A New Cryptic Species of the Adenomera andreae Clade from Southwestern Amazonia (Anura, Leptodactylidae)

Authors: Thiago R. De Carvalho, Ariadne Angulo, Marcelo N.C. Kokubum, Diego A. Barrera, Moisés B. De Souza, et. al.

Source: Herpetologica, 75(3) : 233-246

Published By: The Herpetologists' League

URL: https://doi.org/10.1655/D-18-00049

Herpetologica, 75(3), 2019, 233–246

A New Cryptic Species of the *Adenomera andreae* Clade from Southwestern Amazonia (Anura, Leptodactylidae)

Thiago R. de Carvalho[1,6,7], Ariadne Angulo[2], Marcelo N.C. Kokubum[3], Diego A. Barrera[4], Moisés B. de Souza[5], Célio F.B. Haddad[1], and Ariovaldo A. Giaretta[6]

[1] Laboratório de Herpetologia, Departamento de Zoologia e Centro de Aquicultura (CAUNESP), Universidade Estadual Paulista, Rio Claro, SP, Brazil
[2] IUCN SSC Amphibian Specialist Group, Toronto, Canada
[3] Laboratório de Herpetologia, Unidade Acadêmica de Ciências Biológicas, Universidade Federal de Campina Grande, Patos, PB, Brazil
[4] Departamento de Herpetología, (MUSM), Lima, Peru
[5] Centro de Ciências Biológicas e da Natureza, Universidade Federal do Acre, Rio Branco, AC, Brazil
[6] Laboratório de Anuros Neotropicais. Faculdade de Ciências Integradas do Pontal (FACIP), Universidade Federal de Uberlândia, Ituiutaba, MG, Brazil

Abstract: We describe a new Amazonian species of *Adenomera* that corresponds to one of the acoustic signals and morphs from Tambopata National Reserve (*Adenomera* "Forest Call I") and to a confirmed candidate species (*Adenomera* sp. E) in the most recently published phylogeny for the genus. The new species is distinguished from all 19 described congeners by its unique advertisement call, consisting of a single multipulsed note, formed by 22–35 partly fused pulses (greatest pulse number recorded for the genus among species with partly fused notes). The new species is further distinguished from most congeners in having toe tips expanded into small discs. Its distribution is associated with the Amazonian region encompassing western Brazil (state of Acre), southeastern Peru, and north-central Bolivia.

Key words: Amazon rain forest; Bioacoustics; Biodiversity; Integrative taxonomy; Leptodactylinae; Tambopata

Leptodactylid frogs of the genus *Adenomera* are widely distributed in South America east of the Andes. The genus is currently comprised of 19 described species (Carvalho et al. 2019), in addition to the molecular-based lineages (candidate species) of Fouquet et al. (2014). For *Adenomera*, vocalizations are informative in species identification and reliable evidence for the recognition and resolution of the intricate taxonomy of clades comprising complexes of morphologically cryptic species subsumed under valid nominal species (Kwet et al. 2009; Angulo and Icochea 2010; Carvalho and Giaretta 2013a; Carvalho et al. 2019).

The *Adenomera andreae* clade is restricted to Amazonia and comprises two nominal species and four candidate lineages (sensu Fouquet et al. 2014) of forest-dwelling frogs. *Adenomera andreae* was described from eastern Amazonia in the Brazilian state of Pará (Müller 1923) and is widely distributed throughout Amazonian forests (Fouquet et al. 2014). The other nominal species, *A. simonstuarti*, was described from Camisea, in the Peruvian region of Cusco (Angulo and Icochea 2010). All four candidate species assigned to the *A. andreae* clade (species C, D, E, and T) were found in central or southeastern Peru. *Adenomera* sp. D and *Adenomera* sp. T have single records in Peru and have no known associated call data, so both are considered to be unconfirmed candidate species. On the other hand, *Adenomera* sp. C and *Adenomera* sp. E were found in some localities in Peru and adjacent countries (Bolivia and Brazil), and have associated call data. These were indicated as confirmed candidate species (Fouquet et al. 2014).

Angulo et al. (2003) described four distinct call patterns for *Adenomera* species from an Amazonian region in southeastern Peru (Tambopata National Reserve), referred to as Forest Calls I to III, plus *A. hylaedactyla* (open-formation species), and associated them with morphotypes. Later, Fouquet et al. (2014) assigned the candidate species *Adenomera* sp. E and *Adenomera* sp. C to two taxonomic units of Angulo et al. (2003): *Adenomera* Forest Call Types I and II, respectively. Here, *Adenomera* Forest Call I is described as a new species of the *A. andreae* clade from southwestern Brazilian and Peruvian Amazonia on the basis of morphological, acoustic, and molecular evidence. On the basis of mitochondrial deoxyribonucleic acid (mtDNA) sequences from type specimens, the new species corresponds to *Adenomera* sp. E of Fouquet et al. (2014).

Material and Methods

Study Area and Institutional Acronyms

We conducted fieldwork over a span of 2 yr: in 2017, at Parque Zoobotânico (9.955752°S, 67.870476°W; ~165 m above sea level [a.s.l.]; in all cases datum = WGS84), a forest fragment and protected property of Universidade Federal do Acre (UFAC), municipality of Rio Branco, state of Acre, northwestern Brazil; in 2018, at the Tambopata National Reserve, within the limits of Explorer's Inn Lodge (12.83782°S, 69.29551°W; 242 m a.s.l.), district and province of Tambopata, region of Madre de Dios, southeastern Peru. Type specimens were deposited in the following amphibian collections in Brazil: Universidade Federal de Uberlândia, Minas Gerais (AAG-UFU), Universidade Estadual Paulista, São Paulo (CFBH), and Universidade Estadual de Campinas, São Paulo (ZUEC). We examined multiple other specimens that had been collected in Brazil and Peru (Appendix I). Institutional abbreviations followed Sabaj (2016). For those not included there, abbreviations are Coleção Zoológica Paulo Bührnheim, Universidade Federal do Amazonas, Manaus, Amazonas, Brazil (CZPB-AA) and the herpetological collection of the Universidade Federal de Campinas Grande, in Patos, Paraíba, Brazil (LHUFCG).

[7] Correspondence: e-mail, thiago_decarvalho@yahoo.com.br

Morphology

We measured the following morphometric traits using digital calipers (±0.1 mm): snout–vent length (SVL), thigh length (THL), tibia length (TL), foot length (FL). Other morphometric traits were measured using an ocular micrometer (±0.1 mm) fitted to a stereomicroscope: hand length (HAL), head length (HL), head width (HW), eye diameter (ED), tympanum diameter (TD), eye–nostril distance (EN), and internarial distance (IND). We followed the definitions and terminology of Watters et al. (2016) for eight morphometric measurements (SVL, THL, TL, FL, ED, TD, EN, IND). Three measurements were taken as follows: HAL, as the distance between the base of inner metacarpal tubercle to the tip of finger III; HL, as the distance between the tip of the snout and the midpoint (center) of the tympanum; and HW, as the distance between the midpoint of tympani. Snout shape was assessed according to Heyer et al. (1990). Toe tip development (character states) followed Heyer (1973): specifically, a species bears toe discs (character state D) when the toe tips are fully expanded into small discs. We classified a species into character state D when toes II–IV were fully expanded into small discs, given that tips of toes I and V vary from unexpanded to partially or fully expanded.

Sound Recordings and Acoustic Analysis

Calls from Parque Zoobotânico (Brazil) were recorded by TRC and MNCK using either a Marantz PMD 670 digital recorder (sampling rate = 44.1 kHz; sample size = 16 bits) and a Sennheiser K6/ME67 unidirectional microphone, or a Sony-MD minidisc recorder (sampling rate = 22.05 kHz; sample size = 8 bits) and an Audiotechnica ATR55 condenser shotgun microphone. Calls from the Tambopata National Reserve (Peru) were recorded by TRC using a Marantz PMD 671 digital recorder (sampling rate = 44.1–48.0 kHz; sample size = 16 bits) and Sennheiser K6/ME67 unidirectional microphones. Recordings were stored as monochannel uncompressed wave files. Sound files were deposited in the acoustic repositories of AAG-UFU and CFBH collections (Appendix II). In addition, calls from Angulo et al. (2003) recorded from the Tambopata National Reserve and adjacent buffer zone were reanalyzed. These were obtained either from Macaulay Library collection (Cornell Lab of Ornithology) or recorded by AA; voucher specimens are housed at the National Museum of Natural History, Smithsonian Institution (USNM), Washington, DC, USA, and the Royal Ontario Museum (ROM), Toronto, Canada. Acoustic analyses were conducted in Soundruler (Gridi-Papp 2007), built as a package interfacing with Matlab scripts (Matlab 2004) through automated procedures that allow for unbiased quantification of acoustic traits. Grand means (±1 SD) for pulse duration were obtained from mean values of pulse duration for each multipulsed call. Parameters were set as follows: fast Fourier transform (FFT) size = 1024 samples, FFT overlap = 90%, window type = Hanning, contrast = 70%. Settings for automated recognition of pulses (in sample sizes) were defined as follows: pulse detection (smoothing = 250, resolution = 1); pulse delineation (smooth factor = 1, smoothing = 25, resolution = 1). Critical amplitude ratio = 1.0. Note rate (per minute) was quantified manually in Audacity v2.1.1 (Audacity Team 2017). A 400-Hz high-pass filter and a 6000-Hz low-pass filter were applied to sound files in Soundruler before conducting the acoustic analyses to reduce background noise. Sound figures were produced using seewave v2.1.0 (Sueur et al. 2008) and tuneR v1.3.2 (Ligges et al. 2017) in R v3.5.0 (R Core Team 2018). Settings for these were: window Hann, FFT size = 256 samples, FFT overlap = 90%; the intensity of frequency components is indicated by their darkness in a relative 36-dB scale. Acoustic definitions and terminology are given in Appendix III.

Molecular Data Acquisition and Phylogenetic Analysis

A data set of mtDNA cytochrome *c* oxidase subunit I (*COI*) sequences was compiled from GenBank for all *Adenomera* individuals reported by Fouquet et al. (2014: Appendix S1a), with the addition of new sequences for four type specimens of the new species (holotype = CFBH 43562; paratypes = AAG-UFU 5862–5864; GenBank accession numbers MK659563–MK659566, respectively). The data of Fouquet et al. (2014) are the most recent and comprehensive currently available for the genus, as they include sequences of all 19 described species of *Adenomera*, as well as undescribed candidate species. The following species (and corresponding GenBank accession nos.) were included as outgroup taxa: *Leptodactylus rhodomystax* (KC603993), *Hydrolaetare* cf. *caparu* (KC603988), *Lithodytes lineatus* (KC604003), *Physalaemus nattereri* (KC603984), and rooted on *Rupirana cardosoi* (KC603987).

For the new sequences from type specimens, genomic DNA was extracted from ethanol-preserved muscle tissue samples using a standard ammonium acetate extraction protocol adapted from Maniatis et al. (1982). Primers used in amplification (*COI* gene) and polymerase chain reaction enzymatic reaction conditions follow those of Lyra et al. (2017). The resulting amplified fragments were sequenced by Macrogen, Inc. (Seoul, South Korea). Chromatograms were checked manually, and then assembled and edited using Geneious v7 (Biomatters, Ltd.).

We completed a multiple sequence alignment using the Muscle algorithm (Edgar 2004) in MEGA v7 (Kumar et al. 2016). PartitionFinder v2.1.1 (Lanfear et al. 2017) was used to select the optimal partition scheme and nucleotide substitution models using the linked model of branch lengths with a greedy search algorithm. For this analysis, three partitions were previously defined. The best model was selected through Bayesian information criterion. A Bayesian phylogenetic inference analysis was undertaken using MrBayes v3.2.6. (Ronquist et al. 2012), implemented in the online CIPRES Science gateway portal (Miller et al. 2010), with two independent runs of 1.0×10^7 generations, with four Markov chains (one cold), sampled every 1000 generations. Twenty-five percent of generations and trees were discarded as burn-in, and the run was performed with unlinked character-state frequencies, and substitution rates under SYM + I + G, HKY + I, and GTR + I + G models for the three partitions, respectively. The convergence and minimal effective sample sizes (>200) of the parameters were verified in Tracer v1.6 (Rambaut et al. 2014). We calculated uncorrected pairwise distances using the packages ape v3.4 (Paradis et al. 2004) and spider v1.3-0 (Brown et al. 2012) in R v3.5.0 (R Core Team 2018). Sites with gaps were pairwise deleted.

Table 1.—Values for measurements (mm) for the type series of *Adenomera chicomendesi* sp. nov. Measurements are reported as mean ± 1 SD (range), except for ratios in relation to SVL for the holotype in brackets [%]. Abbreviations are defined in Materials and Methods.

	Adenomera chicomendesi sp. nov.		
Trait	Holotype (CFBH 43562)	Male paratypes $n = 12$	Female paratypes $n = 3$
SVL	23.8	22.7 ± 0.8 (21.3–24.0)	23.2 ± 1.0 (22.1–23.8)
HL	7.6 [31.9]	7.6 ± 0.3 (7.1–8.0)	7.5 ± 0.3 (7.2–7.7)
HW	8.4 [35.3]	8.4 ± 0.2 (8.0–8.8)	8.5 ± 0.4 (8.1–8.9)
ED	2.0 [8.4]	2.1 ± 0.2 (1.7–2.3)	1.8 ± 0.1 (1.7–1.8)
TD	1.4 [5.9]	1.4 ± 0.1 (1.2–1.6)	1.5 ± 0.2 (1.2–1.6)
EN	2.0 [8.4]	1.9 ± 0.2 (1.6–2.4)	1.9 ± 0.2 (1.8–2.1)
IND	2.1 [8.8]	2.2 ± 0.1 (2.1–2.5)	2.3 ± 0.1 (2.2–2.4)
HAL	5.1 [21.4]	5.3 ± 0.3 (4.9–5.7)	5.4 ± 0.2 (5.3–5.6)
THL	10.9 [45.8]	10.6 ± 0.4 (10.0–11.2)	10.9 ± 0.3 (10.5–11.1)
TL	11.6 [48.7]	11.0 ± 0.5 (10.4–11.7)	11.3 ± 0.3 (11.0–11.6)
FL	12.1 [50.8]	12.1 ± 0.5 (11.4–13.5)	12.1 ± 0.8 (11.3–12.9)

Species Description

Adenomera ***chicomendesi*** sp. nov.
(Tables 1–3; Figs. 1–3)

Adenomera "Forest Call I"; Angulo et al. (2003).
Adenomera sp. E; Fouquet et al. (2014).

Holotype.—CFBH 43562 (formerly AAG-UFU 5861; Fig. 1A,B), adult male, from Parque Zoobotânico (9.955752°S, 67.870476°W; 167 m a.s.l.), campus of UFAC, municipality of Rio Branco, state of Acre, northwestern Brazil, collected by T.R. de Carvalho, B.F.V. Teixeira, D.L. Bang, and M.B. de Souza on 2 February 2017. Sound recordings: Adenomera_chicomendesiRioBrancoAC1a–bTRC_AAGm671.

Paratopotypes.—Eight adult males collected within Parque Zoobotânico: AAG-UFU 5862–5864 on 10 February 2017 by the same collectors of the holotype; CFBH 43563 (former LHUFCG 193) on 19 December 2005; and ZUEC 24528–24531 (formerly LHUFCG 220–223, respectively), 22–23 December 2005, collected by M.N.C. Kokubum and M.B. de Souza.

Paratypes.—Four adult males (Museo de Historia Natural de San Marcos [MUSM] 39462, 39463, 39467, 39473; field nos. TRC 249, 250, 254, and 260, respectively) and three adult females (MUSM 39468, 39472, 39474; field nos. TRC 255, 259, and 261, respectively) from Explorer's Inn Lodge (12.83782°S, 69.29551°W; 242 m a.s.l.), Tambopata National Reserve, district and province of Tambopata, region of Madre de Dios, southeastern Peru, collected by T.R. de Carvalho and D.A. Barrera on 25–27 November 2018.

Referred specimens.—Other Tambopata specimens: adult males, call vouchers (ROM 40110, 40111, 40321; USNM 342983, 342984); gravid female (ROM 40324).

Generic placement.—The new species is assigned to the genus *Adenomera* on the basis of the following combination of features (Heyer 1973): (1) small body size (relative to other leptodactylids; up to 34.1 mm; Kok et al. 2007); (2) fringing and webbing between toes absent; (3) thumb spines in adult males absent; (4) first and second fingers of approximately equal length.

Diagnosis.—Within *Adenomera*, *A. chicomendesi* is characterized by the following combination of character states: (1) medium size (adult male SVL = 21.3–24.0 mm); (2) toe tips expanded into discs (character state D of Heyer [1973]); (3) absence of antebrachial tubercle; (4) absence of nearly solid dark-colored stripe on underside of forearm; (5) throat light gray, belly cream colored; (6) advertisement call consisting of a single multipulsed note; (7) advertisement notes formed by 22–35 partly fused pulses.

Comparisons with other species.—*Adenomera chicomendesi* is a member of the *A. andreae* clade on the basis primarily of molecular evidence (Fig. 4), which comprises only Amazonian species. *Adenomera chicomendesi* has mid-sized adult males (SVL = 21.3–24.0 mm; Table 1) and differs from some congeners in having a larger SVL (i.e., Araucaria Forest species *A. araucaria* [maximum male SVL = 18.8 mm; Kwet and Angulo 2002], Atlantic Forest species *A. ajurauna* [17.2–20.0 mm; Berneck et al. 2008], and *A. nana* [16.3–19.4 mm; Kwet 2007]), or by a smaller SVL in relation to the Amazonian species *A. lutzi* (25.7–33.5 mm; Kok et al. 2007) and *A. simonstuarti* (25.9–26.2 mm; Angulo and Icochea 2010). The presence of toe tips fully expanded into small discs (character state D of Heyer [1973]) distinguishes *A. chicomendesi* from congeners having toe tips unexpanded or slightly expanded (character states A and B of Heyer [1973]), or moderately expanded (character state C of Heyer [1973]): *A. araucaria*, *A. bokermanni*, *A. coca*, *A. cotuba*, *A. diptyx*, *A. engelsi*, *A. hylaedactyla*, *A. juikitam*, *A. martinezi*, *A. phonotriccus*, *A. saci*, and *A. thomei*. *Adenomera chicomendesi* is further distinguished from *A. cotuba* (Carvalho and Giaretta 2013b), *A. lutzi* (Kok et al. 2007), and *A. phonotriccus* (Carvalho et al. 2019) by the absence of an antebrachial tubercle. The new species can also be distinguished from *A. simonstuarti* (Angulo and Icochea 2010) by the absence of a nearly solid dark-colored stripe on the underside of forearm. *Adenomera chicomendesi* is distinguished from *A. heyeri* (Boistel et al. 2006) and *A. lutzi* (Kok et al. 2007) by having a light-gray throat and cream-colored belly (with hints of yellow in some specimens)

Table 2.—Advertisement call traits of *Adenomera chicomendesi* sp. nov. from the type locality (Rio Branco, Acre, Brazil), and Tambopata National Reserve and its buffer zone (southeastern Peru). Values are given as mean ± 1 SD (range).

Call traits	Type locality (Brazil), $n = 4$ males	Tambopata (Peru), $n = 5$ males
Note duration (ms)	192.5 ± 4.5 (170–213), $n = 25$	200.8 ± 28.6 (154–247), $n = 37$
Note rate/min	20.1 ± 10.0 (5–29), $n = 5$	14.6 ± 7.5 (8–25), $n = 5$
Note rise time (%)	30.8 ± 5.1 (16–52), $n = 25$	19.8 ± 1.8 (11–33), $n = 37$
Pulses per note	30.1 ± 2.5 (25–35), $n = 25$	28.1 ± 3.5 (22–35), $n = 37$
Pulse duration (ms)	6.1 ± 0.8 (2–77), $n = 774$	7.1 ± 0.6 (4–47), $n = 1021$
Pulse rate/s	165.7 ± 11.1 (129–198), $n = 25$	155.6 ± 8.1 (117–173), $n = 37$
Fundamental frequency (Hz)	2109.1 ± 47.4 (2008–2221), $n = 25$	2368.7 ± 104.7 (2223–2525), $n = 37$
Dominant frequency (Hz)	4219.6 ± 114.9 (3941–4500), $n = 25$	4730.5 ± 252.1 (4285–5060), $n = 37$
Frequency modulation (Hz)	159.3 ± 187.0 (−108 to 560), $n = 25$	452.1 ± 187.5 (86–1034), $n = 37$

FIG. 1.—Live specimens (adult males) of *Adenomera chicomendesi* from the type locality (Rio Branco, state of Acre, northwestern Brazil). (A, B) Holotype (CFBH 43652). (C, D) Paratopotypes (AAG-UFU 5863–5864). A color version of this figure is available online.

TABLE 3.—Acoustic information on *Adenomera* with special reference to advertisement call (= note) duration, pulse number, and peak frequencies in the first two harmonics. The new species is indicated in bold. Abbreviations: N = nonpulsed; H0 = fundamental harmonic; H1 = 2nd harmonic.

Species	Note duration (ms)	Pulses/note	H0 frequency (kHz)	H1 frequency (kHz)	Reference
A. ajurauna	130–190	N	3.72–5.43	—	Berneck et al. (2008)
A. andreae	45–86	4	2.32–2.69	4.56–5.49	Boistel et al. (2006)
A. araucaria	86–140	5–11	1.72–3.36	4.63–5.40	Kwet and Angulo (2002)
A. bokermanni[a]	99–152	N	1.79–1.83	3.40–3.57	Kwet (2007)
A. chicomendesi	154–247	22–35	2.01–2.52	3.94–5.06	Present study
A. coca	110–145	10–15	1.69–1.91	3.45–3.75	Angulo and Reichle (2008)
A. cotuba	69–191	8–14	1.73–1.83	3.33–3.80	Carvalho and Giaretta (2013b)
A. diptyx[b]	56–88	Pulsed	2.18–2.28	4.20–4.50	Márquez et al. (1995)
A. engelsi	96–163	N	ca. 2.00	3.46–4.29	Kwet et al. (2009)
A. heyeri	137–185	9.5	1.82–1.88	3.57–3.84	Boistel et al. (2006)
A. hylaedactyla	35–62	4–6	1.95–2.21	3.96–4.48	Angulo et al. (2003)
A. juikitam	148–202	16–21	1.88–2.11	3.70–4.17	Carvalho and Giaretta (2013b)
A. lutzi	41–61	N	1.64–1.81	3.27–3.62	Kok et al. (2007)
A. marmorata	100	N	4.50–5.60	—	Straughan and Heyer (1976)
A. martinezi	63–151	15–21	1.88–2.06	3.38–4.13	Carvalho and Giaretta (2013a)
A. nana	67–122	N	2.30–2.70	4.62–5.44	Kwet (2007)
A. phonotriccus	213–433	14–26	1.86–2.00	3.38–4.13	Carvalho et al. (2019)
A. saci	90–241	N	1.69–2.25	3.38–4.41	Carvalho and Giaretta (2013a)
A. simonstuarti	57–71	3–4	1.81–2.03	3.71–4.05	Angulo and Icochea (2010)
A. thomei	120–210	10–21	2.15–2.81	4.57–5.56	Almeida and Angulo (2006)

[a] *Adenomera* sp. 2 from Joinville.

[b] Reported as *A. andreae* (De la Riva, personal communication to A. Angulo).

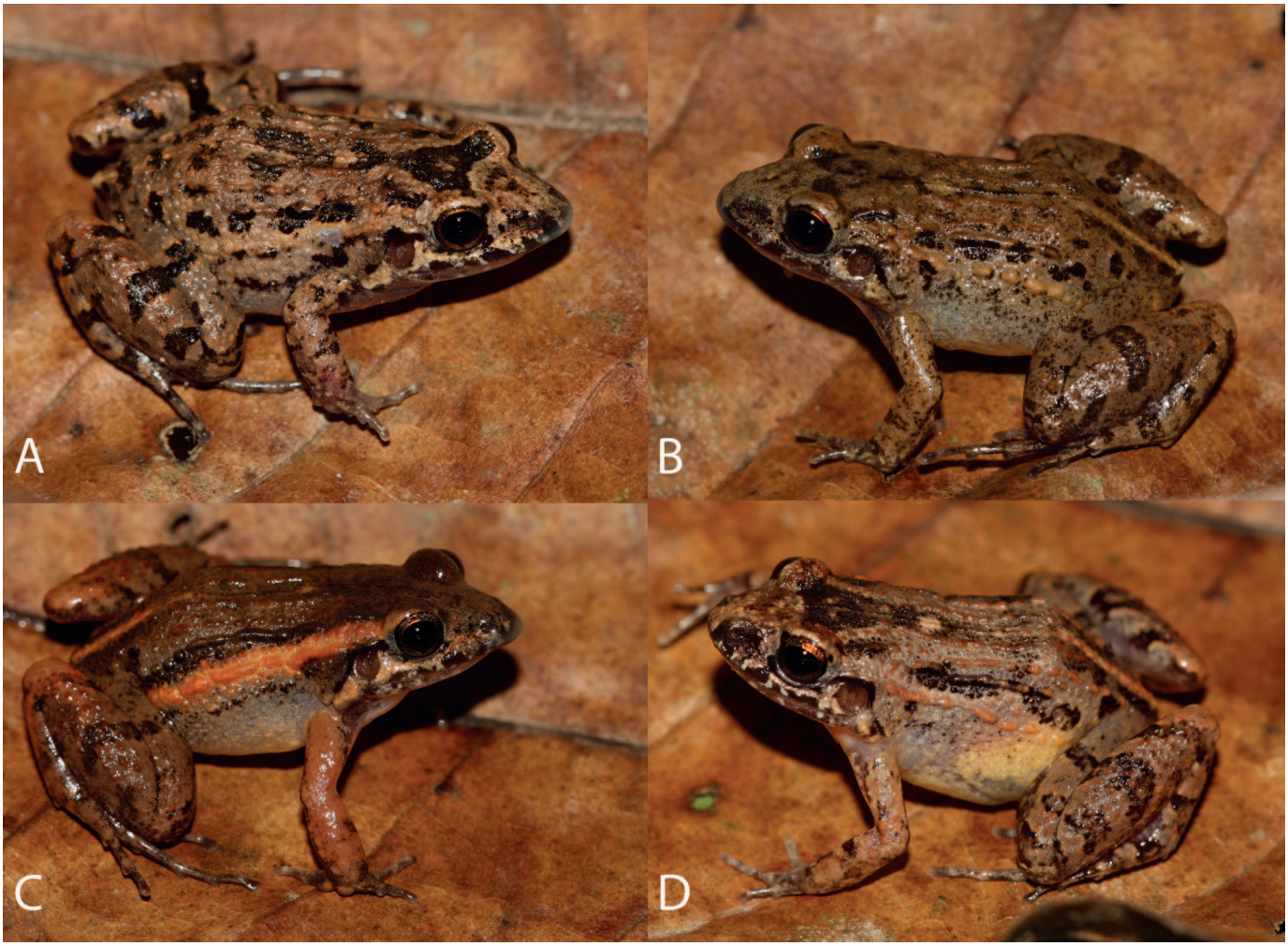

Fig. 2.—Live specimens (adults) of *Adenomera chicomendesi* from the Tambopata National Reserve (Madre de Dios, southeastern Peru). (A–C) Male paratypes (MUSM 39462, 39463, and 39467, respectively). (D) Gravid female paratype (MUSM 39468). A color version of this figure is available online.

in life, whereas *A. heyeri* and *A. lutzi* have uniformly yellow and brown-spotted throat and belly. *Adenomera chicomendesi*, *A. marmorata*, and *A. andreae* are morphologically cryptic species. Although adult specimens of *A. chicomendesi* appear to attain larger SVL values than the Atlantic Forest species, *A. marmorata* (Heyer 1973), there is overlap in SVL ranges between these species. Regarding the Pan-Amazonian *A. andreae* (Heyer 1973, 1977; Appendix I), SVL range is similar in comparison with that of the new species (Table 1). Nevertheless, the calls of these three species are distinct from each other. The advertisement call of *A. chicomendesi* (Table 2; Fig. 5) consists of a single multipulsed call. The new species is distinguished from all congeners with pulsed call (except *A. phonotriccus*) by virtue of having the greatest number of pulses (22–35) in a call (Tables 2 and 3). *Adenomera chicomendesi* has advertisement notes made up of partly fused pulses; advertisement notes of *A. phonotriccus* are made up of complete pulses that are separated by silent gaps in between (Carvalho et al. 2019). Moreover, the new species differs from nonpulsed-call species by having a multipulsed call (Table 3). The new species can be further distinguished from *A. cotuba* (Carvalho and Giaretta 2013b) and *A. simonstuarti* (T.R. de Carvalho, personal observation) by the absence of calling bouts (i.e., multinote calls). Morphological and acoustic comparisons with the three candidate species in the *A. andreae* clade indicated in Fouquet et al. (2014:sp. C, sp. D, and sp. T) are limited because of the lack of phenotypic information associated with these genetic lineages. The first of these (sp. C) was associated by those authors to *Adenomera* "Forest Call II" (Angulo et al. 2003), which is clearly distinguished from *A. chicomendesi* by having its advertisement notes made up of complete pulses as in *A. phonotriccus*, whereas the note pulses are partly fused in *A. chicomendesi*.

Description of holotype.—Adult male, body relatively robust (Fig. 3A). Snout subovoid in dorsal view, acuminate in lateral view (Fig. 3C). Nostrils closer to the snout tip than to the eyes (ED = EN, EN = 95% IND; Table 1); fleshy ridge on snout tip; canthus rostralis rounded; loreal region concave; tympanic annulus well defined, mostly circular (TD = 70% ED); tympanic membrane partially translucent; supratympanic fold from the posterior corner of the eye, over the dorsal edge of the tympanic annulus, to the base of the arm; postcommissural gland ovoid; vocal sac subgular with a fold from jaw extending to arm; vocal slits present; vomerine teeth in two nearly straight rows medial and

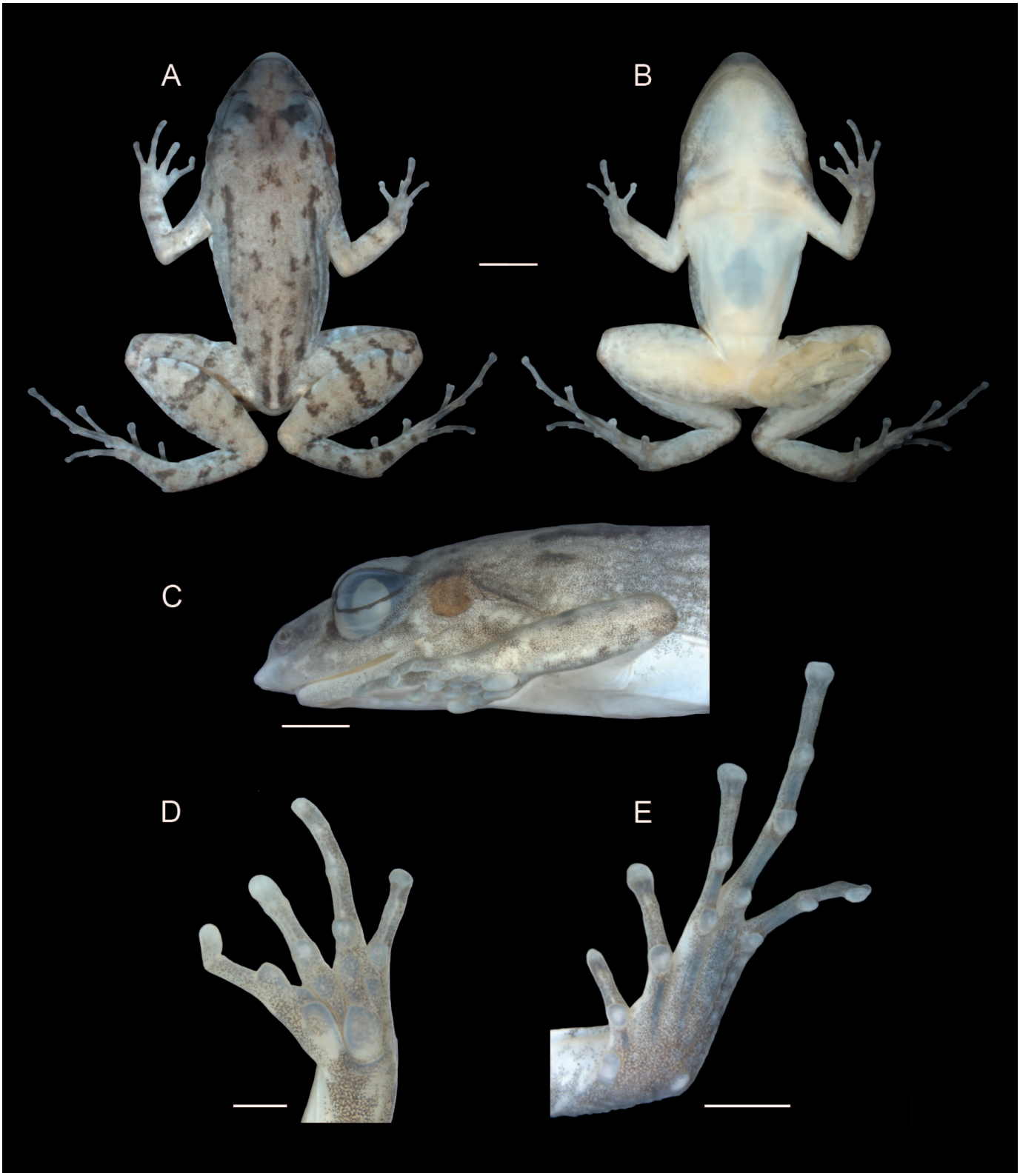

FIG. 3.—Preserved holotype of *Adenomera chicomendesi* (adult male, CFBH 43652; snout–vent length = 23.8 mm). (A, B) Dorsal and ventral body, respectively (scale = 5 mm). (C) Lateral head (scale = 2 mm). (D) Hand (scale = 1 mm). (E) Foot (scale = 2 mm). A color version of this figure is available online.

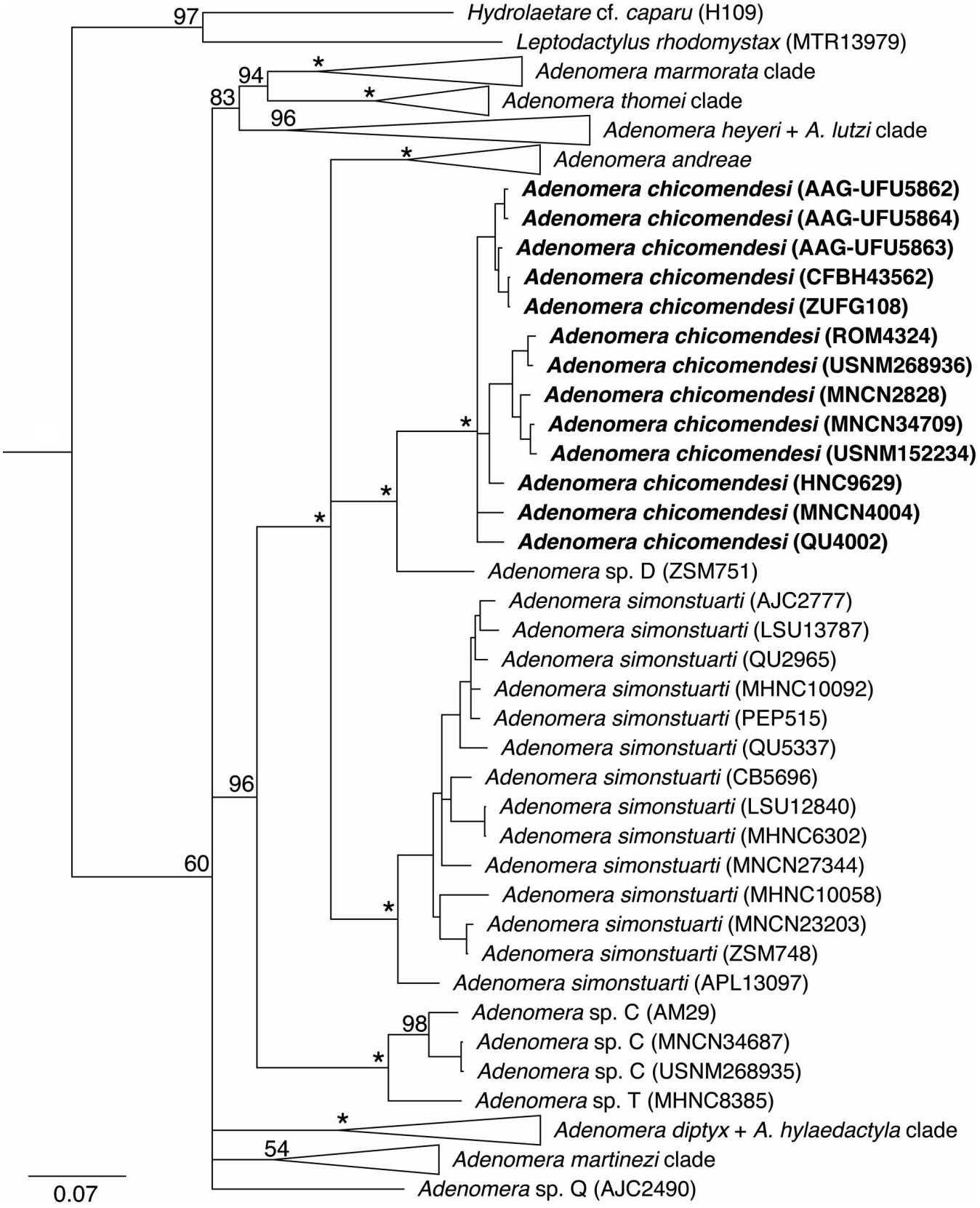

Fig. 4.—The 50% majority rule consensus tree from Bayesian inference analysis based on 657 base pairs of the mitochondrial deoxyribonucleic acid *cytochrome c oxidase subunit I* gene for all sequences of *Adenomera* from Fouquet et al. (2014) and new sequences for the holotype and paratypes of *A. chicomendesi* (vouchers following species names). Posterior probabilities (%) are given near the nodes; asterisks (*) indicate maximum probability (within-species posterior probabilities are not shown). Only the nominal species and candidate species in the *Adenomera andreae* clade (sensu Fouquet et al. 2014) are expanded in this figure (other clades are collapsed; more distantly related outgroups are not shown). Scale indicates number of base substitutions per site.

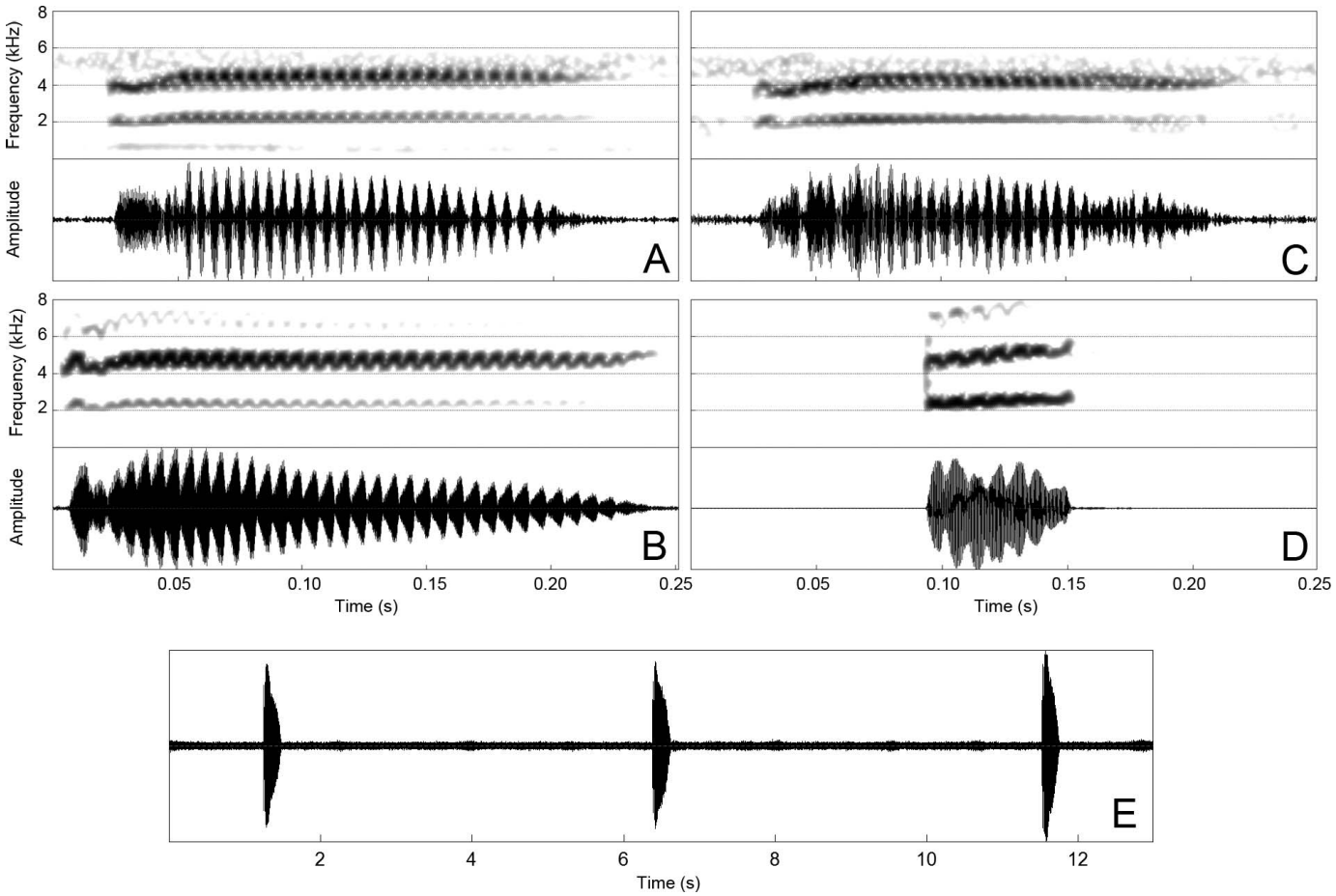

FIG. 5.—Advertisement call of (A–C) *Adenomera chicomendesi* recorded at (A, C) the type locality (Parque Zoobotânico/UFAC; Rio Branco, Acre, Brazil) and (B, E) Tambopata, Peru, and (D) a comparative call of *Adenomera andreae* from Reserva Florestal Humaitá (Porto Acre, Acre, Brazil). (A) Paratopotype (AAG-UFU 5862): an initial low-amplitude portion, followed by 27 pulses. (B) Nontype male (USNM 342983): an abrupt peak in the first pulse, followed by a decay phase in the second pulse, followed by 33 pulses. (C) Holotype (CFBH 43562): a note containing over 20 pulses, plus irregular amplitude modulations in-between pulses. (D) Unvouchered male (recording FNJV 11210): a seven-pulse note. (E) Time section containing three calls of the male in B. Spectrograms and oscillograms were produced on the same timescale (ca. 250 ms).

posterior to choanae and almost parallel to sagittal plane. Tongue elongated, free from the posterior third. Relative finger lengths IV < I ≅ II < III; fingers without ridges or fringes; finger tips rounded, expanded, especially fingers I and II, without flattening; inner metacarpal tubercle elongated; outer metacarpal tubercle ovoid (Fig. 3D). Subarticular tubercles rounded; supernumerary tubercles rounded. Antebrachial tubercle on distal edge of forearm absent. Dorsum mostly smooth, warty on flanks and inguinal region. Dorsolateral folds extending posteriorly from scapular region until 2/3 of the body length. Dorsal folds indistinct. Sacral region, dorsal surface of tibia, and posterior surface of tarsus covered with white-tipped tubercles. THL = 94% of TL and 90% of FL. Ventral surface of body and limbs smooth, underside of thigh areolate (Fig. 3B). Posterior surface of thigh with conspicuous, nearly rounded paracloacal gland. Relative toe lengths I < II < V < III < IV; lateral ridges, fringing, and webbing between toes absent; tips of toes II–IV expanded into discs; tip of toe I unexpanded and that of toe V slightly expanded. Inner and outer metatarsal tubercles ovoid, inner tubercle twice in maximum diameter than outer tubercle (Fig. 3E). Tarsal fold extending 2/3 of tarsus length, from the inner metatarsal tubercle toward the heel. Subarticular tubercles nearly rounded or subconical; supernumerary tubercles rounded.

Color of holotype in life.—Snout tip with a faded white coloration (coincident with fleshy ridge). Iris copper. Outer margin of the upper eyelid pale yellow. White to light-gray blotches on the upper lip. Tubercles have vivid colors; those on the dorsal surface of tibia are orange/light brown; tubercles on flanks and dorsum have yellow shades. Postcommissural gland yellow, merged with melanophores. Tympanum reddish brown. Dorsal surface of body, hind limbs, and flank grayish brown with dark-brown specks and spots (Fig. 1A). Discontinuous black interorbital bar. Arm and forearm lighter brown with dark specks. Dorsal surface of hand and foot grayish brown, covered with patches of melanophores. Black dorsolateral stripelike blotches extending posteriorly from the scapular region to mid-body length. Thigh, shank, and tarsus with black transverse bars. Vertebral stripe restricted to sacral region, light yellow. Black lumbar spot. Posterior surface of thigh covered with melanophores on a dull yellow background, including the coloration of paracloacal gland. Throat, chest, and underside of limbs translucent (Fig. 1B). Throat light gray, chest gray (darker than throat). Ventral surface of limbs tan brown to

reddish; thigh with orange tubercles and patches of melanophores posteroventrally. Belly cream-colored with hints of yellow, partially translucent (parietal peritoneum visible through skin). White and light-gray blotches surrounding the lower jaw, yellow stains in the background. Melanophores concentrated laterally (coincident with vocal slits). Underside of forearm (outer margin), palm of hand, sole of foot, and digits and subarticular tubercles mostly dark-colored merged with a light background. Ventral surface of hand and foot partially translucent (phalanges visible through skin). Finger and toe tips lack coloration. Posterior surface of arm and groin yellow orange.

Color of holotype in preservative.—Snout tip with a faded white coloration (coincident with fleshy ridge). Light blotches on the upper lip (Fig. 3C). Tubercles on dorsal surface of body and limbs, flank, and ventral surface of thigh lack coloration. Postcommissural gland mostly covered with melanophores. Tympanum light brown. Dorsal surface of body and limbs and flank grayish brown. Specks, blotches, interorbital bar, inguinal spot, and transverse bars on hind limbs dark brown (Fig. 3A). Dorsal surface of forelimbs pale brown with dark specks. Dorsal surface of hand and foot grayish brown, covered with patches of melanophores. Vertebral stripe restricted to sacral region and grayish brown (but lighter than dorsal coloration). Posterior surface of thigh covered with melanophores on a cream background. Paracloacal gland darkened on the lower margin. Throat, chest, belly, and underside of limbs translucent yellowish cream (Fig. 3B). Throat and belly lighter than chest and hind limbs. Melanophores line the lower jaw and have greater density around the vocal slits. Underside of forearm (outer margin), palm of hand, sole of foot, and digits and subarticular tubercles mostly covered with melanophores interspersed with lighter sections of subarticular tubercles and finger/toe tips (Fig. 3D,E). Yellow-orange coloration of posterior surface of arm and groin in life completely faded.

Variation (Table 1).—Dorsum can be smooth or warty (Fig. 1C,D). Dorsolateral and dorsal folds are discontinuous, conspicuous (extending longitudinally along the body length; Fig. 1C,D) or short/flattened (the latter observed under magnification). Fingers I and II unexpanded or slightly expanded. Toe I slightly expanded in AAG-UFU 5864 and MUSM 39473. Females (MUSM 39468, 39472, 39474) have a rounded snout in dorsal and lateral views, lack a fleshy ridge at the tip of the snout (secondary sexual character), and are relatively more robust than males. Two gravid females (MUSM 39468, 39472) had eggs visible through skin (Fig. 2D). Coloration of the dorsal surfaces varies from light grayish brown, uniformly brown in the oldest specimens collected in 2005 (CFBH 43563, ZUEC 24528–30), to dark gray. Color patterns in the interorbital region vary from a discontinuous horizontal bar, an inverted triangle, to an hourglass-shaped blotch. Dorsolateral stripe (cream, yellow, or orange) present in AAG-UFU 5863, MUSM 39467, and ZUEC 24531. Sacral stripe (cream to yellow) absent in MUSM 39462 and ZUEC 24528.

Etymology.—The new species is named after Francisco Alves Mendes Filho, better known as Chico Mendes, in recognition of his efforts and sacrifice as an environmentalist in the Amazon rain forest, especially in the Brazilian state of Acre, his homeland. He also fought for the human rights of indigenous peoples and rubber tapper communities of the region. As a consequence of his activism, Chico Mendes was assassinated on 22 December 1988 in his hometown of Xapuri, in Acre. Suggested vernacular name: Chico Mendes' Terrestrial Nest-Building Frog.

Advertisement call.—This description is based on recordings of nine males from Brazil and Peru (Appendix II). Sample sizes for each acoustic trait and descriptive statistics (mean ± 1 SD) for each population are given in Table 2. The call (Fig. 5) consists of a single multipulsed note, which is emitted at a rate of 5–29 calls/min. Note duration varies from 154 to 247 ms. Note onset comprises an initial low amplitude increase followed by an abrupt peak, then a gradual amplitude decay for the ensuing pulses (Fig. 5A). Other notes might have an abrupt peak in the first pulse, followed by a decay phase (one to three pulses), a second amplitude peak that is sustained around the middle of the note, and then a gradual decrease for the ensuing pulses (Fig. 5B). Rise time is 11–52% of the notes' length. Pulse number varies from 22 to 35; pulse duration from 2–77 ms, emitted at a rate of 117–198 pulses/s. Initial and final sections of notes sometimes have weak modulation so pulses might not have discernible limits. As a result, these instances have higher values for pulse duration (Fig. 5C). The fundamental frequency of the note occupies a narrow bandwidth harmonic at 2008–2525 Hz; the dominant frequency varies from 3941 to 5060 Hz and corresponds to the second harmonic. Notes usually have a slight or well-marked frequency upsweep throughout their duration, but a few notes do not have any modulation or a weak negative modulation. Linear frequency modulation varies from −108 to 1034 Hz.

Phylogenetic position and genetic distance.—*Adenomera chicomendesi* corresponds to *Adenomera* Forest Call I of Angulo et al. (2003) on the basis of acoustic and molecular evidence. The phylogenetic reconstruction based on *COI* mtDNA sequences recovered the holotype and three paratypes of *A. chicomendesi* (Fig. 4) nested in the clade of the confirmed candidate species *Adenomera* sp. E of Fouquet et al. (2014). The molecular voucher "ZUFG 108" of Fouquet et al. (2014) corresponds to a paratype of *A. chicomendesi* (CFBH 43563). Genetic distances (in *COI*) within *A. chicomendesi* varied from zero to 5.4% (see Table 4 for genetic distances within and between species in the *A. andreae* clade).

Habitat and natural history.—At the type locality (Parque Zoobotânico, Brazil), *A. chicomendesi* could be heard within primary and secondary forests, and along forest edges. Males called from exposed positions or under leaf litter from late afternoon to well into the night. The vocalization of *A. chicomendesi* was easily recognized when heard in the field. There were two sympatric species of *Adenomera* at *A. chicomendesi*'s type locality, a forest fragment in an urban center: *A. andreae* and *A.* aff. *hylaedactyla*. *Adenomera andreae* was syntopic with *A. chicomendesi*; both vocalized exclusively in forest areas. In contrast to *A. andreae* and *A. chicomendesi*, *A.* aff. *hylaedactyla* used every kind of open area, including altered habitats (e.g., gardens). Field observations suggest that there is possibly some temporal segregation between the forest-dwelling *A. andreae* and *A. chicomendesi*, even though there are a few hours of overlap between their calling activity. *Adenomera andreae* often calls during the daytime, whereas

TABLE 4.—Uncorrected pairwise genetic distances (%, reported as minimum–maximum) of partial cytochrome c oxidase subunit I within and between nominal and candidate species of the *Adenomera andreae* clade. Within-species distances are indicated in bold.

	A. andreae	*A. chicomendesi*	*A. simonstuarti*	*Adenomera* sp. C	*Adenomera* sp. D	*Adenomera* sp. T
A. andreae	**0.0–10.7 (*n* = 72)**					
A. chicomendesi	10.4–15.2	**0.0–5.4 (*n* = 13)**				
A. simonstuarti	9.4–15.3	10.2–14.3	**0.0–7.5 (*n* = 14)**			
Adenomera sp. C	13.2–17.2	14.2–17.4	13.5–15.7	**0.2–4.1 (*n* = 3)**		
Adenomera sp. D	10.5–13.5	9.1–10.7	11.7–13.4	15.2–15.8	**(*n* = 1)**	
Adenomera sp. T	12.8–16.1	14.9–17.6	14.9–16.8	7.7–8.4	15.6	**(*n* = 1)**

A. chicomendesi begins calling activity usually at dusk. On the other hand, *A.* aff. *hylaedactyla* can be heard calling during the daytime and throughout night, especially after rain showers.

At Explorer's Inn in Tambopata (Peru), *A. chicomendesi* and *Adenomera* Forest Call II (sensu Angulo et al. 2003) were syntopic species and could be heard synchronously. This site was distinct from *A. chicomendesi*'s type locality in consisting of a seasonally flooded forest (as described in Angulo et al. 2003) in comparison with the well-drained forest remnant at the type locality. Calling shifts at Tambopata were similar to our field observations at the type locality (calling activity beginning at dusk).

Behavior.—During field playbacks conducted in the evening of 24 February 1999 at Bahuaja Lodge in the then Tambopata Candamo Reserve Zone (now split into the Tambopata National Reserve and its associated buffer zone), Peru, one of us (AA) documented the following behavior: a male was observed calling and a speaker was set up for playbacks of advertisement calls, including the conspecific call of *A. chicomendesi*. Within the first two to three playbacks of its call, the male reacted by hopping to the speaker, underneath it, and around it, producing agonistic/territorial calls throughout. On two occasions, it stood on tiptoes, widened its legs, and inflated its lungs as it hopped toward the speaker. Shortly thereafter, another larger frog approached the speaker and was chased away by the first frog who displayed the same kind of behavior described above. The second frog returned, only to be chased away again. Upon capture, we determined that the second frog was a gravid female (ROM 40324, field no. AA 9972) and the first (posturing) frog was a male.

Longevity.—ROM 40324 was part of a small number of specimens kept alive in an attempt to encourage reproduction in a laboratory setting. Unfortunately, captive breeding was unsuccessful, but this female was kept alive thereafter. Her identity was initially confirmed through playback experiments (see above) and subsequently through DNA sequences (Fouquet et al. 2014). She was found deceased in her enclosure on 20 August 2008, which makes her time in captivity 9 yr, 5 mo, and 28 d. She was already a reproductively mature adult when she was captured. Although the reproductive mode of this species is as yet unknown, available data for other members of the genus with exotrophic tadpoles indicate that development from egg to metamorph can take 77 d (*A. thomei*; Almeida and Angulo 2002), whereas it can take 11–13 d in endotrophic species (*A.* aff. *hylaedactyla*; Kokubum and Souza 2008). Depending on this species' reproductive mode and age to reproductive maturity, this female could have exceeded 10 yr of age.

Distribution.—*Adenomera chicomendesi* is associated with forest habitats in northwestern Brazil's state of Acre, as well as over a vast region of eastern Peru and north-central Bolivia (specimens assigned to *Adenomera* sp. E by Fouquet et al. 2014; see Fig. 6). The species' type locality lies on the eastern portion of the state of Acre, but populations in western Acre correspond to *A. chicomendesi* on the basis of sound recordings from Marechal Thaumaturgo (Fonoteca Neotropical Jacques Vielliard (FNJV) 11225, 31058, and 31535). The distribution of *A. chicomendesi* is mostly associated with the ecoregion of Southwest Amazon Moist Forests (sensu Dinerstein et al. 2017), even though three occurrence points in Bolivia and the Peruvian border with Bolivia coincide with the Bolivian Yungas (Fig. 6).

Conservation.—On the basis of a combination of acoustic and molecular data, *A. chicomendesi* is found to occur over a wide swath of the southwestern Amazon (Fig. 6), with an estimated extent of occurrence (IUCN 2012) of 392,436.231 km^2 (sensu Bachman et al. 2011), inclusive of both lowland Amazon rain forest and the foothills of the Andes of Peru and Bolivia (however, note that call data are still needed to further confirm species identities of samples from the foothills of the Andes). It is known from both outside and within protected areas (Tambopata National Reserve, Amboró National Park, and the type locality is found within the Universidade Federal do Acre's Parque Zoobotânico which, in practice, operates like a conservation area) and it is likely to occur in other protected areas with suitable habitat in between known geographic records. It is relatively common where it occurs.

Although illegal mining and illegal logging threaten the habitat of *A. chicomendesi* in this part of the Amazon, the species has a widespread distribution. Thus, it is recommended that the species' extinction risk be assessed as Least Concern following the IUCN (2012) Red List categories and criteria.

Remarks.—A common in situ pattern is the partly or completely syntopic occurrence of two or three *Adenomera* species, a pattern documented both in Acre and Tambopata (personal observations). *Adenomera chicomendesi* and *A. andreae* comprise a case of syntopic occurrence in forest habitats of the Brazilian state of Acre, whereas *A. chicomendesi* and *Adenomera* Forest Call II are syntopic in forest habitats of Tambopata. Their calls are distinct enough from each other, however, that the species can co-occur assuming acoustic niche partitioning (see Fig. 5D; Angulo et al. 2003). In addition to the type locality of *A. chicomendesi*, the syntopic occurrence of the new species and *A. andreae* is acoustically confirmed for another area of the region: Reserva Florestal Humaitá (RFH; circa 9.716667°S, 67.550000°W, municipality of Porto Acre; recordings FNJV

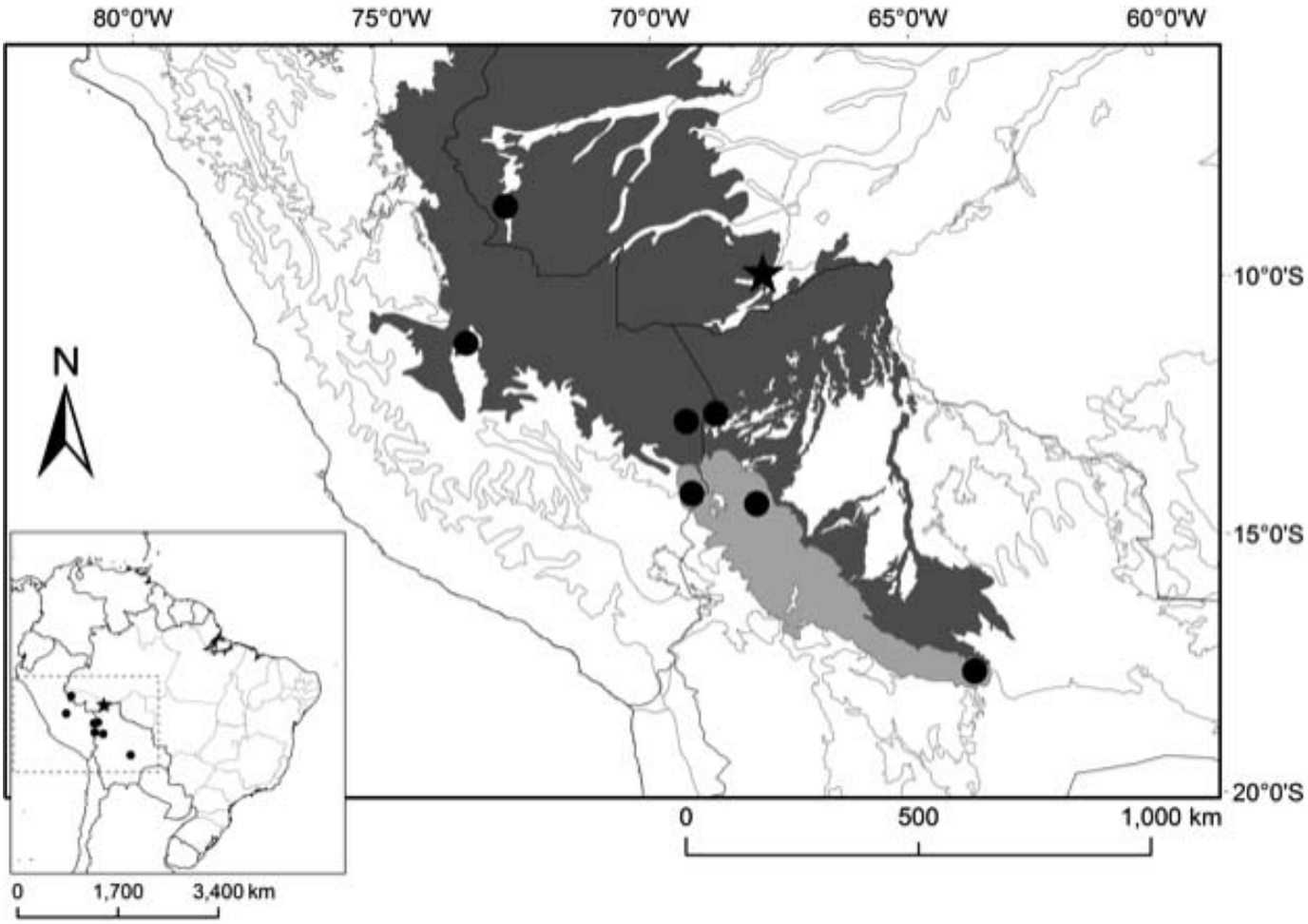

Fig. 6.—Geographic distribution of *Adenomera chicomendesi*. Type locality (Parque Zoobotânico, Rio Branco, Acre, Brazil) represented by a star. The species' distribution occurs within two ecoregions (sensu Dinerstein et al. 2017): the Southwest Amazon Moist Forests (dark gray) and the Bolivian Yungas (light gray). Light gray lines indicate other ecoregions; black lines indicate country boundaries.

11210 [*A. andreae*] and FNJV 11215 [*A. chicomendesi*]). It is also important to note that distinguishing these species is possible on the basis of their calls (Fig. 5D), given that they are quite similar in overall morphology. To avoid designating a composite type series, only those specimens that were recorded or at least collected in reproductive activity were assigned to *A. chicomendesi*.

Given that the overall morphology, size, and coloration of *A. chicomendesi* and *A. andreae* are similar, and that they are syntopic, Acre specimens without acoustic information or DNA sequence were conservatively assigned to *Adenomera* sp. of the *A. andreae* clade (see localities in Appendix I). The unequivocal identification of these species in the region should rely either on vocalizations or tissue samples for DNA barcoding.

Discussion

We have described one of many morphologically similar members of the *A. andreae* clade of leptodactyline frogs in western Amazonia. A detailed characterization of the other candidate species in this clade is still necessary for the confirmation of additional undescribed Amazonian species. It would be productive for future efforts to obtain different sources of data to focus attention on southwestern Amazonia, especially in the case of those species currently lacking acoustic information (e.g., *Adenomera* sp. D and *Adenomera* sp. T of Fouquet et al. [2014]). Another aspect that deserves attention is research into the reproductive biology and larval morphology of *Adenomera*, because available data are scarce and remain undescribed for most species. To date in this clade, only the nominal *A. andreae* has a description of its reproductive biology and tadpole available (Menin and Rodrigues 2013).

On the basis of current knowledge of the *A. andreae* clade, it is expected that the unnamed species might share certain morphological (e.g., toe discs) and ecological (e.g., forest dwellers with terrestrial reproduction and endotrophic larval development) features with the two nominal species of the clade (i.e., *A. andreae* and *A. simonstuarti*) and *A. chicomendesi*. Acoustic patterns, on the other hand, are reliable indicators of species identity, so vocalizations for these species might be, together with molecular data, the strongest evidence for their recognition as undescribed species. Pairwise distances (in *COI* gene) in the *A. andreae* clade (Table 4) constitute pieces of evidence supporting the hypothesis that the high levels of genetic diversity between nominal and candidate species in this Amazonian clade might reflect distinct acoustic patterns obscured within

morphologically conservative lineages, thus representing potentially additional unnamed *Adenomera* species in southwestern Amazonia.

As for candidate species *Adenomera* sp. C, its assignment to *Adenomera* Forest Call II by Fouquet et al. (2014) is being assessed on the basis of call vouchers recently obtained by two of us (TRC, DAB) in the 2018 field expedition to Tambopata, and this taxonomic unit should be formally described once its phylogenetic position has been assessed (TRC and DAB, personal observations). Assuming that the assignment by Fouquet et al. (2014) is correct, one additional species of the *A. andreae* clade (*Adenomera* sp. C) would be named, leaving two candidate species (namely, *Adenomera* sp. D and *Adenomera* sp. T) to be further investigated and identified.

The third forest-dwelling species from Tambopata (*Adenomera* Forest Call III; Angulo et al. 2003) was tentatively assigned by Fouquet et al. (2014) to *A. andreae*. If so, *A. chicomendesi* and *A. andreae* would be sympatric species also in southeastern Peru. At the study site in Tambopata, *Adenomera* Forest Call III was found in a different location and forest habitat from *A. chicomendesi* and *Adenomera* Forest Call II, even though a few calling males of *A. chicomendesi* were heard in that location of *Adenomera* Forest Call III. Ongoing research into the integrated approach of acoustic information and DNA sequences could shed more light on the identity of *Adenomera* Forest Call III in relation to *A. andreae*. Nominal *A. andreae* had its call described at least twice from Central Amazonia (Zimmerman and Bogart 1984) and French Guiana (Boistel et al. 2006), but acoustic information from the type locality in Brazil's eastern Amazonia remains undescribed. The redescription of *A. andreae* from the type locality, including calls and DNA sequences, might elucidate the taxonomic identity of many Amazonian populations currently subsumed into *A. andreae*.

Recent studies on the taxonomy of *Adenomera* have highlighted the importance of acoustic signals for species differentiation among members of this mostly cryptic leptodactylid genus (Angulo et al. 2003; Kwet 2007; Carvalho and Giaretta 2013a,b). The recognition and description of *A. chicomendesi* is another case of species-level resolution in *Adenomera* based mainly on an integrative taxonomy approach (Dayrat 2005). Thus, the characterization and formal description of undescribed species is a required step for the understanding of species richness in *Adenomera*, enabling the assessment of extinction risk and if needed, targeted conservation action.

Acknowledgments.—Financial support was received through research grants from the National Council for Scientific and Technological Development (CNPq Grant #446935/2014-0 to AAG, and #302518/2013-4 to CFBH) and São Paulo Research Foundation (FAPESP #2013/50741-7; CFBH). The taxonomic review of *Adenomera* species was part of the first author's Ph.D. dissertation, and he has received financial support from a doctoral and postdoctoral fellowships from FAPESP (#2012/15763-7 and #2017/08489-0, respectively). Collecting permits were granted by Instituto Chico Mendes (ICMBio/SISBIO #30059-9; Brazil) and by the Peruvian Government (#97-98-INRENA-DGANPFS-DANP; #17-2018-SERNANP-JEF). B.F.V. Teixeira and D.L. Bang provided companionship during the 2017 field expedition to Rio Branco (Acre); M.L. Lyra assisted with DNA sequencing protocols; P. Lemes assisted with generating the map; C. Aguilar-Puntriano provided logistical support for the duration of the visit in Lima. The following individuals granted access to specimens under their care: F.P. Werneck and A. Silva (INPA-H), N. Pupin and D.B. Delgado (CFBH), L.F. Toledo and K. Rabelo (ZUEC), M. Menin (CZPB-AA), A.L.C. Prudente and F. Sarmento (MPEG), T. Grant and A.S. Benetti (MZUSP), G.F. Pontes (MCP), and C. Aguilar-Puntriano (MUSM). The FNJV and the Macaulay Library at the Cornell Lab of Ornithology enabled access to *Adenomera* sound files.

Literature Cited

Almeida, A.P., and A. Angulo. 2002. Natural history notes: *Adenomera* aff. *marmorata* (reproduction). Herpetological Review 33:197–198.

Almeida, A.P., and A. Angulo. 2006. A new species of *Leptodactylus* (Anura: Leptodactylidae) from the state of Espírito Santo, with remarks on the systematics of associated populations. Zootaxa 1334:1–25.

Angulo, A., and J. Icochea. 2010. Cryptic species complexes, widespread species and conservation: Lessons from Amazonian frogs of the *Leptodactylus marmoratus* group (Anura: Leptodactylidae). Systematics and Biodiversity 8:357–370.

Angulo, A., and S. Reichle. 2008. Acoustic signals, 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 152:59–77.

Angulo, A., R.B. Cocroft, and S. Reichle. 2003. Species identity in the genus *Adenomera* (Anura: Leptodactylidae) in southeastern Peru. Herpetologica 59:490–504.

Audacity Team. 2017. Audacity(R): Free Audio Editor and Recorder, version 2.2.1. Available at https://www.audacityteam.org. Archived by WebCite at http://www.webcitation.org/732btrNLV on 9 October 2018.

Bachman, S., J. Moat, A.W. Hill, J. De la Torre, and B. Scott. 2011. Supporting Red List threat assessments with GeoCAT: Geospatial conservation assessment tool. ZooKeys 150:117–126.

Berneck, B.V.M., C.O.R. Costa, and P.C.A. Garcia. 2008. A new species of *Leptodactylus* (Anura: Leptodactylidae) from the Atlantic Forest of São Paulo State, Brazil. Zootaxa 1795:46–56.

Boistel, R., G.C. Massary, and A. Angulo. 2006. Description of a new species of the genus *Adenomera* (Amphibia, Anura, Leptodactylidae) from French Guyana. Acta Herpetologica 1:1–14.

Brown, S.D.J., R.A. Collins, S. Boyer, M.C. Lefort, J. Malumbres-Olarte, C. Vink, and R.H Cruickshank. 2012. Spider: An R package for the analysis of species identity and evolution, with particular reference to DNA barcoding. Molecular Ecology Resources 12:562–565.

Carvalho, T.R., and A.A. Giaretta. 2013a. Taxonomic circumscription of *Adenomera martinezi* (Bokermann, 1956) (Anura: Leptodactylidae: Leptodactylinae) with the recognition of a new cryptic taxon though a bioacoustic approach. Zootaxa 3701:207–237.

Carvalho, T.R., and A.A. Giaretta. 2013b. Bioacoustics reveals two new syntopic species of *Adenomera* Steindachner (Anura: Leptodactylidae: Leptodactylinae) in the Cerrado of central Brazil. Zootaxa 3731:533–551.

Carvalho, T.R., A.A. Giaretta, A. Angulo, C.F.B. Haddad, and P.L.V. Peloso. 2019. A new Amazonian species of *Adenomera* (Anura: Leptodactylidae) from the Brazilian state of Pará: A tody-tyrant voice in a frog. American Museum Novitates 3919:1–21.

Dayrat, B. 2005. Towards integrative taxonomy. Biological Journal of the Linnean Society 85:407–415.

Dinerstein, E., D. Olson, A. Joshi, ... M. Saleem. 2017. An ecoregion-based approach to protecting half the terrestrial realm. BioScience 67:534–545.

Edgar, R.C. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32:1792–1797.

Fouquet, A., C.S. Cassini, C.F.B. Haddad, N. Pech, and M.T. Rodrigues. 2014. Species delimitation, patterns of diversification and historical biogeography of the Neotropical frog genus *Adenomera* (Anura, Leptodactylidae). Journal of Biogeography 41:855–870.

Gridi-Papp, M. 2007. Soundruler: Acoustic analysis for research and teaching. Available at http://soundruler.sourceforge.net. Archived by WebCite at http://www.webcitation.org/732cA62V3 on 9 October 2018.

Heyer, W.R. 1973. Systematics of the marmoratus group of the frog genus *Leptodactylus*. Contributions in Science, Los Angeles County Natural History Museum 251:1–50.

Heyer, W.R. 1977. A discriminant analysis of the frogs of the genus *Adenomera* (Amphibia: Leptodactylidae). Proceedings of the Biological Society of Washington 89:581–592.

Heyer, W.R., A.S. Rand, C.A.G. Cruz, O.L. Peixoto, and C.E. Nelson. 1990. Frogs of Boracéia. Arquivos de Zoologia 31:231–410.

IUCN (International Union for Conservation of Nature). 2012. IUCN Red List Categories and Criteria, version 3.1, 2nd ed. IUCN, Switzerland.

Kok, P.J.R., M.N.C. Kokubum, R.D. MacCulloch, and M. Lathrop. 2007.

Morphological variation in *Leptodactylus lutzi* (Anura, Leptodactylidae) with description of its advertisement call and notes on its courtship behavior. Phyllomedusa 6:45–60.
Kokubum, M.N.C., and M.B. de Souza. 2008. Reproductive ecology of *Leptodactylus* aff. *hylaedactylus* (Anura, Leptodactylidae) from an open area in northern Brazil. South American Journal of Herpetology 3:15–21.
Kumar, S., G. Stecher, and K. Tamura. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33:1870–1874.
Kwet, A. 2007. Bioacoustic variation in the genus *Adenomera* in southern Brazil, with revalidation of *Leptodactylus nanus* Müller, 1922 (Anura, Leptodactylidae). Mitteilungen aus dem Museum für Naturkunde in Berlin, Zoologische Reihe Supplement 83:56–68.
Kwet, A., and A. Angulo. 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 20:28–43.
Kwet, A., J. Steiner, and A. Zillikens. 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 44:93–107.
Lanfear, R., P.B. Frandsen, A.M. Wright, T. Senfeld, and B. Calcott. 2017. PartitionFinder 2: New methods for selecting partitioned models of evolution for molecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34:772–773.
Ligges, U., S. Krey, O. Mersmann, and S. Schnackenberg. 2017. tuneR: Analysis of music and speech. Available at https://CRAN.R-project.org/package=tuneR. Archived by WebCite at http://www.webcitation.org/75LSCIE5h on 11 January 2019.
Lyra, M.L., C.F.B. Haddad, and A.M.L. Azeredo-Espin. 2017. Meeting the challenge of DNA barcoding Neotropical amphibians: Polymerase chain reaction optimization and new COI primers. Molecular Ecology Resources 17:966–980.
Maniatis, T., E.F. Fritsch, and J. Sambrook. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, USA.
Márquez, R., I. de la Riva, and J. Bosch. 1995. Advertisement calls of Bolivian Leptodactylidae (Amphibia, Anura). Journal of Zoology 237:313–336.
Matlab. 2004. The Language of Technical Computing, version 6.5.2. The MathWorks, Inc., USA.
Menin, M., and D.J. Rodrigues. 2013. The terrestrial tadpole of *Leptodactylus andreae* (Anura, Leptodactylidae), a member of the *Leptodactylus marmoratus* group. Zootaxa 3683:92–94.
Miller, M.A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. Proceedings of the Gateway Computing Environments Workshop 2010:1–8.
Müller, L. 1923. Neue oder seltene Reptilien und Batrachier der zoologischen Sammlung des bayerischen Staates. Zoologischer Anzeiger 57:38–42.
Paradis, E., J. Claude, and K. Strimmer. 2004. APE: Analyses of phylogenetics and evolution in R language. Bioinformatics 20:289–290.
R Core Team. 2018. R: A Language and Environment for Statistical Computing. Available at http://www.r-project.org. R Foundation for Statistical Computing, Austria. Archived by WebCite at http://www.webcitation.org/732cRpqcw on 9 October 2018.
Rambaut, A., M.A. Suchard, D. Xie, and A.J. Drummond. 2014. Tracer, version 1.6. Available at http://tree.bio.ed.ac.uk/software/tracer. Archived by WebCite at http://www.webcitation.org/732cIWTxB on 9 October 2018.
Ronquist, F., M. Teslenko, P. van der Mark, D.L. Ayres, A. Darling, S. Höhna, B. Larget, L. Liu, M.A. Suchard, and J.P. Huelsenbeck. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61:539–542.
Sabaj, M.H. (ed.).2016. Standard Symbolic Codes for Institutional Resource Collections in Herpetology and Ichthyology: An Online Reference, version 6.5. American Society of Ichthyologists and Herpetologists, USA. Available at http://www.asih.org/sites/default/files/documents/symbolic_codes_for_collections_v6.5_2016.pdf. Archived by WebCite at http://www.webcitation.org/74ilEB3Nr on 16 December 2018.
Straughan, I.R., and W.R. Heyer. 1976. A functional analysis of the mating calls of the Neotropical frog genera of the *Leptodactylus* complex (Amphibia, Leptodactylidae). Papéis Avulsos de Zoologia, São Paulo 29:221–245.
Sueur, J., T. Aubin, and C. Simonis. 2008. Seewave: A free modular tool for sound analysis and synthesis. Bioacoustics 18:213–226.
Watters, J.L., S.T. Cummings, R.L. Flanagan, and C.D. Siler. 2016. Review of morphometric measurements used in anuran species descriptions and recommendations for a standardized approach. Zootaxa 4072:477–495.
Zimmerman, B.L., and J.P. Bogart. 1984. Vocalizations of primary forest frog species in the Central Amazon. Acta Amazonica 14:473–519.

Accepted on 20 March 2019
ZooBank.org registration LSID: E253373A-F58E-4A1F-91AF-1BCEB59AE73C
Published on 24 September 2019
Associate Editor: Julian Faivovich

Appendix I

Specimens Examined

Adenomera ajurauna.—BRAZIL: São Paulo: Santo André: Paranapiacaba (AAG-UFU 5024; MCP 13115).

Adenomera andreae.—BRAZIL: Amapá: Serra do Navio (AAG-UFU 5994, 6006–07, CFBH 43259, 43265); Amazonas: Manaus (INPA-H 34045, 34048, 34073–74, 34076, 34081–82, 34084–86, 34090; LHUFCG 189, 194, 197–204; ZUEC 3937, 3969, 3973–74, 7799); Pará: Belém (AAG-UFU 2797–98); Nova Timboteua (AAG-UFU 2788–94); ECUADOR: Orellana: Yasuní (QCAZ-A 64180–84, 64186–87, 64191–92, 64194–99).

Adenomera araucaria.—BRAZIL: Rio Grande do Sul: São Francisco de Paula (holotype: MCP 2421; paratypes: MCP 3208, 3345, 3463, 3672, 3676).

Adenomera bokermanni.—BRAZIL: Santa Catarina: Garuva (CFBH 43152, 43154).

Adenomera cotuba.—BRAZIL: Goiás: Teresina de Goiás (holotype: AAG-UFU 1400; paratypes: AAG-UFU 0808, 1397–99, 1401–04).

Adenomera diptyx.—BRAZIL: Mato Grosso: Cáceres (AAG-UFU 5366); Santo Antônio de Leverger: São Vicente (AAG-UFU 1435–38).

Adenomera engelsi.—BRAZIL: Santa Catarina: Florianópolis (holotype: MCP 6415; paratypes: MCP 6379, 6439–40, 7704–05, 8255–56, 8266–67).

Adenomera heyeri.—BRAZIL: Pará: Oriximiná: ESEC-Grão Pará (MPEG 30099–101).

Adenomera hylaedactyla.—BRAZIL: Acre: Cruzeiro do Sul (AAG-UFU 5907–11); Feijó (AAG-UFU 5895–97); Amazonas: Manaus (INPA-H 22410–13, 26606–09); São Gabriel da Cachoeira (AAG-UFU 3859–66); Roraima: Cantá (AAG-UFU 5540–43).

Adenomera juikitam.—BRAZIL: Goiás: Teresina de Goiás (holotype: AAG-UFU 1406; paratypes: AAG-UFU 0807, 1405).

Adenomera lutzi.—BRAZIL: Roraima: Upper Maú River (INPA-H 6247); GUYANA: Potaro-Siparuni (MZUSP 150799–804).

Adenomera marmorata.—BRAZIL: Rio de Janeiro: Tijuca (MZUSP 83374–76, 84079–81, 84083).

Adenomera martinezi.—BRAZIL: Pará: Novo Progresso: Cachimbo (holotype: MZUSP 73695; allotype: MZUSP 73684; topotypes: AAG-UFU 1515–25).

Adenomera nana.—BRAZIL: Santa Catarina: Jaraguá do Sul (MCP 8149–50); Joinville (MCP 8633); São Bento do Sul (MCP 8751–55).

Adenomera phonotriccus.—BRAZIL: Pará: Palestina do Pará (holotype: MPEG 41155; paratypes: CFBH 43130–31, MPEG 41156).

Adenomera saci.—BRAZIL: Goiás: Alto Paraíso de Goiás (holotype: AAG-UFU 1339; paratypes: AAG-UFU 0108–09, 0762–63; ZUEC 3287).

Adenomera simonstuarti.—PERU: Cusco, La Convención, Echarate, Río Camisea (holotype: MUSM 18218; paratypes: MUSM 18220–21, 18229).

Adenomera thomei.—BRAZIL: Espírito Santo: Linhares: Povoação (AAG-UFU 6185–86).

Adenomera sp. (*A. andreae* clade).—BRAZIL: Acre: Brasiléia (UFAC 5870); Capixaba (UFAC 5215); Cruzeiro do Sul (UFAC 2352); Feijó (UFAC 3445); Marechal Thaumaturgo (ZUEC 8440–43); Parque Nacional da Serra do Divisor (UFAC 1574, 4114); Porto Acre: Reserva Florestal Humaitá (UFAC 1132; ZUEC 11324); Rio Branco (CFBH 43564–66; UFAC 3475; ZUEC 24532); Tarauacá (UFAC 4412).

Appendix II

Information on Sound Recordings Analyzed

Sound recordings #1–4 are from *Adenomera chicomendesi*'s type locality (Parque Zoobotânico/UFAC, Rio Branco, Acre, Brazil); recordings #5–9 are from Tambopata National Reserve and adjacent buffer zone (Puerto

Maldonado, Madre de Dios, Peru):

(1) Voucher: male, CFBH 43652 (holotype)
  (1a) Sound file—Adenomera_chicomendesiRioBrancoAC1aTR-C_AAGm671; recorded by Thiago R. de Carvalho on 2 February 2017 at 1948 h, temperature not measured.
  (1b) Sound file—Adenomera_chicomendesiRioBrancoAC1bTR-C_AAGm671; recorded by Thiago R. de Carvalho on 2 February 2017 at 1951 h, temperature not measured.
(2) Voucher: male, AAG-UFU 5862
  Sound file—Adenomera_chicomendesiRioBrancoAC2TR-C_AAGm671; recorded by Thiago R. de Carvalho on 10 February 2017 at 1942 h, air temperature = 25°C.
(3) Voucher: male, ZUEC 24528
  Sound file—Adenomera_sp2_canto009; recorded by Marcelo N. C. Kokubum on 22 December 2005 at 1838 h, air temperature = 25°C.
(4) Voucher: male, ZUEC 24531
  Sound file—Adenomera_sp2_canto014; recorded by Marcelo N. C. Kokubum on 23 December 2005 at 1603 h, air temperature = 26°C.
(5) Voucher: male, USNM 342983
  Sound file—ML 198620; recorded by Reginald B. Cocroft on 30 December 1988 at 0007 h, air temperature = 22°C.
(6) Voucher: male, USNM 342984
  Sound file—ML 198622; recorded by Reginald B. Cocroft on 30 December 1988 at 0045 h, air temperature = 22°C.
(7) Voucher: male, ROM 40110
  Sound file—AA 9904; recorded by Ariadne Angulo on 6 January 1999 at 1720 h, air temperature = 24°C.
(8) Voucher: male, MUSM 39463
  Sound file—TRC 176; recorded by Thiago R. de Carvalho on 25 November 2018 at 2308 h, air temperature = 25°C.
(9) Unvouchered recording
  Sound file—TRC 177; recorded by Diego Barrera on 26 November 2018 at 2143 h, air temperature = 19°C.

Appendix III

Acoustic Definitions and Terminology

Acoustic traits	Brief description
Time domain	
Call traits	
Note duration (s)	From initial 10% amplitude (first pulse) to final 10% of note amplitude (last pulse)
Note rise time (%)	Point of maximum amplitude relative to note duration
Note rate (notes/min)	Note number minus 1, divided by the duration between the onset of first and last notes
Pulse traits	
Pulse duration (ms)	From initial 10% to final 10% of pulse amplitude
Pulse rate (pulses/s)	Pulse number minus 1, divided by duration of peak-to-peak from first to last pulse of the note
Frequency domain	
Call traits	
Fundamental frequency (Hz)	Peak frequency of the first harmonic
Dominant frequency (Hz)	Frequency containing the greatest energy in the note
Linear frequency modulation (Hz)	Difference between the peak frequency from final 10% (last pulse) to initial 10% (first pulse) of note amplitude