (1960) A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16: 183–190. https://www.jstor.org/stable/3890061

Haddad CFB, Prado CPA (2005) Reproductive modes in frogs and their unexpected diversity in the Atlantic Forest of Brazil. Bioscience 55(3): 207–217. https://doi.org/10.1641/0006-3568(2005)055[0207:RMIFAT]2.0.CO;2

Heyer WR (1973) Systematics of the *marmoratus* group of the frog genus *Leptodactylus* (Amphibia, Leptodactylidae). Contributions in Science, Los Angeles County Museum of Natural History 251: 1–50. https://doi.org/10.5962/p.241234

Heyer WR (1974) Relationships of the *marmoratus* species group (Amphibia, Leptodactylidae) within the subfamily Leptodactylinae. Contributions in Science, Los Angeles County Museum of Natural History 253: 1–45. https://doi.org/10.5962/p.241236

Heyer WR (1975) *Adenomera lutzi* (Amphibia: Leptodactylidae), a new species of frog from Guyana. Proceedings of the Biological Society of Washington 88: 315–318. https://biostor.org/reference/65701

Heyer WR (1998) The relationships of *Leptodactylus diedrus* (Anura, Leptodactylidae). Alytes 16: 1–24.

Heyer WR, Silverstone PA (1969) The larva of *Leptodactylus hylaedactylus* (Amphibia, Leptodactylidae). Fieldiana. Zoology 51: 141–145.

Heyer WR, Rand AS, Cruz CAG, Peixoto OL, Nelson CE (1990) Frogs of Boraceia. Arquivos de Zoologia 31: 231–410.

Hoang DT, Chernomor O, Haeseler AV, Minh BQ, Vinh LS (2018) UFBoot2: Improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35(2): 518–522. https://doi.org/10.1093/molbev/msx281

Jorge RF, Ferrão M, Lima AP (2020) Out of bound: A new threatened harlequin toad (Bufonidae, *Atelopus*) from the outer borders of the Guiana Shield in central Amazonia described through integrative taxonomy. Diversity 12(310): 1–25. https://doi.org/10.3390/d12080310

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28: 1647–1649. https://doi.org/10.1093/bioinformatics/bts199

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16(2): 111–120. https://doi.org/10.1007/BF01731581

Köhler G (2012) Color catalogue for field biologists. Herpeton. [ISBN9783936180404]

Köhler J, Jansen M, Rodriguez A, Kok PJR, Toledo LP, Emmrich M, Glaw F, Haddad CFB, Rödel MO, Vences M (2017) The use of bioacoustics in anuran taxonomy: theory, terminology, methods and recommendations for best practice. Zootaxa 4251: 1–124. https://doi.org/10.11646/zootaxa.4251.1.1

Kok PJR, Kokubum MNC, MacCulloch RD, Lathrop A (2007) Morphological variation in *Leptodactylus lutzi* (Anura, Leptodactylidae) with description of its advertisement call and notes on its courtship behavior. Phyllomedusa 6(1): 45–60. https://doi.org/10.11606/issn.2316-9079.v6i1p45-60

Kokubum MNC, Giaretta AA (2005) Reproductive ecology and behaviour of a species of *Adenomera* (Anura, Leptodactylidae) with endotrophic tadpoles: Systematic implications. Journal of Natural History 39(20): 1745–1758. https://doi.org/10.1080/00222930400021515

Kokubum MNC, Sousa MB (2008) Reproductive ecology of *Leptodactylus* aff *hylaedactylus* (Anura, Leptodactylidae) from an open area in northern Brazil. South American Journal of Herpetology 3(1): 15–21. https://doi.org/10.2994/1808-9798(2008)3[15:REOLAH]2.0.CO;2

Kwet A (2007) Bioacoustic variation in the genus *Adenomera* in southern Brazil, with revalidation of *Leptodactylus nanus* Müller, 1922 (Anura: Leptodactylidae). Zoologische Reihe 83: 56–68. https://doi.org/10.1002/mmnz.200600027

Kwet A, Angulo A (2002) A new species of *Adenomera* (Anura, Leptodactylidae) from the Araucaria forest of Rio Grande do Sul (Brazil), with comments on the systematic status of southern populations of the genus. Alytes 20(1–2): 28–43.

Kwet A, Steiner J, Zillikens A (2009) A new species of *Adenomera* (Amphibia: Anura: Leptodactylidae) from the Atlantic rain forest in Santa Catarina, southern Brazil. Studies on Neotropical Fauna and Environment 44(2): 93–107. https://doi.org/10.1080/01650520902901659

Lamarre GPD, Amoretti S, Baraloto C, Bénéluz F, Mesones I, Fine PV (2016) Phylogenetic overdispersion in Lepidoptera communities of Amazonian white-sand forests. Biotropica 48(1): 101–109. https://doi.org/10.1111/btp.12294

Lanfear R, Frandsen PB, Wright AM, Senfeld T, Calcott B (2017) PartitionFinder 2: new methods for selecting partitioned models of Evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34: 772–773. https://doi.org/10.1093/molbev/msw260

Lavilla EO, Scrocchi GJ (1986) Mofometría laval de los géneros de Telmatobiinae (Anura: Leptodactylidae) de Argentina y Chile. Physis (Rio de Janeiro, Brazil) 44: 39–43.

Ligges U, Krey S, Mersmann O, Schnackenberg S (2017) tuneR: Analysis of Music and Speech, Version 1.3.2. https://CRAN.R-project.org/package=tuneR [Accessed on 20 November 2023]

Lima AP, Simões PI, Kaefer IL (2015) A new species of *Allobates* (Anura: Aromobatidae) from Parque Nacional da Amazônia, Pará State, Brazil. Zootaxa 3980(4): 501–525. https://doi.org/10.11646/zootaxa.3980.4.3

Lima AP, Ferrão M, Silva DH (2020) Not as widespread as thought: Integrative taxonomy reveals cryptic diversity in the Amazonian nurse frog *Allobates tinae* Melo-Sampaio, Oliveira & Prates, 2018 and description of a new species. Journal of Zoological Systematics and Evolutionary Research 58(4): 1173–1194. https://doi.org/10.1111/jzs.12406

Lins AC, Magalhaes RF, Costa RN, Brandão RA, Py-Daniel TR, Miranda NE, Maciel NM, Nomura F, Pezzuti TL (2018) The larvae of two species of *Bokermannohyla* (Anura, Hylidae, Cophomantini) endemic to the highlands of central Brazil. Zootaxa 4527(4): 501–520. https://doi.org/10.11646/zootaxa.4527.4.3

Lutz A (1930) Segunda memoria sobre especies brasileiras do genero *Leptodactylus*, incluindo outras aliadas. Memorias do Instituto Oswaldo Cruz 23(1): 1–59. https://doi.org/10.1590/S0074-02761930000100001

Menin M, Rodrigues DJ (2013) The terrestrial tadpole of *Leptodactylus andreae* (Anura, Leptodactylidae), a member of the *Leptodactylus marmoratus* species group. Zootaxa 3683(1): 92–94. https://doi.org/10.11646/zootaxa.3683.1.7

Menin M, Almeida AP, Kokubum MNC (2009) Reproductive aspects of *Leptodactylus hylaedactylus* (Anura: Leptodactylidae), a member of the *Leptodactylus marmoratus* species group, with a description of tadpoles and calls. Journal of Natural History 43: 2257–2270. https://doi.org/10.1080/00222930903097707

Mônico AT, Ferrão M, Chaparro JC, Fouquet A, Lima AP (2022) A new species of rain frog (Anura: Strabomantidae: *Pristimantis*) from the Guiana Shield and amended diagnosis of *P. ockendeni* (Boulenger, 1912). Vertebrate Zoology 72: 1035–1065. https://doi.org/10.3897/vz.72.e90435

Mônico AT, Ferrão M, Moravec J, Fouquet A, Lima AP (2023) A new species of *Pristimantis* (Anura: Strabomantidae) from white-sand forests of central Amazonia, Brazil. PeerJ 11: e15399. https://doi.org/10.7717/peerj.15399

Moraes LJCL, Werneck FP, Réjaud A, Rodrigues MT, Prates I, Glaw F, Kok PJR, Ron SR, Chaparro JC, Osorno-Muñoz M, Vechio FD, Recoden RS, Marques-Souza S, Rojas RR, Demay L, Hrbek T, Fouquet A (2022) Diversification of tiny toads (Bufonidae: *Amazophrynella*) sheds light on ancient landscape dynamism in Amazonia. Biological Journal of the Linnean Society. Linnean Society of London 136(1): 1–17. https://doi.org/10.1093/biolinnean/blac006

Nguyen LT, Schmidt AH, von Haeseler A, Minh BQ (2015) IQ-TREE: A fast and effective stochastic algorithm for estimating Maximum-Likelihood phylogenies. Molecular Biology and Evolution 32(1): 268–274. https://doi.org/10.1093/molbev/msu300

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2020) vegan: Community Ecology Package. R package version 2.5-7. https://CRAN.R-project.org/package=vegan

Parker HW (1932) XXXVII.—The systematic status of some frogs in the Vienna Museum. Annals & Magazine of Natural History 10(58): 341–344. https://doi.org/10.1080/00222933208673582

Pyron RA, Wiens JJ (2011) A large-scale phylogeny of Amphibia including over 2800 species, and a revised classification of extant frogs, salamanders, and caecilians. Molecular Phylogenetics and Evolution 61(2): 543–583. https://doi.org/10.1016/j.ympev.2011.06.012

R Core Team (2021) A language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. http://www.r-project.org [Acessed 25 Nov. 2022]

Schulze A, Jansen M, Köhler G (2015) Tadpole diversity of Bolivia's lowland anuran communities: Molecular identification, morphological characterization, and ecological assignment. Zootaxa 4016(1): 1–111. https://doi.org/10.11646/zootaxa.4016.1.1

Steindachner F (1867) Reise der österreichischen Fregatte Novara um die Erde in den Jahren 1857, 1858, 1859 unter den Befehlen des Commodore B. von Wüllerstorf-Urbair. Pt. 9, Bd. 1, Abt. 4, Zoologischer Theil. Amphibien. Wien: K. K. Hof- und Staatsdruckerei.

Sueur J, Aubin T, Simonis C (2008) Seewave, a free modular tool for sound analysis and synthesis. Bioacoustics 18: 213–226. https://doi.org/10.1080/09524622.2008.9753600

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Molecular Biology and Evolution 30(12): 2725–2729. https://doi.org/10.1093/molbev/mst197

Trifinopoulos J, Nguyen LT, von Haeseler A, Minh BQ (2016) W-IQ-TREE: A fast online phylogenetic tool for maximum likelihood analysis. Nucleic Acids Research 44(W1): 232–235. https://doi.org/10.1093/nar/gkw256

Vacher J-P, Chave J, Ficetola FG, Sommeria-Klein G, Tao S, Thébaud C, Blanc M, Camacho A, Cassimiro J, Colston TJ, Dewynter M, Ernst R, Gaucher P, Gomes JO, Jairam R, Kok PJR, Lima JD, Martinez Q, Marty C, Noonan BP, Nunes PMS, Ouboter P, Recoder R, Rodrigues MT, Snyder A, Marques-Souza S, Fouquet A (2020) Large scale DNA-based survey of frogs in Amazonia suggests a vast underestimation of species richness and endemism. Journal of Biogeography 47(8): 1781–1791. https://doi.org/10.1111/jbi.13847

Vicentini A (2016) The evolutionary history of *Pagamea* (Rubiaceae), a white-sand specialist lineage in tropical South America. Biotropica 48(1): 58–69. https://doi.org/10.1111/btp.12295

Watters JL, Cummings ST, Flanagan RL, Siler CD (2016) Review of morphometric measurements used in anuran species descriptions and recommendations for a standardized approach. Zootaxa 4072(4): 477–495. https://doi.org/10.11646/zootaxa.4072.4.6

Zaracho VH, Kokubum MNC (2017) Reproduction and larval morphology of *Adenomera diptyx* (Anura: Leptodactylidae) from the Argentinean humid Chaco and Brazilian Pantanal. Salamandra (Frankfurt) 53: 1–9.

Zaracho VH, Lavilla EO, Carvalho TR, Motte M, Basso NG (2023) Redescription of *Adenomera diptyx* (Boettger, 1885) (Anura, Leptodactylidae) and description of a closely related new species. European Journal of Taxonomy 888: 1–45. https://doi.org/10.5852/ejt.2023.888.2205

Appendix 1

Material examined.

***Adenomera simonstuarti*.** BRAZIL: ACRE: Tarauacá (ZUEC-AMP 25698–700; INPA-H 40967, 44903–12; MPEG 44653–55).

Appendix 2

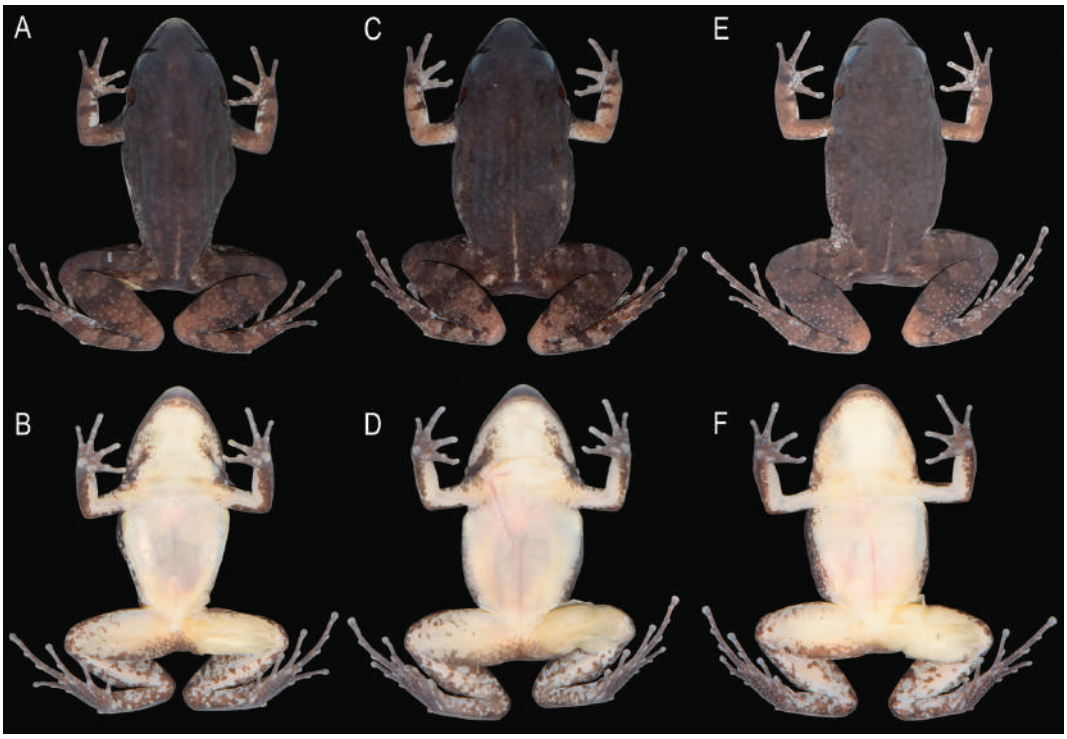

Figure A1. *Adenomera simonstuarti* from the Municipality of Tarauacá, State of Acre, Brazil. Dorsal and ventral views of males (**A–D**) and females (**E–F**). **A, B.** INPA-H 44905, SVL 25.2 mm; **C, D** INPA-H 44912, SVL 24.5 mm; **E, F.** INPA-H 44909, SVL 23.0 mm. Photographs: L. R. Mendonça.

Supplementary material 1

Additional information

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Data type: xlsx

Explanation note: **table S1.** Morphometric raw data. **table S2.** Bioacoustic raw data. **table S3.** dataset containing sequences retrieved from GenBank. **table S4.** minimum–maximum values of genetic distances.

Link: https://doi.org/10.3897/zse.100.110133.suppl1