R e s e a r c h   a r t i c l e

urn:lsid:zoobank.org:pub:D2F73DB1-D4EF-4708-ADEC-7BD341240F54

Redescription of *Adenomera diptyx* (Boettger, 1885) (Anura, Leptodactylidae) and description of a closely related new species

Víctor H. ZARACHO [1,*], Esteban O. LAVILLA [2], Thiago R. CARVALHO [3], Martha MOTTE [4] & Néstor G. BASSO [5]

[1] Laboratorio de Herpetología, Departamento de Biología, Facultad de Ciencias Exactas y Naturales y Agrimensura, Universidad Nacional del Nordeste, Av. Libertad 5470, (3400) Corrientes, Argentina.
[2] Unidad Ejecutora Lillo (UEL), CONICET-FML, Miguel Lillo 251, (4000) S.M. Tucumán, Argentina.
[3] Departamento de Zoologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.
[4] Museo Nacional de Historia Natural del Paraguay, Km 10,5 Ruta Mcal. Estigarribia, San Lorenzo, Paraguay.
[5] Instituto de Diversidad y Evolución Austral (IDEAus-CONICET), Bvd. Brown 2915; and Facultad de Ciencias Naturales y Ciencias de la Salud, Universidad Nacional de la Patagonia "San Juan Bosco", (9120) Puerto Madryn, Chubut, Argentina.

[*] Corresponding author: victorzaracho@exa.unne.edu.ar
[2] Email: eolavilla@gmail.com
[3] Email: carvalho.thiagoribeiro@gmail.com
[4] Email: marthamottep@gmail.com
[5] Email: nesbasso@gmail.com

[1] urn:lsid:zoobank.org:author:5DAFD855-5520-4FAC-A578-84628225B861
[2] urn:lsid:zoobank.org:author:1CAEDA07-7C9E-436A-A9C2-93A09A3D8B48
[3] urn:lsid:zoobank.org:author:A1286A8F-C01A-421B-8135-A333D321AC95
[4] urn:lsid:zoobank.org:author:28A331B6-8083-4F40-BA9F-40BC3DD27B90
[5] urn:lsid:zoobank.org:author:F3BFB896-7F2C-4971-B274-E23A48978DD3

Abstract. *Adenomera* is a genus of Neotropical leaf-litter frogs widely distributed in South America and regarded taxonomic-wise challenging. One of these is the open-habitat *Adenomera diptyx* from Paraguay, which may correspond to a species complex. An integrative analysis of morphological variation, in combination with acoustic and molecular data of several populations from Argentina, Paraguay, and Brazil resulted in the recharacterization of nominal *A. diptyx* and the description of a new, closely related species. *Adenomera diptyx* is recognized by its advertisement call given at a high repetition rate (176–299 per minute), and the dorsal color pattern consisting of a light mid-dorsal line and a dark brown interorbital bar with irregular black edges. In comparison with *A. diptyx*, the new species, *Adenomera guarani* sp. nov., produces its advertisement call at a lower repetition rate (73–147 per minute), and the dorsal color pattern consists of a light, broad, mid-dorsal stripe and a mask-like patch in the interorbital region. The redefinition of *A. diptyx* will contribute to future studies focusing on the taxonomic status of other genetic lineages tentatively assigned to this species complex, which could represent additional unnamed species in the open-habitat *Adenomera* clade.

Introduction

Adenomera Steindachner, 1867 is a genus of Neotropical leaf-litter frogs widely distributed in South America. This frog genus contains 29 species, most of them described or revalidated in the past twenty years (Carvalho *et al.* 2021). This genus of leptodactylid frogs has been considered as a taxonomically challenging group, mainly because of considerable intraspecific variation allied to repeated cases of sympatric occurrence among species (Angulo *et al.* 2003; Carvalho *et al.* 2019a; Cassini *et al.* 2020). This renders interspecific discrimination difficult based on morphological characters alone. Furthermore, the taxonomy of some species has not been addressed because of the lack of precise information on type localities and preservation conditions of type specimens (see Carvalho *et al.* 2019a for an example). Fouquet *et al.* (2014) conducted a phylogenetic analysis of *Adenomera* based on a DNA sequence dataset containing 320 samples collected across South America. The phylogenetic and lineage delimitation analyses uncovered 31 confirmed candidate species. Some of these genetic lineages were subsequently named and described as new species of *Adenomera* in a series of taxonomic contributions to the genus (Carvalho *et al.* 2019b, 2019c, 2019d, 2020a), including lineages that had not been sampled previously (see Carvalho *et al.* 2020c, 2021).

One of the problematic species is the open-habitat *Adenomera diptyx* (Boettger, 1885) from Paraguay. Since its original description, this species name has been applied to Chacoan populations (e.g., Nieden 1923; A. Lutz 1930; Freiberg 1942; Cei 1956; Gallardo 1966). *Adenomera diptyx* was placed into the synonymy of *A. marmorata* Steindachner, 1867 by Cochran (1955), and then *A. hylaedactyla* (Cope, 1868) by Heyer (1973). Both names were widely used in the literature in the mid-to-late 20th century to refer to *A. diptyx* (e.g., Gorham 1966, 1974; Cei 1980, 1987; Gallardo 1986, 1987a, 1987b; Mcdiarmid & Foster 1987; Gallardo & Varela de Olmedo 1992; Lavilla 1992; Gavetti & Andreone 1993; Yanosky *et al.* 1993; Mercolli *et al.* 1995).

Adenomera hylaedactyla and *A. marmorata* have non-feeding larvae that complete their development in terrestrial foam nests (e.g., Heyer *et al.* 1990; Kokubum & Sousa 2008), but the tadpoles of the Chacoan populations have functional mouthparts and a spiracle, completing their development in temporary aquatic habitats, such as rain puddles and seasonally flooded plains (De la Riva 1995; Zaracho & Kokubum 2017). Based on the reproductive mode with exotrophic tadpoles, De la Riva (1996) removed *A. diptyx* from the synonymy of *A. hylaedactyla*. Since its revalidation, the name *A. diptyx* has been applied to several populations distributed in Bolivia, Brazil, Paraguay, and Argentina.

Fouquet *et al.* (2014) found that *A. diptyx* has a deep genetic structure in mitochondrial DNA sequences, comprising four lineages occurring in Bolivia, Brazil, Paraguay, and Argentina. Those authors suggested that *A. diptyx* likely corresponds to more than one species. We compared several populations of *Adenomera* from Argentina, Paraguay, and Brazil, based on morphological, acoustic, and DNA sequence data, and confirmed the existence of a species complex, as already suggested in previous studies (De la Riva 1996; Lavilla & Cei 2001; Brusquetti & Lavilla 2006; Fouquet *et al.* 2014). The existence of two distinct lineages in Paraguay is relevant since the name-bearing type of *A. diptyx* was collected in Paraguay, but all Paraguayan populations of *Adenomera* have historically been assigned to a single taxon. The examination of the type material of *A. diptyx* and data retrieved from its original description allowed us to identify populations belonging to *A. diptyx* of Boettger (1885), the nominal species, and

distinguish them from those of another species also occurring in Paraguay and adjacent countries. This latter species was identified as distinct from nominal *A. diptyx* and is named and described herein as a new species.

Material and methods

Examined specimens

We analyzed 181 individuals of *Adenomera* from 29 localities in northeastern Argentina and Paraguay, housed at UNNEC, MACN, MNHNP, and CZCEN (Fig. 1). Most of the examined specimens were collected in the field for this study. Specimens were euthanized with benzocaine solution, fixed in 10% formalin, and stored in 70% ethanol. Additionally, we examined other specimens of *Adenomera* housed at LHUFCG, AAG-UFU, and MCP herpetological collections. The list of examined specimens appears in the Results and/or Appendix 1. In addition to these, three type specimens of *A. diptyx*, housed at NHM and NMW collections, were analyzed based on high-quality photographs provided by curators and collection managers of the two natural history museums.

Abbreviations

|		
AAG-UFU	=	Amphibian Collection of the Museu de Biodiversidade do Cerrado, Universidade Federal de Uberlândia, Ituiutaba, Brazil
CFBH	=	Herpetological Collection of the Universidade Estadual Paulista, Rio Claro campus, Rio Claro, Brazil
CHUNB	=	Coleção Herpetológica da Universidade de Brasília, DF, Brazil
CZCEN	=	Colección Zoológica de la Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Asunción, San Lorenzo, Paraguay
IIBPH	=	Herpetological Collection of the Instituto de Investigación Biológica del Paraguay, Asunción, Paraguay
INPA-H	=	Herpetological Collection of the Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil
LGE	=	Laboratorio de Genética Evolutiva, Instituto de Biología Subtropical, Posadas, Misiones, Argentina
LHUFCG	=	Herpetological collection of the Universidade Federal de Campina Grande, Patos, Brazil
LSU	=	Louisiana State University, Museum of Natural Science, Baton Rouge, Louisiana, USA
MACN	=	Museo de Ciencias Naturales Bernardino Rivadavia, Buenos Aires, Argentina
MCP	=	Herpetological collection of the Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil
MHNC	=	Museo de Historia Natural Alcide d'Orbigny, Cochabamba, Bolivia
MNCN	=	Museo Nacional de Ciencias Naturales, Madrid, Spain
MNHNP	=	Museo Nacional de Historia Natural del Paraguay, San Lorenzo, Paraguay
MPEG	=	Herpetological Section of the Museu Paraense Emílio Goeldi, Belém, Brazil
MUSM	=	Herpetological Section of the Museo de Historia Natural de la Universidad Nacional Mayor de San Marcos, Lima, Peru
MZUSP	=	Herpetological Section of the Museu de Zoologia da Universidade de São Paulo, São Paulo, Brazil
NHM	=	Natural History Museum (formerly British Museum of Natural History = BMNH), London, England
NMW	=	Naturhistorisches Museum Wien (Natural History Museum Vienna), Vienna, Austria
ROM	=	Royal Ontario Museum, Department of Herpetology, Toronto, Ontario, Canada
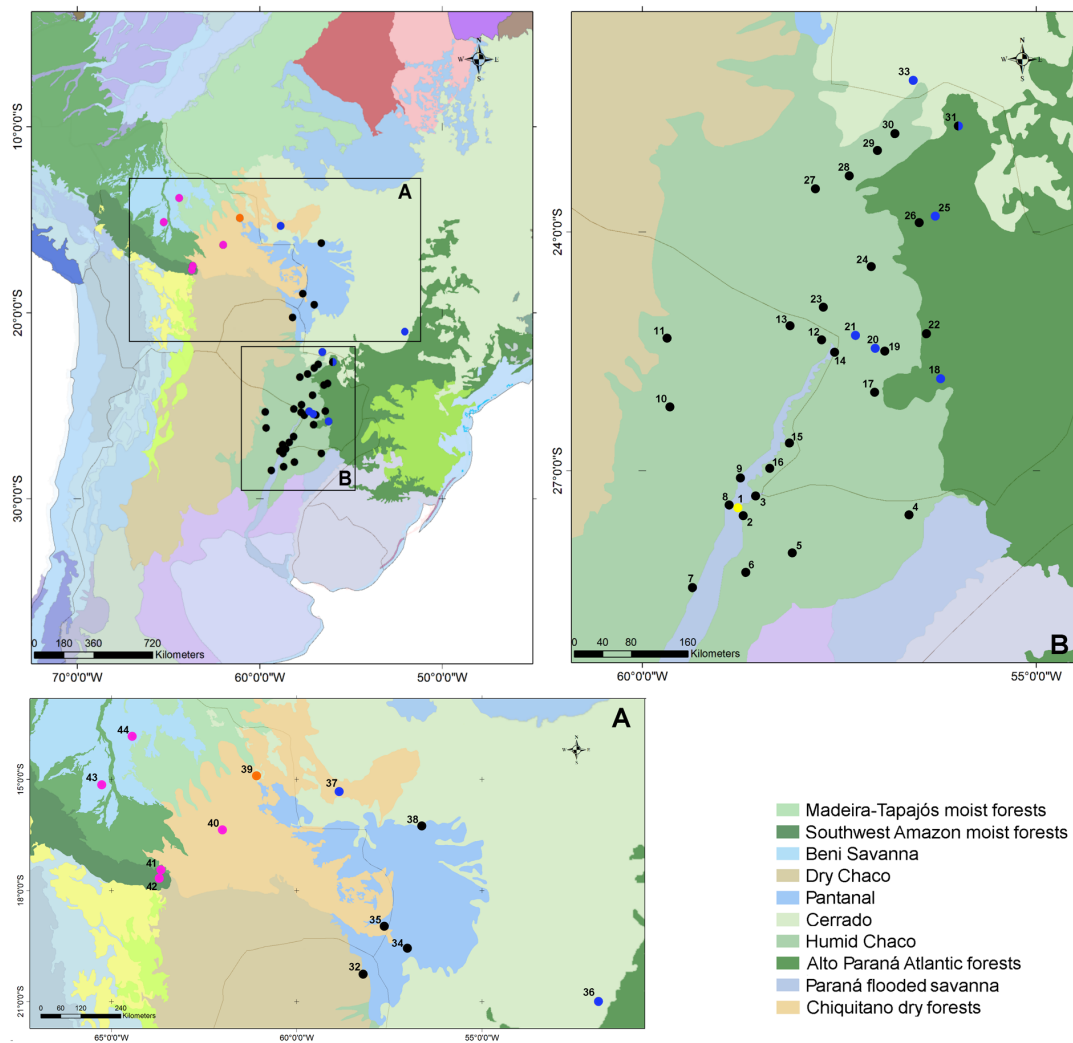
Fig. 1. Geographic distribution of *Adenomera diptyx* (Boettger, 1885) (blue circles) and *Adenomera guarani* sp. nov. (black circles and yellow circle = type locality). Subclade 3 (pink circles) and subclade 4 (orange circle) according Fouquet *et al.* (2014) are also represented. ARGENTINA: *Corrientes*: 1) Corrientes; 2) San Cayetano; 3) Paso de la Patria; 4) Ituzaingó; 5) Parque Nacional Mburucuyá; 6) Saladas (acoustic record by VHZ no voucher); *Santa Fe*: 7) Villa Ocampo (acoustic record by VHZ, no voucher); *Chaco*: 8) Resistencia (acoustic record by VHZ, no voucher), 9) La Leonesa; 10) San Martín; *Formosa*: 11) Fontana; 12) Clorinda; 13) Laguna Naick Neck. PARAGUAY: *Central*: 14) Villeta; *Ñeembucú*: 15) 26 km NE of Pilar; 16) 19,8 km SSW of Pilar; *Paraguarí*: 17) Parque Nacional Ybycuí; *Guairá*: 18) Reserva de Recursos Manejados Ybyturuzú (Airaldi *et al.* 2013); *Cordillera*: 19) Saltos de Piraretá; 20) Piribebuy; *Central*: 21) Lago de Ypacaraí (Fouquet *et al.* 2014); *Caaguazú*: 22) 24 km N of Coronel Oviedo; *Presidente Hayes*: 23) Ea. La Golondrina; *San Pedro*: 24) Villa del Rosario; 25) Laguna Blanca; 26) Lima; *Presidente Hayes*: 27) 44 km W of Concepción, Riacho Negro; *Concepción*: 28) 19 km NE of Concepción; 29), Rancho Z; 30) 85 km W of Concepción, Arroyo Capitigo; *Amambay*: 31) Parque Nacional Cerro Corá; *Alto Paraguay*: 32) Ea. Doña Julia. BRAZIL: *Mato Grosso do Sul*: 33) Bela Vista; 34) Pantanal Research Station (= Nhecolândia, Passo do Lontra); 35) Corumbá (Fouquet *et al.* 2014); 36) Três Lagoas; *Mato Grosso*: 37) UHE Guaporé; 38) Poconé (Fouquet *et al.* 2014). BOLIVIA: *Santa Cruz*: 39) Caparú, Velasco; 40) San Sebastián; 41) Buena Vista; 42) Parque Nacional Amboró; *Beni*: 43) Santa Anita; 44) Paraparau.

UFMG-AMP = Collection of Amphibians of Centro de Coleções Taxonômicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil
UFMT = Herpetological Collection of the Universidade Federal de Mato Grosso, Cuiabá, Brazil
UNNEC = Colección Herpetológica de la Universidad Nacional del Nordeste, Corrientes, Argentina
ZMB = Museum für Naturkunde, Berlin, Germany

External morphology and color patterns

Measurements (in millimeters) were taken with digital calipers to the nearest 0.01 mm. Abbreviations used are: (1) SVL: snout-vent length (from tip of snout to posterior margin of vent); (2) HL: head length (from tip of snout to jaw articulation); (3) HW: head width (greatest width of the head measured between jaw articulations); (4) ED: eye diameter (distance between anterior and posterior corners of eye); (5) TD: horizontal tympanum diameter (between anterior and posterior borders of tympanum); (6) EN: eye-nostril distance (from anterior corner of eye to posterior border of nostril); (7) IOD: interorbital distance (shortest distance between the medial margins of upper eyelids); (8) IND: internarinal distance (between internal borders of nostrils); (9) UAL: upper arm length (from arm insertion to elbow); (10) LAR: lower arm length (from elbow to base of outer metacarpal tubercle); (11) HAL: hand length (from the base of the outer metacarpal tubercle to tip of Finger III; (12) THL: thigh length (from vent to knee); (13) TIL: tibia length (from knee to heel); (14) TAL: tarsal length (from heel to base of inner metatarsal tubercle); and (15) FL: foot length (from base of inner metatarsal tubercle to tip of toe IV). The toe tip development (character states A–D) was assessed according to Heyer (1973) and modified by Carvalho *et al.* (2019a). Morphological comparisons were based on original species descriptions, redescriptions, and taxonomic reviews of species of *Adenomera* (Heyer 1973, 1975; Kwet & Angulo 2002; Almeida & Angulo 2006; Kok *et al.* 2007; Kwet 2007; Angulo & Reiche 2008; Berneck *et al.* 2008; Kwet *et al.* 2009; Angulo & Icochea 2010; Carvalho & Giaretta 2013a, 2013b; Carvalho *et al.* 2019a, 2019b, 2019c, 2019d, 2020a, 2020c, 2021; Cassini *et al.* 2020), as well as specimens morphologically examined by us (see Appendix 1).

Acoustic data acquisition and analysis

Advertisement calls of 10 males of *Adenomera diptyx* from two localities in Paraguay and two males from one locality in Brazil, and 22 males of the new species from five localities in Argentina and one male in Brazil were analyzed in this study (see Appendix 2 for locality and call vouchers). Calls were recorded using a Sony TCM 200-DV tape recorder equipped with built-in microphones and a Marantz PMD 222 tape recorder (with metal tapes) or M-Audio Micro Track II digital recorder and a Sennheiser ME66/K6 unidirectional microphone. Call recordings were made using a sampling rate of 44.1 kHz and a sample size of 16 bits. Recordings were deposited in the Fonoteca Zoológica de la Universidad Nacional del Nordeste (FZ-UNNE) and CFBH/AAG-UFU (see Appendix 2 for locality data). Calls were analyzed using automated procedures in a customized version (ver. 0.9.6.1) of Soundruler interfacing with Matlab (MathWorks, Inc.) scripts. Note repetition rate was assessed using Audacity ver. 2.1.1 (Audacity Team 2017). Acoustic definitions and terminology follow those of Carvalho *et al.* (2019b). A 300-Hz high-pass filter was applied to sound files in Soundruler before conducting the acoustic analysis to reduce background noise. Descriptive statistics are reported as minimum–maximum (mean ± standard deviation). Grand means and standard deviations for pulse duration were obtained from mean values of pulse duration for each multi-pulsed call. Analytical parameters were set as follows: fast Fourier transform (FFT) size = 1024 samples, FFT overlap = 90%, window = Hann, contrast = 70%. Settings for automated recognition of pulses (in sample sizes) were defined as follows: pulse detection (smoothing = 300, resolution = 1); pulse delineation (smooth factor = 1, smoothing = 20, resolution = 1). Critical amplitude ratio = 0.9. Sound figures were produced in seewave 2.1.0 (Sueur *et al.* 2008) and tuneR

ver. 1.3.2 (Ligges *et al.* 2017), implemented in R ver. 3.5.0 (R Core Team 2018), using the following spectrogram settings: window Hann, FFT size = 256 samples, FFT overlap = 90%; the darkness of frequency components indicates intensity in a relative 36-dB scale (0 dB = maximum amplitude of the signal).

Acoustic comparisons were based on the acoustic traits of species of *Adenomera* summarized in Carvalho *et al.* (2021: table 3), additional data obtained from the references cited therein (included in our list of references), and three additional references (Carvalho *et al.* 2020a, 2020b, 2020c).

Chromosome analysis

Chromosome spreads of an individual of *Adenomera diptyx* (UNNEC 13936) were obtained from intestinal epithelium and testes, following Schmid (1978). They were conventionally stained with Giemsa diluted in phosphate-buffered saline solution, pH 6.8. Silver staining of the NORs (Ag-NOR) was obtained following Howell & Black (1980).

Molecular analysis

Liver or muscle samples of eight specimens from five localities were extracted and preserved in 96% ethanol (Appendix 1). DNA was extracted using the standard salt-extraction method (Aljanabi & Martinez 1997) or DNEasy Tissue Kit (QIAGEN, Valencia, CA). A partial region of the cytochrome-*b* gene (cyt-*b*) for seven specimens and cytochrome oxidase I (*COI*) for eight specimens from the mitochondrial genome, and recombinase activating protein 1 (*RAG1*) for seven specimens from nuclear genome were amplified and sequenced using the primer sets MVZ-15/MVZ-16 (Moritz *et al.* 1992), T3-AnF1/T7-AnR1 (Lyra *et al.* 2017), F1GFF/F1GFR (Faivovich *et al.* 2005), respectively. Amplification was carried out in a 25 μl – volume reaction using standard PCR conditions with the following thermal cycle profiles: 2 min at 94ºC, followed by 40 cycles of 94ºC for 30 s, 45ºC for 45 s, and 72ºC for 120 s, and a final extension step at 72ºC for 6 min for cyt-*b*; 3 min at 94ºC, followed by 35 cycles of 95ºC for 20 s, 50ºC for 20 s, and 60ºC for 60 s, and a final extension step at 72ºC for 5 min for *COI*; and 2 min at 94ºC, followed by 35 cycles of 94ºC for 30 s, 52ºC for 60 s, and 72ºC for 60 s, and a final extension step at 72ºC for 6 min for *RAG1*.

Amplified products were purified using Geneclean III PCR Purification Kit (MP biomedical). The products were sequenced in both directions using the standard protocol for Big DyeTerminator ver. 3.1 chemistry in an ABI 3130 Genetic Analyser (Applied Biosystems, Inc). We obtained ~760 bp for cyt-*b*, ~650 bp of *COI*, and ~415 bp for *RAG-1*. Additional sequences were obtained from GenBank (see Appendix 3 for Genbank accession number linked to sequences included in the analysis). We included partial fragments of cyt-*b*, *COI*, *RAG1*, and proopiomelanocortin (*POMC*) of Fouquet *et al.* (2014) for all individuals assigned to *A. diptyx* (four genetic lineages) and one individual of each of the eleven lineages of *A. hylaedactyla* because of their close relationship. We included at least one species of the other clades of *Adenomera* (sensu Fouquet *et al.* 2014). We used *Lithodytes lineatus* (Schneider, 1799) as the outgroup taxon.

Each marker was aligned using the Muscle option in Mega X (Kumar *et al.* 2018) under default parameters, and subsequently concatenated using SequenceMatrix ver. 1.8 (Vaidya *et al.* 2011). The molecular dataset resulted in 3420 bp. To find the best-fitting model for each marker, we used Partition Finder ver. 2 (Lanfear *et al.* 2017) with a greedy searching scheme (Lanfear *et al.* 2012). The four fragments are protein-coding genes, thus treated as separate partitions. The best partition scheme was selected through the corrected Akaike Information Criterion (AICc; Hurvich & Tsai 1989), which is as follows: *COI*1 (SYM+I+G), *COI*2 (F81+I), *COI*3 (GTR+G), cyt-*b* 1 and *POMC*3 (SYM+I+G), cyt-*b* 2 (GTR+I), cyt-*b* 3 (GTR+G), *RAG* 1 (GTR+I), *RAG* 2 (HKY+I), *RAG* 3 (HKY+G), *POMC* 1 (GTR+I+G), and *POMC* 2 (HKY+I).

We inferred the phylogeny through a Bayesian inference optimality criterion. The Bayesian analysis was conducted using MrBayes ver. 3.2.7a (Ronquist *et al.* 2012), in two independent runs with default priors' values, each composed of four independent Monte Carlo Markov chains of 20 million generations, sampled every 1000 generations with a burn-in of 25%. We examined the stationarity of parameters by checking the effective sample size (ESS $>$ 200), average standard deviation of split frequencies ($<$ 0.01), and potential scale reduction factor (PSRF the closest possible to 1.0; Gelman & Rubin 1992). Both the analysis of nucleotide substitution models and phylogenetic reconstruction were run on the CIPRES Science Gateway website (Miller *et al.* 2011). The alignment and tree files are provided as supplemental files (Supp. file 1, Supp. file 2).

Genetic distances (uncorrected *p*-distances) in the COI gene were calculated within and among lineages of *A. diptyx* using Mega X, with uniform rates among sites and the same (homogeneous) pattern among lineages. Missing data were pairwise deleted.

Results

Taxonomic history

Adenomera diptyx (originally *Leptodactylus diptyx* Boettger, 1885) was described based on five individuals (syntypes) from "Paraguay, Amer. Merid.". They were part of a collection of amphibians and reptiles obtained by H. Rohde in Paraguay and loaned to Boettger by the Naturhistorisches Institut Linnaea (G. Köhler, pers. com. to V.H.Z.). "Linnaea" was a commercial institution based in Frankfurt am Main and in Berlin after 1885. This institution no longer exists but used to purchase animals from various collectors. Linnaea identified and sold specimens to many museums, including those of London, Vienna, and St Petersburg, among others (Milto 2010).

The Natural History Museum houses two specimens identified originally as *Leptodactylus marmoratus* cotypes 1947.2.17.47-48 "Linnaea" (P. Campbell, pers. com. to V.H.Z.), one of which (BMNH 1947.2.17.47) was designated as the lectotype of *Leptodactylus diptyx* Boettger by Heyer (1973). It is an adult male, characterized as follows: "the tips of the toes are pointed, the dorsum is spotted, there are narrow lateral stripes on the sides of the body, and there is a mid-dorsal pinstripe from above the vent extending beyond the sacral region" (Heyer op. cit.).

More than thirty years later, Kwet (2007) mentioned that the specimen ZMB 10595 should correspond to a paralectotype, because the specimen is marked as the type of *A. diptyx* in the catalog of the Museum für Naturkunde Berlin, and also indicated on the label of the jar. This specimen (Kwet 2007: fig. 10) is similar to that described by Boettger (e.g., the presence of an evident mid-dorsal pin stripe).

One new paralectotype was found in the Natural History Museum Vienna: NMW 4475. The specimen was cataloged originally as "*Leptodactylus diptyx*", but is currently identified as "*Adenomera hylaedactyla*, Coll Linnaea 1885.17" (G. Gassner pers. com. to V.H.Z). The specimen is well-preserved, but the color pattern is faded and the glands are flattened.

The location of the one remaining individual of the type series of *Adenomera diptyx* was tracked down in other collections. In the Forschungsinstitut und Naturmuseum Senckenberg collection, where Boettger worked, there are no types or other specimens of *Adenomera diptyx* (G. Köhler, pers. com. to V.H.Z.). Boettger (1892) carried out a catalog of amphibians of this museum, including several specimens from "Linnaea" Institute, but no specimens of *Adenomera* are mentioned in Boettger's catalog. The Saint Petersburg museum also houses specimens collected by Rhode in Paraguay and described by Boettger, but its amphibian collection does not contain any specimens of *Adenomera diptyx* (N. Ananjeva pers. com. to V.H.Z).
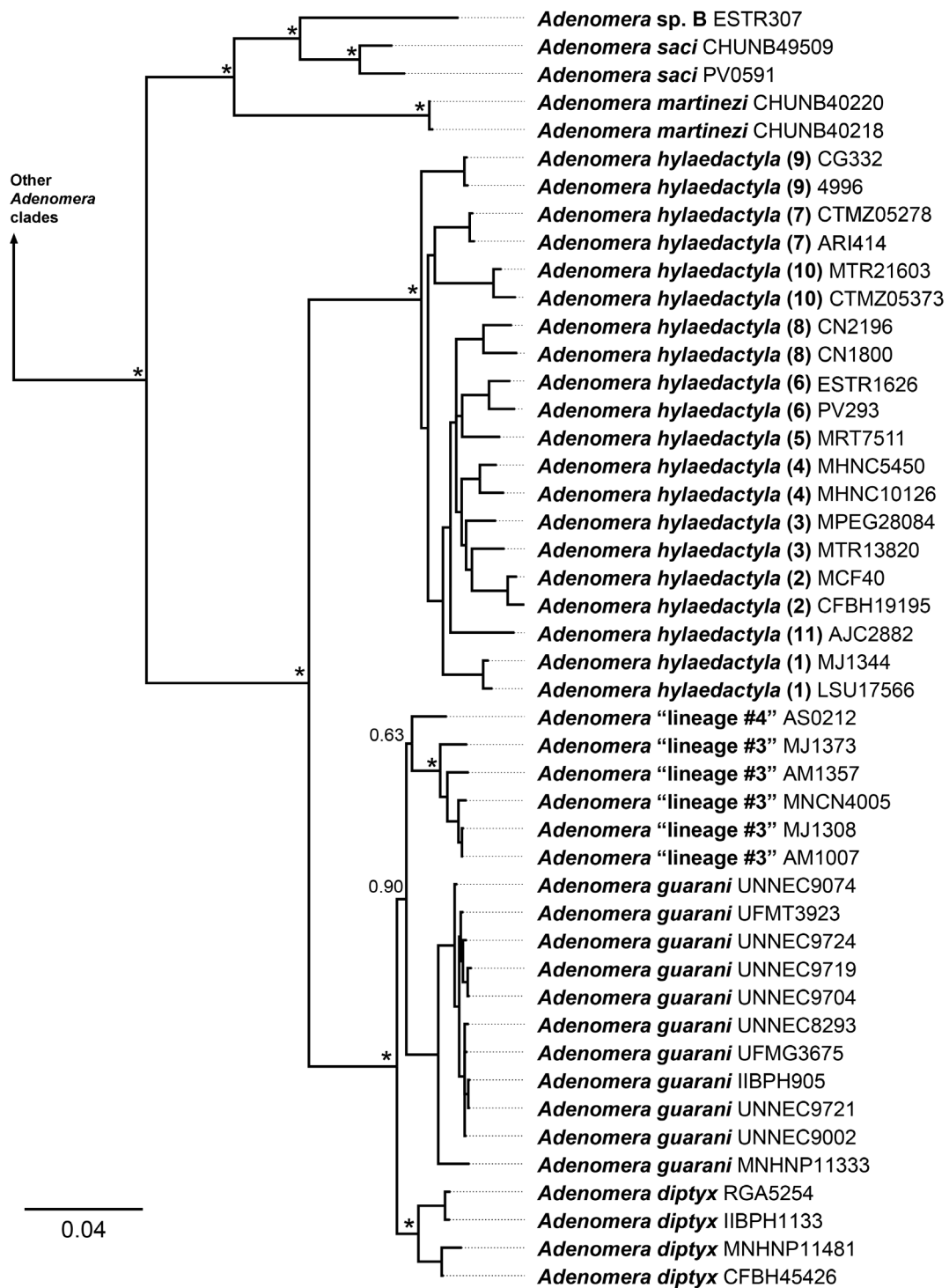
Fig. 2. Phylogenetic relationships of the *Adenomera* Steindachner, 1867 open-habitat clade, based on the 50% majority-rule consensus tree from Bayesian inference of a concatenated dataset of four (two mitochondrial + two nuclear) genes. Outgroup sampling and molecular dataset are presented in the Material and Methods and Appendix 3. Terminals are species names, followed by voucher specimens. Numbers in parentheses across *A. hylaedactyla* (Cope, 1868) terminals indicate the lineage number (sensu Fouquet *et al.* 2014). Posterior probabilities (PP) are provided near the nodes (not shown below species level). Asterisks denote PP = 1.0. Scale bar is given as nucleotide substitution rate.

Table 1. Uncorrected pairwise genetic distances (%; reported as minimum–maximum) of partial COI gene within and among the four subclades of the *Adenomera diptyx* species complex. Within-species distances are indicated in bold (sample sizes). Subclade 1 = *A. guarani* sp. nov., subclade 2 = *A. diptyx* (Boettger, 1885).

	A. diptyx	*A. guarani* sp. nov.	Subclade 3	Subclade 4
A. diptyx	**0.0–0.5 (4)**			
A. guarani sp. nov.	6.7–7.9	**0.0–1.0 (11)**		
Subclade 3	5.1–6.8	5.9–6.9	**0.0–3.0 (5)**	
Subclade 4	6.4–6.7	5.9–6.6	5.5–6.4	**− (1)**

Phylogenetic affinities and genetic diversity of nominal Adenomera diptyx

Our phylogenetic analysis of DNA sequence data focusing on the *Adenomera* open-habitat clade recovered the four lineages of *A. diptyx* that had been previously identified by Fouquet *et al.* (2014). Lineage #2 is linked to the nominal species. Nominal *A. diptyx* is sister to the other three lineages. Lineage #1 is the one named and described as a new species in this study, which is sister to the other two lineages (3 and 4). Relationships within the *A. diptyx* complex can be visualized in Fig. 2.

The maximum genetic distance in the COI gene among the four lineages of *A. diptyx* is 7.9%. The genetic diversity within lineage #2 (i.e., nominal *A. diptyx*) ranges from none to 0.5%, and that within lineage #1 (i.e., the species described herein as new) from none to 1.0%. The genetic diversity between *A. diptyx* and lineage #1 ranges from 6.7 to 7.9%. See Table 1 for genetic distances in the COI gene within and among the four lineages of *A. diptyx*.

Species accounts

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

Adenomera diptyx (Boettger, 1885), redescription
Figs 3–6; Tables 2–3

Diagnosis

Adenomera diptyx is recognized within *Adenomera* by the following combination of character states: (1) body slender; (2) medium size (adult male SVL = 18.2–23.1 mm); (3) a long vertebral pin stripe, extending from the interorbital region to the vent;(4) a distinctive interorbital bar; (5) toe tips unexpanded, tending to a tapered point; (6) antebrachial tubercle absent; (7) single-note advertisement call; (8) call notes formed by incomplete pulses; (9) short note duration (28–49 ms); (10) high note repetition rate (176–299 per minute); and (11) low pulse number (2–6 per note).

Type material

    Lectotype
PARAGUAY • ♂ (Fig. 3A–C); "Paraguay, Amer. Merid" (exact locality unknown); 1985; H.R. Rhode leg.; BMNH 1947.2.17.47.
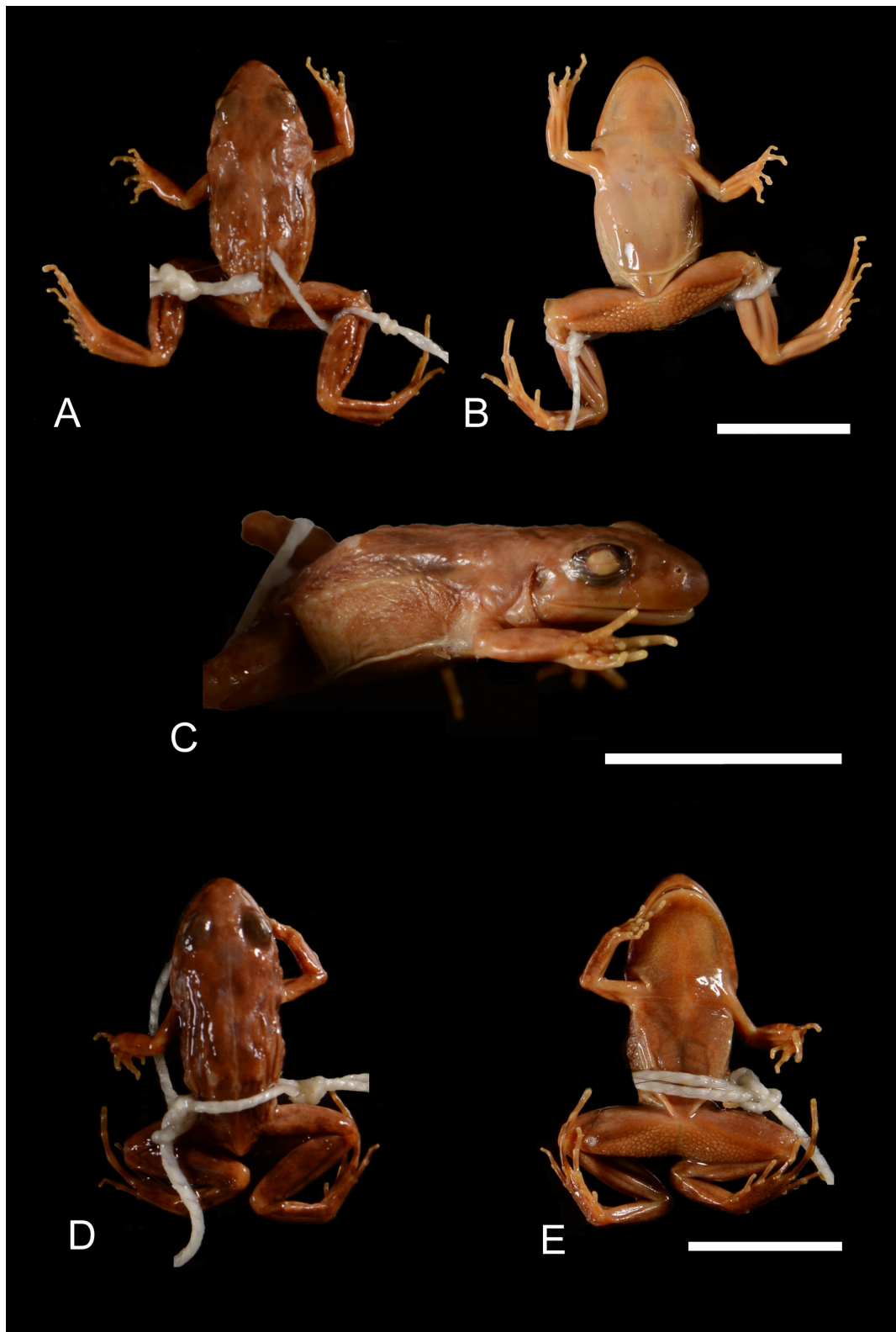
9

Fig. 3. *Adenomera diptyx* (Boettger, 1885), type specimens housed at the Natural History Museum. **A–C**. Lectotype, ♂ (BMNH 1947.2.17.47). **A**. Dorsal view. **B**. Ventral view. **C**. Lateral view. **D–E**. Paralectotype (BMNH 1947.2.17.48). **D**. Dorsal view. **E**. Ventral view. (Photographic credit: NHM, edited by VHZ.) Scale bars = 1 cm.

Paralectotypes (3 specimens examined; one specimen not examined, possibly lost)
PARAGUAY • 1 spec.; same collection data as for lectotype; BMNH 1947.2.17.48 (Fig. 3D–E) • 1 spec.; same collection data as for lectotype; ZMB 10595 • 1 spec.; same collection data as for lectotype; NMW 4475 (Fig. 4).

Material examined

PARAGUAY – **Amambay** • 6 ♀♀; Parque Nacional Cerro Corá; 22°39′56″ S, 56°00′24″ W; MNHNP 0416, 0429, 1221, 4420, 4492, 5709 • 1 ♀; same collection data as for preceding; UNNEC 13937 • 2 ♂♂; same collection data as for preceding; MNHNP 0418, 0438 • 1 ♂; same collection data as for preceding; UNNEC 13936. – **Cordillera** • 2 ♀♀; Piribebuy; 25°28′06″ S, 57°02′33″ W; CZCEN 0646, 0647, 0650 • 8 ♂♂; same collection data as for preceding; CZCEN 0640 to 0645, 0648, 0649.

Redescription of *A. diptyx*

The original description of the syntypes of *Adenomera diptyx* by Boettger (1885) included the main morphological characters of the species, some of which were later highlighted by Heyer (1973) when he designated the species' lectotype. The following re-description is based on all the specimens seen and examined by us, including specimens recently collected and linked to calls and DNA sequences.

Morphology

Body medium, slender (SVL = 18.2–25.9 mm); head nearly as broad as long (HW/HL = 1.06). Measurements of males and females are given in Table 2. Sexual dimorphism in head shape: males with snout sub-elliptical dorsally and acuminate laterally due to the presence of a developed, shovel-shaped, fleshy ridge, and females with snout rounded laterally, without a fleshy ridge (Fig. 5D). Canthus rostralis round, indistinct; loreal region slightly concave; eye dorsolateral, its diameter about 34% of head length; pupil horizontal, elliptical; palpebral membrane translucent, bordered in its upper edge by a black streak; tympanum distinct, its diameter about 47% eye diameter; tympanic membrane translucent; supratympanic fold well developed, extended from eye to arm insertion; nostrils anterolateral, closer to the tip of the snout than to the eye; internarial distance about 28% head width; postcommissural gland oval; lower lip

Fig. 4. *Adenomera diptyx* (Boettger, 1885), paralectotype housed at the Natural History Museum Vienna (NMW 4475). Dorsal and ventral views. (Photographic credit: A. Schumacher.) Scale bar = 5 mm.

Table 2. Morphological measurements (mm) of males and females of *Adenomera guarani* sp. nov. from Argentina and Paraguay, and *A. diptyx* (Boettger, 1885) from Paraguay. Mean ± SD (minimum–maximum). Abbreviations: ED = eye diameter; EN = eye–nostril distance; FL = foot length; HAL = hand length; HL = head length; HW = head width; IND = internarinal distance; IOD = interorbital distance; LAR = lower arm length; SVL = snout–vent length; TAL = tarsus length; TD = horizontal tympanum diameter; THL = thigh length; TIL = tibia length; UAL = upper arm length.

	Adenomera guarani sp. nov.		*Adenomera diptyx*	
	Males (N = 75)	**Females (N = 37)**	**Males (N = 11)**	**Females (N = 8)**
SVL	23.1 ± 1.3 (20.1–26.4)	22.9 ± 2.5 (18.4–27.6)	20.8 ± 1.4 (18.2–23.1)	22.1 ± 1.8 (20.6–25.9)
HL	7.7 ± 0.4 (6.9–8.7)	7.5 ± 0.7 (6.3–8.7)	7.0 ± 0.6 (6.0–8.0)	7.1 ± 0.5 (6.5–7.8)
HW	8.3 ± 0.5 (6.6–9.2)	8.1 ± 0.8 (6.7–9.5)	7.4 ± 0.4 (6.7–8.1)	7.5 ± 0.5 (7.3–8.4)
ED	2.6 ± 0.2 (2.2–3.1)	2.5 ± 0.3 (2.0–3)	2.4 ± 0.2 (1.9–2.6)	2.5 ± 0.3 (2.3–3.2)
TD	1.4 ± 0.1 (1.1–1.8)	1.4 ± 0.2 (1.1–1.9)	1.1 ± 0.2 (0.8–1.4)	1.2 ± 0.2 (0.9–1.5)
EN	1.9 ± 0.1 (1.6–2.2)	2.0 ± 0.2 (1.7–2.4)	1.9 ± 0.1 (1.8–2.0)	2.0 ± 0.1 (1.9–2.1)
IOD	2.2 ± 0.2 (1.4–2.6)	2.2 ± 0.2 (1.6–2.5)	1.9 ± 0.1 (1.8–2.0)	2.0 ± 0.2 (1.8–2.3)
IND	2.3 ± 0.1 (2.0–2.6)	2.3 ± 0.3 (1.8–2.8)	2.1 ± 0.1 (2.0–2.2)	2.2 ± 0.2 (2.0–2.4)
UAL	3.7 ± 0.5 (2.96–6.31)	3.8 ± 0.4 (3.1–5.0)	3.4 ± 0.1 (3.3–3.5)	3.6 ± 0.2 (3.4–3.9)
LAR	4.4 ± 0.4 (3.6–5.5)	4.5 ± 0.5 (3.7–5.6)	4.1 ± 0.2 (4.0–4.3)	4.3 ± 0.2 (4.1–4.5)
HAL	5.3 ± 0.3 (4.6–5.8)	5.2 ± 0.5 (4.3–6.4)	4.7 ± 0.4 (3.9–5.4)	4.8 ± 0.3 (4.3–5.2)
THL	9.3 ± 0.6 (7.7–11.0)	9.4 ± 1.0 (7.3–11.9)	8.8 ± 0.6 (8.0–9.9)	8.9 ± 0.4 (8.4–9.5)
TIL	10.1 ± 0.5 (8.8–11.3)	10.5 ± 0.8 (8.9–11.8)	9.5 ± 0.7 (8.5–11.0)	9.5 ± 0.3 (8.9–10.0)
TAL	5.7 ± 0.6 (2.8–7.7)	5.9 ± 0.5 (4.8–6.8)	5.5 ± 0.3 (5.0–6.0)	5.5 ± 0.3 (5.0–6.0)
FL	11.5 ± 0.5 (10.2–12.8)	11.5 ± 0.8 (9.7–13.0)	10.6 ± 0.6 (9.3–11.6)	10.6 ± 0.5 (9.9–11.4)

with a medial projection that fits into a notch in the upper; tongue ovoid; males with vocal sac subgular with a fold parallel to jaw extending to forearm; maxillary and premaxillary teeth present; vomerine teeth present, located posterior to and between choanae, arranged in straight transverse rows. Dorsum glandular; with numerous, small, white-tipped tubercles posteriorly; two pairs (dorsal and lateral) of glandular rows starting behind the eyes and running towards the posterior part of dorsum; ventral surface smooth, except on thigh, areolate; pectoral fold absent, lateral and abdominal folds distinct; axillary gland small; arm short and robust, finger tips rounded, not expanded; relative finger length IV < I = II < III; inner metacarpal tubercle oval and ½ to ⅔ smaller than the outer metacarpal tubercle, circular; palmar tubercles present; subarticular tubercles prominent, rounded, the one on the thumb remarkably larger than the others; shank slightly longer than thigh; thigh length about 41% SVL; relative toe length I < II < V < III < IV, with rounded tips, neither expanded nor flattened (character state A); toes without webbing or lateral fringing; metatarsal tubercles oval, the outer smaller than the inner; inner tarsal fold distinct, with a single row of small, white-tipped tubercles on the edge, running about two thirds length of the tarsus; dorsal surface of the shank and ventral surface of the heel and sole of foot with numerous, small, white-tipped tubercles; most specimens with a paracloacal gland circular on each side of the vent.

Coloration

In life (Fig. 5G–H), dorsum medium brown with dark brown blotches; yellowish vertebral pinstripe in most specimens, sometimes interrupted anteriorly, continuous and broader posteriorly. Brown spot pattern on the head, defined by an anterior interorbital bar, a medial X-shaped spot, and a lateral oblique
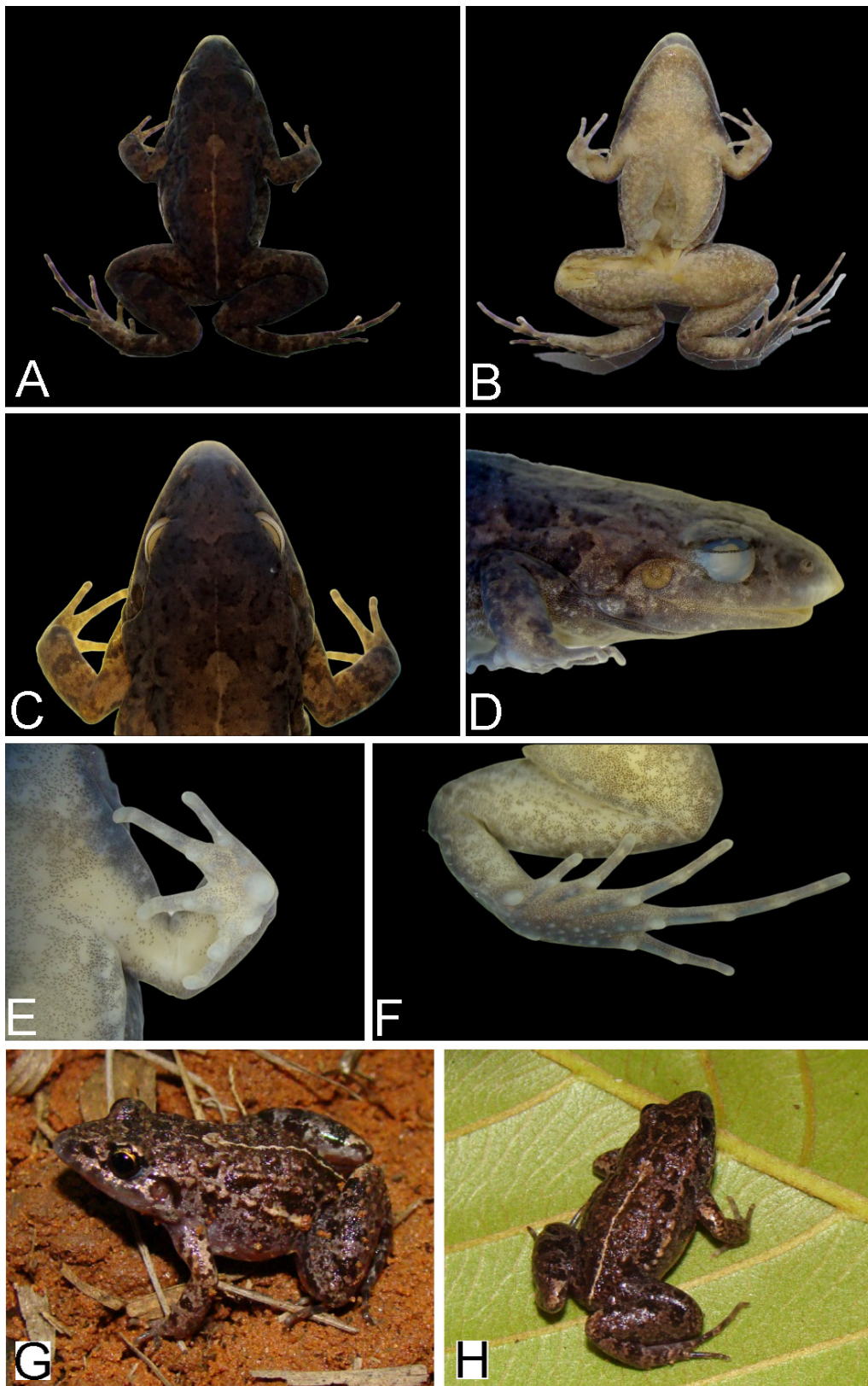
Fig. 5. *Adenomera diptyx* (Boettger, 1885), ♂ (UNNEC 13936, SVL: 22.6 mm) from Parque Nacional Cerro Corá, Amambay Department, Paraguay. **A–B**. Dorsal and ventral views. **C–D**. Dorsal and lateral views of the head. **E–F**. Ventral views of the left hand and foot. **G–H**. Coloration in life.

13

postorbital spot. Canthus rostralis highlighted by a dark brown band. Posterior part of the supratympanic fold and border of the upper eyelids highlighted in dark brown. Dorsal glandular row highlighted by coloration dark brown and cream. Upper surface of the arm light brown, with irregular spots; forearm with darker cross-bars. Upper surface of the thigh and shank light brown, in most with distinct, dark cross-bars on the shank. Posterior surface of the thigh with distinct darker blotches of poorly outlined borders. Dorsal surface of the shank-heel articulation cream-colored. Venter grayish white; in males lateral fold of the vocal sac dark brown. In preservative, color patterns similar to those described for the specimen in life, except for a duller coloration.

Variation

Number of small white-tipped tubercles highly variable, absent on the dorsal surface of the body in some specimens; paracloacal gland poorly defined in some specimens, only with a light-colored, small spot, or even absent. Lateral and abdominal folds indistinct occasionally. Palmar tubercles variable in number. Dorsal and lateral glandular rows sometimes unclear, but most are formed by few or several segments.

Vertebral pinstripe of variable length: from vent to the interorbital or inter-scapular region (long) or restricted to the pelvic region (short); indistinct in two specimens of 31 analyzed specimens (Fig. 6). Number, size, and distribution of the dark marks on the dorsum are quite variable, as well as the contrast between the background color and that of the marks. Glandular rows are highlighted by a single color or a combination of cream and dark color shades. Dark cross-bars on the dorsal surface of limbs are variable in number, in some specimens appear as spots with variable size and are distributed irregularly. The coloration of the paracloacal gland varies from cream-colored, dark-colored, or a combination of both. A few individuals have a dark-colored pigmentation at the base of the lower lip, coinciding with the vocal sac (Fig. 6).

Sexual dimorphism evident in head shape (see above), presence of a subgular vocal sac with distinct folds lateral to the jaw and extending to the forearm, highlighted by dark coloration in males, and females slightly larger than males, averaging 22.1 mm SVL in females and 20.8 mm SVL in males (Table 2).

Comparisons with congeners

Adenomera diptyx is distinguished from all congeners, except *A. martinezi* (Bokermann, 1956) and *A. saci* Carvalho & Giaretta, 2013, by its slender body, as opposed to the generally robust body of its congeners, including the morphologically similar but allopatric *A. bokermanni* (Heyer, 1973), *A. coca* (Angulo & Reichle, 2008), *A. hylaedactyla*, and *A. thomei* (Almeida & Angulo, 2006). The body size of *A. diptyx* (male SVL = 18.2–23.1 mm) differs from that of large-sized species of the genus, especially *A. glauciae* Carvalho, Simões, Gagliardi-Urrutia, Rojas-Runjaic, Haddad & Castroviejo-Fisher, 2020 (male SVL = 27.6–30.4 mm) and *A. lutzi* Heyer, 1975 (male SVL = 27.5–33.5 mm). *Adenomera diptyx* is also distinguished from all congeners, except *A. martinezi* and *A. saci*, by having a long vertebral pinstripe extending from the interorbital region to the vent, which is absent or rarely present in other species of *Adenomera*. *Adenomera martinezi* and *A. saci* have a complete pinstripe, extending from the tip of the snout. *Adenomera diptyx* is also distinguished from *A. martinezi* and *A. saci* by the presence of an interorbital bar and the absence of longitudinal rows of black spots on the dorsum (Carvalho & Giaretta 2013b). *Adenomera diptyx* has toe tips unexpanded with a tapered point (character state A), whereas the following 14 species have moderately to fully expanded toe tips (states C–D, i.e., swollen, knob-shaped toe tips and toe discs, respectively): *A. ajurauna* (Berneck, Costa & Garcia, 2008), *A. amicorum* Carvalho, Moraes, Lima, Fouquet, Peloso, Pavan, Drummond, Rodrigues, Giaretta, Gordo, Neckel-Oliveira & Haddad, 2021, *A. andreae* (Müller, 1923), *A. chicomendesi* Carvalho, Angulo, Kokubum, Barrera, Souza, Haddad & Giaretta, 2019, *A. engelsi* Kwet, Steiner & Zillikens, 2009, *A. gridipappi* Carvalho *et al.*, 2021, *A. guarayo* Carvalho, Angulo, Barrera, Aguilar-Puntriano & Haddad, 2020, *A. heyeri* Boistel, Massary & Angulo, 2006, *A. inopinata* Carvalho *et al.* 2021, *A. lutzi*,
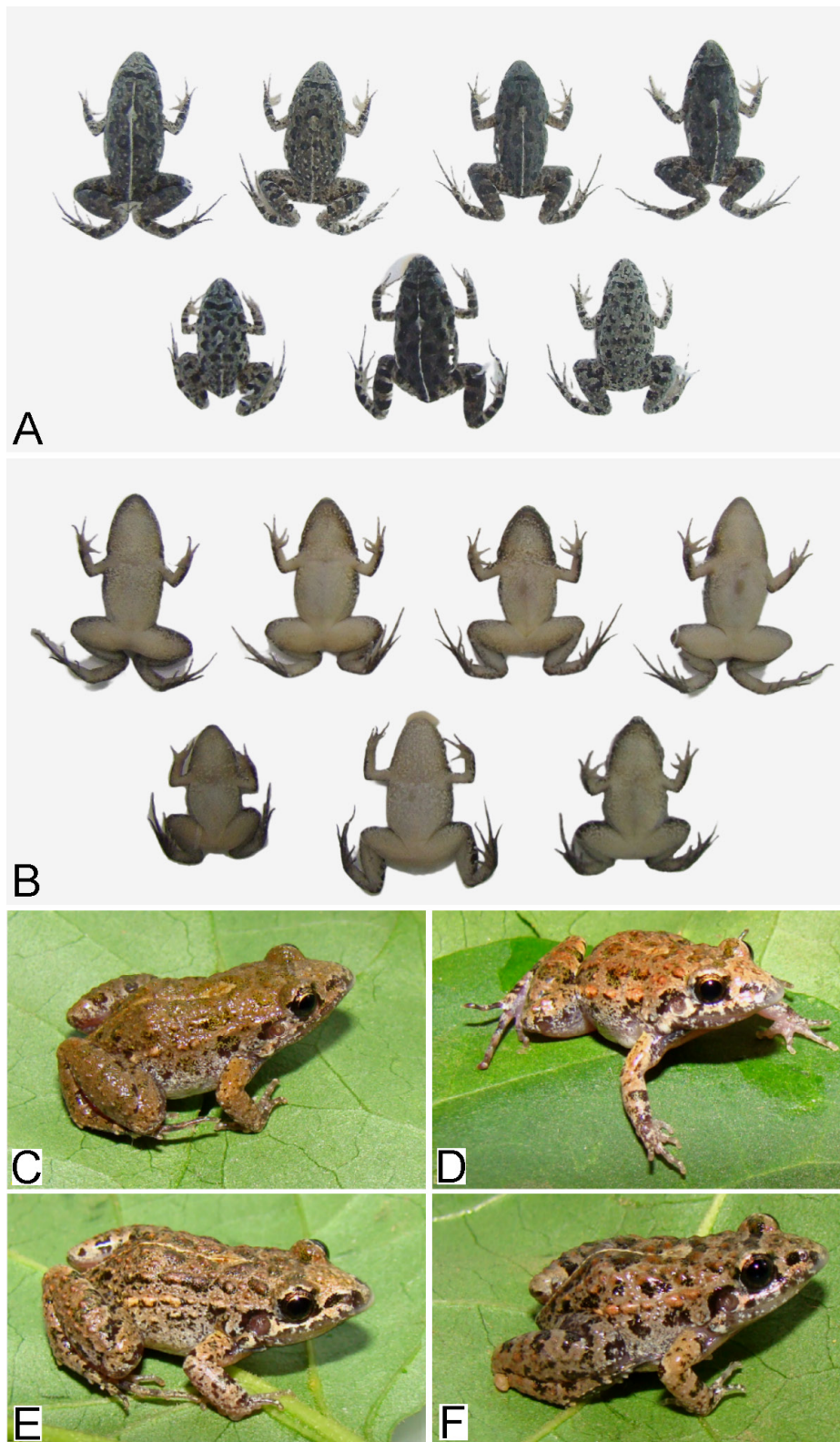
Fig. 6. Coloration patterns of *Adenomera diptyx* (Boettger, 1885) from Piribebuy, Cordillera Department, Paraguay. **A–B**. Coloration in preservative in dorsal and ventral views (from left to right, in the upper row CZCEN 0647, 0644, 0649 and 0648; in the lower row: CZCEN 0645, 0646, and 0650). **C–F**. Coloration in life (CZCEN 0640 to 0643).

A. marmorata, *A. nana* (Müller, 1922), *A. simonstuarti* (Angulo & Icochea, 2010), and *A. tapajonica* Carvalho *et al.*, 2021. *Adenomera diptyx* is distinguished from *A. amicorum*, *A. aurantiaca* Carvalho *et al.*, 2021, *A. cotuba* Carvalho & Giaretta, 2013, *A. glauciae*, *A. gridipappi*, *A. inopinata*, *A. kayapo* Carvalho *et al.*, 2021, *A. lutzi*, *A. phonotriccus* Carvalho, Giaretta, Angulo, Haddad & Peloso, 2019, and *A. tapajonica* by the absence of an antebrachial tubercle.

Adenomera diptyx is distinguished from most congeners in advertisement call traits. The single-note call of *A. diptyx* differs from the multi-note calls of *A. amicorum*, *A. aurantiaca*, *A. cotuba*, *A. glauciae*, *A. gridipappi*, *A. inopinata*, and *A. simonstuarti*. Among *Adenomera* with single-note calls, call notes of *A. diptyx* are formed by incomplete pulses, whereas those of *A. guarayo* and *A. phonotriccus* are formed by complete pulses, and those of *A. ajurauna*, *A. bokermanni*, *A. engelsi*, *A. kweti*, *A. lutzi*, *A. marmorata*, *A. nana*, and *A. saci* are nonpulsed. From congeners also having single-note calls formed by incomplete pulses, *A. diptyx* differs by a higher note repetition rate (176–299 per minute; Table 3) from *A. andreae* (24–68 per minute), *A. araucaria* Kwet & Angulo, 2002 (23–46 per minute), *A. chicomendesi* (5–29 per minute), *A. coca* (66–84 per minute), *A. heyeri* (22–27 per minute), *A. juikitam* (32–52 per minute; retrieved from the raw dataset analyzed and published in Carvalho *et al.* 2021), *A. kayapo* (23–33 per minute), *A. tapajonica* (52–59 per minute), and *A. thomei* (10–23 per minute). *Adenomera diptyx* differs by a lower pulse number per note (2–6; Table 3) from *A. martinezi* (15–21). *Adenomera diptyx* cannot be completely distinguished acoustically from the allopatric *A. hylaedactyla* (characterized by Carvalho *et al.* 2019a). Despite the marginal overlap in value ranges of all analyzed traits, three temporal traits can be of help in their discrimination: note duration (*A. diptyx*: 28–49 ms, X = 38, SD = 5, N = 12 males; *A. hylaedactyla*: 41–89 ms, X = 59, SD = 9, N = 24 males), note repetition rate (*A. diptyx*: 176–299 per minute, X = 227, SD = 36, N = 12 males; *A. hylaedactyla*: 107–242 per minute, X = 154, SD = 32, N = 24 males), and pulse number (*A. diptyx*: 2–6 per note, X = 4, SD = 1, N = 12 males; *A. hylaedactyla*: 4–10 per note, X = 7 SD = 1, N = 24 males).

Advertisement call (Fig. 7, Table 3)

We characterized the advertisement call of *A. diptyx* based on 12 males from Paraguay and Brazil (N = 187 quantified calls and 645 quantified pulses; see Appendix 2 for locality data and voucher specimens). The call consists of single notes given at a repetition rate of 176 to 299 per minute (227 ± 36). Note duration varies from 28 to 49 (38 ± 5) ms, and the rise time is 8 to 74 % (30 ± 9) of note duration. Notes are formed by 2 to 6 (4 ± 1) incomplete pulses (i.e., not separated by silent gaps) emitted at a repetition rate of 65 to 239 per second (142 ± 22). The dominant frequency coincides with the second harmonic, ranging from 3984 to 5060 (4504 ± 237) Hz. Notes can have a modest or pronounced frequency modulation, ranging from -47 to 689 (301 ± 124) Hz.

Chromosome number

One specimen (UNNEC 13936, also genotyped) from Parque Nacional Cerro Corá (Amambay department, Paraguay) showed a diploid number 2n = 26 chromosomes and FN = 34. The karyotype is composed of three submetacentric pairs (pairs 1–3), one metacentric (pair 5), and nine telocentric (pairs 4 and 6–13) (Fig. 8A); pair 7 shows a conspicuous proximal secondary constriction NORs. The diploid number was confirmed in meiotic preparations from testes, in which 13 bivalents were observed (Fig. 8B).

Geographic distribution and habitat (Fig. 1)

Adenomera diptyx is known to occur in a few localities in Paraguay and Brazil. The distribution in Argentina and Bolivia is still uncertain and needs future sampling efforts in the field for the collection of specimens and acoustic data. In Paraguay, the occurrence records are the Parque Nacional Cerro Corá (Amambay Department) and the surroundings of Piribebuy (Cordillera Department). In Brazil,
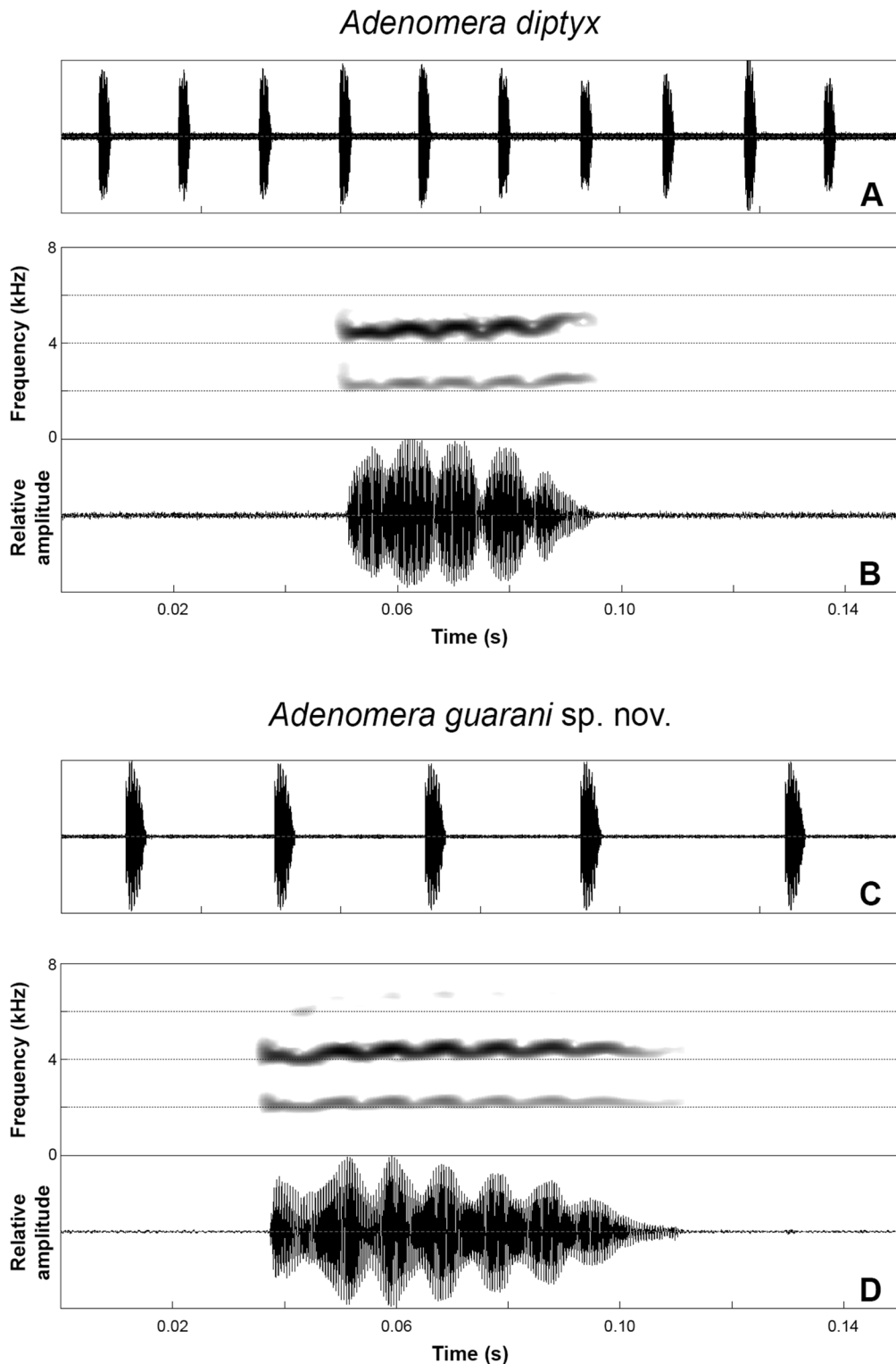
Adenomera diptyx

Adenomera guarani sp. nov.

Fig. 7. Advertisement calls. **A–B**. Sound file FZ-UNNE 0074 of *Adenomera diptyx* (Boettger, 1885). **C–D**. Sound file FZ-UNNE 0284 of *A. guarani* sp. nov. See Appendix 2 for locality data and voucher specimens. Oscillograms (A, C) show the distinctive note repetition rates in the two species. Call sections are equally scaled (ca 3 s; each tick mark corresponds to 0.5 s). Spectrograms and corresponding oscillograms (B, D) of the first note in A and C.

Table 3. Summary of acoustic traits of the five species belonging to the open-habitat *Adenomera* Steindachner, 1867 clade (the new species is highlighted in bold). Data are reported as value ranges (mean ± standard deviation). Species of this *Adenomera* clade have the dominant frequency coinciding with either the first or second harmonic. For this reason, this trait is reported as the peak frequency of the fundamental harmonic (H1) and the second harmonic (H2). Mean values of the acoustic traits of *A. saci* Carvalho & Giaretta, 2013 were provided for each population separately in the original description (see Carvalho & Giaretta 2013a: table 2). Global means and corresponding standard deviations for all four populations of *A. saci* with recorded calls are provided here, calculated from the raw acoustic dataset of Carvalho & Giaretta (2013a).

| Species	Note duration (ms)	Note rate (min)	Pulses/note	H1 frequency (Hz)	H2 frequency (Hz)	Reference
A. diptyx (N = 12)	28–49 (38±5)	176–299 (227±36)	2–6 (4±1)	2018–2519 (2241±122)	3984–5060 (4504±237)	This study
A. guarani sp. nov. (**N = 23**)	45–98 (66±11)	73–147 (102±18)	4–9 (6±1)	2001–2448 (2198±89)	3984–4974 (4329±165)	This study
A. hylaedactyla (N = 30)	41–89 (59±9)	107–242 (154±32)	4–10 (7±1)	1852–2211 (2021±97)	3680–4457 (4071±214)	Carvalho *et al.* (2019a)
A. martinezi (N = 15)	63–151 (117±11)	97–142 (122±15)	15–21 (18±1)	1880–2060 (1930±80)	3380–4130 (3740±180)	Carvalho & Giaretta (2013a)
A. saci (N = 19)	72–241 (141±41)	90–175 (119±22)	Nonpulsed	1690–2250 (1953±125)	3380–4441 (3895±276)	Carvalho & Giaretta (2013a)
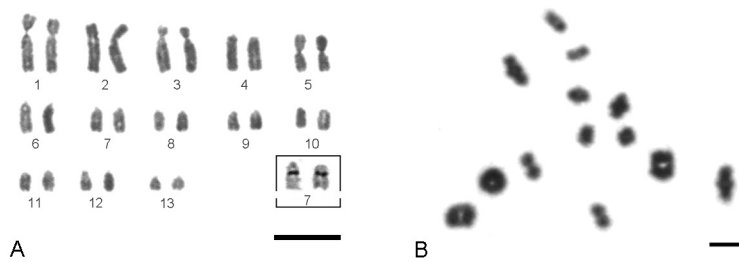
Fig. 8. Karyotype of *Adenomera diptyx* (Boettger, 1885), ♂ (UNNEC 13936) (2n = 26, FN = 34) from Parque Nacional Cerro Corá, Amambay department (Paraguay). **A**. Conventional Giemsa staining. Inset: NOR-bearing chromosome pair after silver staining. **B**. Meiosis showing 13 bivalents. Scale bars = 10 μm.

our record in Três Lagoas (eastern Mato Grosso do Sul, in border of São Paulo State) represents the easternmost range of the distribution of *A. diptyx*. The specimen was collected in a flooded area covered by tall grasses, right next to a river. The area seems to be seasonally flooded by the dynamics of the river. The population from Parque Nacional Cerro Corá (Amambay) was found on the bottom of dry ditches on the margin of road "Ruta N° 5" in a dense and tall (ca 1 m) grassland of *Pennisetum purpureum* Schumach. (elephant grass). Specimens were vocalizing on the ground, hidden in dead organic material. At Piribebuy, individuals were found in a partially urbanized area next to the Piribebuy River.

Additionally, DNA sequences from Lago Ypacaraí (Cordillera Department, Paraguay) and UHE Guaporé (Mato Grosso State, Brazil) provided by Fouquet *et al.* (2014) were also recovered as *Adenomera diptyx*. The presence of a long vertebral pinstripe in specimens from the Reserva de Recursos Manejados de Ybyturuzú (Guairá Department, Paraguay; Airaldi *et al.* 2013) and other specimens examined by us (see Appendix 1) from Laguna Blanca (San Pedro Department, Paraguay) confirm also its occurrence in these localities. The distribution of *A. diptyx* is mostly associated with the Alto Paraná Atlantic Forest and the Humid Chaco ecoregions, and also in the Chiquitano Dry Forest Ecoregion (Dinerstein *et al.* 2017).

Conservation

Adenomera diptyx seems to be a species that can adapt quite well to partially disturbed areas. The Amambay population is protected within the area of Parque Nacional Cerro Corá. However, outside of the park, vast extensions of forests have disappeared, and they were mostly replaced with pasture. The species is part of the open-habitat clade of *Adenomera*. These species are well adapted to open areas, both natural grasslands and savannas, and disturbed areas.

Description of a new species of Adenomera

Simultaneously to the identification and redescription of *A. diptyx*, the morphological, acoustic, and DNA sequence data allowed us to recognize a distinct species, which has been historically confused with the nominal species. Both species are separated by a genetic distance from 6.7 to 7.9%, and also differ in the dorsal color pattern and temporal call traits. In the next section this taxon, closely related to *A. diptyx*, is formally described.

Adenomera guarani sp. nov.
urn:lsid:zoobank.org:act:13EA7E3F-AFF1-4999-985E-EAB9B45109AA
Figs 9–12; Tables 2–3

Diagnosis

Adenomera guarani sp. nov. is recognized within *Adenomera* by the following combination of character states: (1) body robust, (2) medium size (adult male SVL = 20.1–26.4 mm); (3) light-colored, broad

mid-dorsal stripe with diffuse borders, extending from above the vent to the scapular region; (4) a dark mask-like pattern formed by an inverted triangle in the interorbital region; (5) toe tips unexpanded with tapered point; (6) antebrachial tubercle absent; (7) exotrophic larvae; (8) labial tooth row formula (LTRF): 2(2)/3(1); (9) single-note advertisement call; (10) call notes formed by incomplete pulses; (11) relatively high note repetition rate (73–147 per minute); and (12) low pulse number (4–9 per note).

Etymology

The specific name *guarani*, used as a noun in apposition and refers to the Guaraní, a native indigenous group that inhabited in the past a vast region in South America, including areas where the new species occurs. Currently, a few indigenous communities still live in wild areas. However, the Guaraní language (Avañe′ẽ), is usually spoken by people in Paraguay and other neighboring countries and also utilized to name innumerous geographical features (e.g., rivers, wetlands, hills), animals, plants, towns, and cities.

Type material

Holotype
ARGENTINA • ♂ (Fig. 9); Corrientes Province, Capital Department, Corrientes City, Campus Universitario, Universidad Nacional del Nordeste; 27°28′04″ S, 58°46′53″ W; 60 m a.s.l.; 8 Feb. 2007; V.H. Zaracho leg.; UNNEC 9074.

Paratypes
ARGENTINA – **Corrientes** • 1 ♂; Capital Department, Corrientes City; 27°28′04″ S, 58°46′53″ W; 8 Nov. 2016; V.H. Zaracho leg.; UNNEC 8994 • 1 ♂; same collection data as for preceding; LGE 25740 • 2 ♂♂; same collection data as for preceding; Feb. 2011; F. Abreliano leg.; UNNEC 11977, 11978 • 3 ♀♀; Capital Department, Corrientes City; 27°29′26″ S, 58°50′13″ W; 24 Jun. 2005; V.H. Zaracho, J.A. Ruiz García and J.A. Céspedez leg.; UNNEC 8365 to 8367 • 1 ♀; Capital Department, San Cayetano; 27°33′04″ S, 58°40′37″ W; 18 Mar. 2011; J.L. Acosta and M. Ortiz leg.; LGE 25741 • 1 ♀; San Cosme Department, Paso de la Patria; 27°19′27″ S, 58°34′05″ W; 29 Jul. 2007; J.L. Acosta and S. Palomas leg.; UNNEC 9551 • 1 ♂; same collection data as for preceding; 2 Dec 2006; V.H. Zaracho and M.R. Ingaramo leg.; UNNEC 9003. – **Chaco** • 1 ♂; San Martin Department, Paraje Las Tablas; 26°11′40″ S, 59°38′45″ W; 13 Dec. 2005; V.H. Zaracho, R.H. Aguirre and J.A. Céspedez leg.; UNNEC 8531. – **Formosa** • 2 ♂♂; Patiño Department, Comandante Fontana; 25°20′19″ S, 59°41′19″ W; 24 Nov. 2007; V.H. Zaracho leg.; UNNEC 9728, 9729 • 3 ♂♂; Pilcomayo Department, Laguna Naick Neck; 25°10′58″ S, 58°07′29″ W; 29 Feb. 2008; V.H. Zaracho and J.L. Acosta leg.; UNNEC 9856 to 9858 • 1 ♂; Pilcomayo Department, Laguna Naick Neck; 25°10′58″ S; 58°07′29″ W; 15 Oct. 2009; V.H. Zaracho leg.; UNNEC 11964.

PARAGUAY – **Concepción** • 4 ♂♂; 85 km NE of Concepción, Arroyo Capitigo; 22°46′30″ S, 56°47′56″ W; 11 Nov. 1981; L. Fitzgerald, A.L. Aquino and N.J. Scott leg.; MNHNP 0413, 0415, 0421, 0436. – **Presidente Hayes** • 1 ♀; Ea. La Golondrina; 24°57′02″ S, 57°42′07″ W; 9 Sep. 1982; A.L. Aquino and D. Norman leg.; MNHNP 0783. – **Central** • 1 ♂; Villeta; 25°30′48″ S, 57°33′30″ W; 31 Aug. 1982; A.L. Aquino and D. Norman leg.; MNHNP 0417. – **Amambay** • 1 ♂; Parque Nacional Cerro Corá; 22°39′56″ S, 56°00′24″ W; 13 Oct. 2009; V.H. Zaracho and M. Motte leg.; MNHNP 11333. – **Alto Paraguay** • 3 ♀♀; Ea. Doña Julia, 5 km N of Bahía Negra; 20°15′34″ S, 58°12′02″ W; 1 Oct. 1984; A.L. Aquino and D. Norman leg.; MNHNP 0784 to 0786. – **Ñeembucú** • 1 ♂; 19.8 km SSW of Pilar; 26°58′47″ S, 58°22′52″ W; 20 Feb. 1996; P. Freed leg.; MNHNP 10046 • 1 ♂; same collection data as for preceding; UNNEC 13938.

Description

Holotype
Body small, robust; head nearly as broad as long (HW/HL = 1.1); snout sub-elliptical dorsally and acuminate laterally; canthus rostralis round; loreal region slightly concave; eye dorsolateral, its diameter
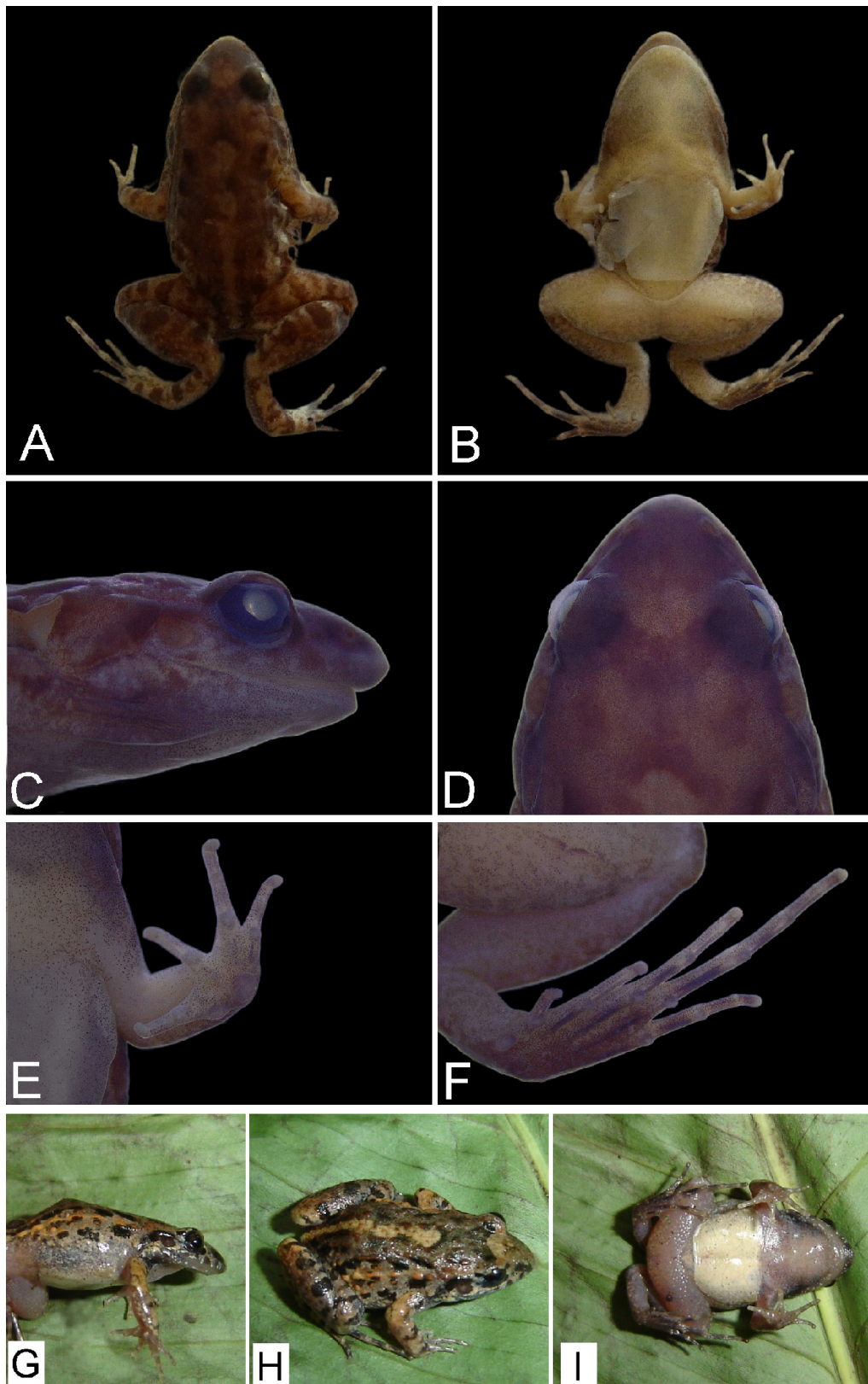
Fig. 9. *Adenomera guarani* sp. nov., holotype, ♂ (UNNEC 9074), Corrientes, Argentina, SVL: 21.6 mm. **A–B**. Dorsal and ventral views. **C–D**. Lateral and dorsal views of the head (note the mask-like dark patch). **E–F**. Ventral views of the left hand and of the right foot. **G–I**. Coloration in life.

about 32% of head length; pupil horizontal, elliptical; palpebral membrane translucent, bordered in its upper edge by a black streak; tympanum distinct, its diameter about 63% of the eye diameter. Tympanic membrane translucent, with some melanophores on the central region; supratympanic fold well developed, extending from the back of the eye to arm insertion; nostrils anterolateral, closer to the tip of the snout than to the eye, flanged in almost all their extension, except on the inner edge; internarial distance about 26% of head width; triangular postcommissural gland; lower lip with a medial projection that fits into a notch in the upper lip; tongue ovoid; vocal sac subgular with a fold parallel to jaw extending to the forearm; maxillary and premaxillary teeth present; vomerine teeth located posterior to and between choanae, arranged in straight transverse rows. Dorsum glandular, with more conspicuous glands placed on the posterior region. A pair (dorsal and lateral) of glandular rows formed by small segments starting behind the eyes and running towards the posterior part of the dorsum; ventral surface of the body smooth, with a distinct discoidal fold (pectoral few distinctive in the specimen in conservation); an axillary gland behind each arm insertion. Arms short and robust; fingertips rounded, not expanded (character state A); relative finger length IV < II ≤ I < III; nuptial asperities absent; inner metacarpal tubercle oval and ½ smaller than the outer, rounded; palmar tubercles present; prominent, rounded and entire subarticular tubercles on fingers, those on the base of each finger the most noticeable. Shank slightly longer than the thigh; thigh length about 43% SVL; relative toe lengths I < II < V < III < IV, with rounded tip, neither expanded nor flattened (character state A); toes without webbing or lateral fringing; metatarsal tubercles oval, not keratinized; outer tubercle smaller than the inner; subarticular tubercles present; plantar and supernumerary tubercles absent; posterior part of dorsum and ventral surface of both heel and metatarsals with dispersed, small and white-tipped tubercles; distinct inner tarsal fold with a single row of small, white-tipped tubercles on the edge, running for about two-thirds of the tarsus length. Ventral surfaces of the legs smooth, except the ventral surface of thighs, which is areolate. Vent fold distinct on the upper anus. A cream, rounded and small paracloacal gland is present on each side of the vent.

Measurements of the holotype (mm)

SVL = 21.6, HL = 7.3, HW = 8.3, ED = 2.4, TD = 1.2, EN = 1.9, IOD = 2.2, IND = 2.2, UAL = 3.1, LAR = 4.3, HAL = 5.2, THL = 9.2, TIL = 9.7, TAL = 5.6 and FL = 11.2.

Coloration of the holotype

In life, dorsum medium grayish-brown, with a black mask-like patch on the interorbital region; chevron shaped mark on the scapular area, and several smaller spots on dorsum. Broad, light mid-dorsal stripe with diffuse borders, running from the chevron-shaped mark to the vent fold. Dorsal glandular row mainly highlighted in dark; lateral glandular row and paracloacal glands cream. Faded orange patches on elbows and heels. Distinct dark longitudinal bars on the dorsal region of lower arms, thighs, and shanks. Dark marks on lateral upper lip and behind nostrils. Posterior part of the supratympanic fold and the border of the upper eyelids highlighted with dark brown coloration. Body ventrally whitish, immaculate; gular region and ventral surface of thighs faded pink; lateral fold of the vocal sac black. In preservative, color patterns as in life, except for a duller coloration.

Comparisons with congeners

Adenomera guarani sp. nov. (robust body shape) is distinguished from three congeners with slender body shapes (*A diptyx*, *A. martinezi*, and *A. saci*). The body size of *A. guarani* (male SVL = 20.1–26.4 mm) differs from that of large-sized species of the genus, especially *A. glauciae* (male SVL = 27.6–30.4 mm) and *A. lutzi* (male SVL = 27.5–33.5 mm). *Adenomera guarani* has a broad mid-dorsal stripe, whereas *A. diptyx*, *A. martinezi*, and *A. saci* have a vertebral pin stripe. Additionally, *A. guarani* is distinguished from *A. diptyx* by a mask-like pattern in the interorbital region (an interorbital bar in *A. diptyx*). *Adenomera guarani* has toe tips unexpanded with a tapered point (character state A), whereas the following 14 species have moderately to fully expanded toe tips (states C–D, i.e., swollen, knob-shaped

toe tips and toe discs, respectively): *A. ajurauna*, *A. amicorum*, *A. andreae*, *A. chicomendesi*, *A. engelsi*, *A. gridipappi*, *A. guarayo*, *A. heyeri*, *A. inopinata*, *A. lutzi*, *A. marmorata*, *A. nana*, *A. simonstuarti*, and *A. tapajonica*. *Adenomera guarani* differs from *A. amicorum*, *A. aurantiaca*, *A. cotuba*, *A. glauciae*, *A. gridipappi*, *A. inopinata*, *A. kayapo*, *A. lutzi*, *A. phonotriccus*, and *A. tapajonica* by the absence of an antebrachial tubercle. The exotrophic larva of *A. guarani* distinguishes the new species from almost all congeners, except *A. saci* and *A. thomei* (Almeida & Angulo 2006; Carvalho & Giaretta 2013b), as well as *A. diptyx* and *A. martinezi* (assumed based on calling habitat, but their tadpoles remain unknown; Carvalho & Giaretta 2013b; T.R. Carvalho, field obs.). The tadpole of *A. guarani* has a labial tooth row formula (LTRF): 2(2)/3(1), whereas that of *A. thomei* has a LTRF: 2(2)/3 (Almeida & Angulo 2006).

Adenomera guarani sp. nov. can also be distinguished from congeners in advertisement call traits. The single-note call of *A. guarani* differs from the multi-note calls of *A. amicorum*, *A. aurantiaca*, *A. cotuba*, *A. glauciae*, *A. gridipappi*, *A. inopinata*, and *A. simonstuarti*. Among *Adenomera* with single-note calls, call notes of *A. guarani* are formed by incomplete pulses, whereas those of *A. guarayo* and *A. phonotriccus* are formed by complete pulses, and those of *A. ajurauna*, *A. bokermanni*, *A. engelsi*, *A. kweti*, *A. lutzi*, *A. marmorata*, *A. nana*, and *A. saci* are nonpulsed. From congeners also having single-note calls formed by incomplete pulses, *A. guarani* differs from *A. andreae* (24–68 per minute), *A. araucaria* (23–46 per minute), *A. chicomendesi* (5–29 per minute), *A. heyeri* (22–27 per minute), *A. juikitam* (30–52 per minute), *A. kayapo* (23–33 per minute), *A. tapajonica* (52–59 per minute), and *A. thomei* (10–23 per minute) by a higher note repetition rate (73–147 per minute; Table 3), and by a lower note rate than that of *A. diptyx* (176–299 per minute; Table 3). *Adenomera guarani* differs from *A. coca* (10–15; Angulo & Reichle 2008) and *A. martinezi* (15–21; Carvalho & Giaretta, 2013a) by a lower pulse number per note (4–9; Table 3). *Adenomera guarani* cannot be consistently distinguished from the allopatric *A. hylaedactyla* in any of the analyzed call traits (Carvalho *et al.* 2019a). Still, these two species are segregated by distinct breeding habitats and reproductive modes. *Adenomera guarani*, endemic to the Humid Chaco and Pantanal Wetlands, has exotrophic larvae, whereas the Amazonian *A. hylaedactyla* has endotrophic larvae (Kokubum & Souza 2008; Carvalho *et al.* 2019a). This is the most reliable character in the discrimination of the two species, in addition to their phylogenetic relationships not sharing an immediate common ancestor (Fig. 2). *Adenomera guarani* is the sister taxon of *A. diptyx*, from which the new species is distinguished by morphological and acoustic traits.

Variation (Figs 10–12)

Measurements of males and females are shown in Table 2. Most specimens show small, white-tipped tubercles on the dorsal surface of the shank, heel, and the sole of the foot, but absent on the dorsal surface of thigh; tubercle density is variable and usually visible under magnification. The supratympanic fold in some specimens is barely visible (may be due to preservation methods). Paracloacal glands generally developed well, but sometimes only a colored spot. Axillary glands are conspicuous in recently fixed specimens (Fig. 12B). Postcommissural gland is variable in size and form. Inner metacarpal tubercle is ⅓ to ⅔ smaller than the outer. Palmar tubercles are variable in number (one or two by finger), varying even between the hands of the same specimen. Dorsal and lateral glandular rows have varying degrees of development (i.e., the number of individual glands and their extent along the body length). When well-developed (as observed in most specimens), the dorsal row extends from behind the eye and the lateral row runs from the posterodorsal region of the tympanum, almost overlapping with the supratympanic fold. Some specimens also have additional small glands posteriorly, sometimes a pair highlighting as a lumbar gland, variable in size and dark colored (Fig. 12A).

A great variation of color patterns is exhibited in living *Adenomera guarani* sp. nov. (Figs 10–11). Dorsal coloration is variable in shades of light to dark brown; even in the same individual exposed to different conditions of luminosity. Sometimes the broad mid-dorsal stripe is poorly evident (Figs 10C, F–G, 11M) and the mask pattern on the interorbital region is easier to distinguish in preserved specimens.
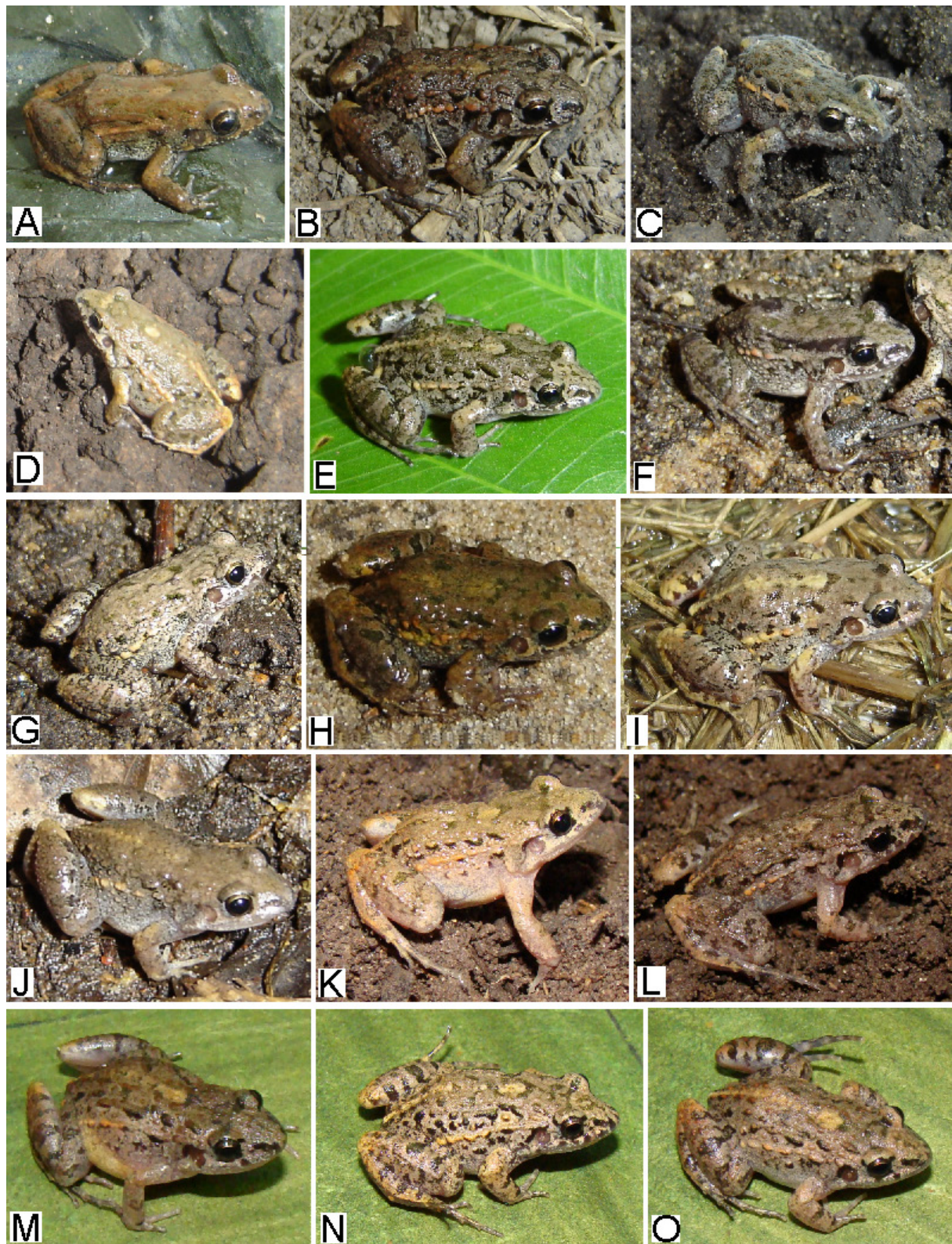
Fig. 10. *Adenomera guarani* sp. nov. Dorsal color patterns. **A–C**. UNNEC 9075 (♂), 9551 (paratype, ♀), 9003 (paratype, ♂) (Paso de la Patria, Corrientes). **D–O**. LGE 25740 (paratype, ♂), UNNEC 9704 (♂), 8366 and 8365 (paratypes, ♀), 8505 (♂), 8293 (♂), 8354 (♂), 11965 to 11969 (♂), (Corrientes city, Corrientes).

Fig. 11. *Adenomera guarani* sp. nov. Dorsal color patterns. **A**. UNNEC 11970 (♂) (Corrientes). **B**. UNNEC 8357 (♂) (Mburucuyá, Corrientes). **C**. UNNEC 8531 (paratype, ♂, Las Tablas, Chaco). **D**. UNNEC 9727 (♂) (Comandante Fontana, Formosa). **E–N**. UNNEC 9718 to 9724 (♂), 9725 and 9726 (♀), 11964 (paratype, ♂, Laguna Naick Neck, Formosa). **O**. MNHNP 11333 (paratype, ♂, Parque Nacional Cerro Corá, Amambay, Paraguay).
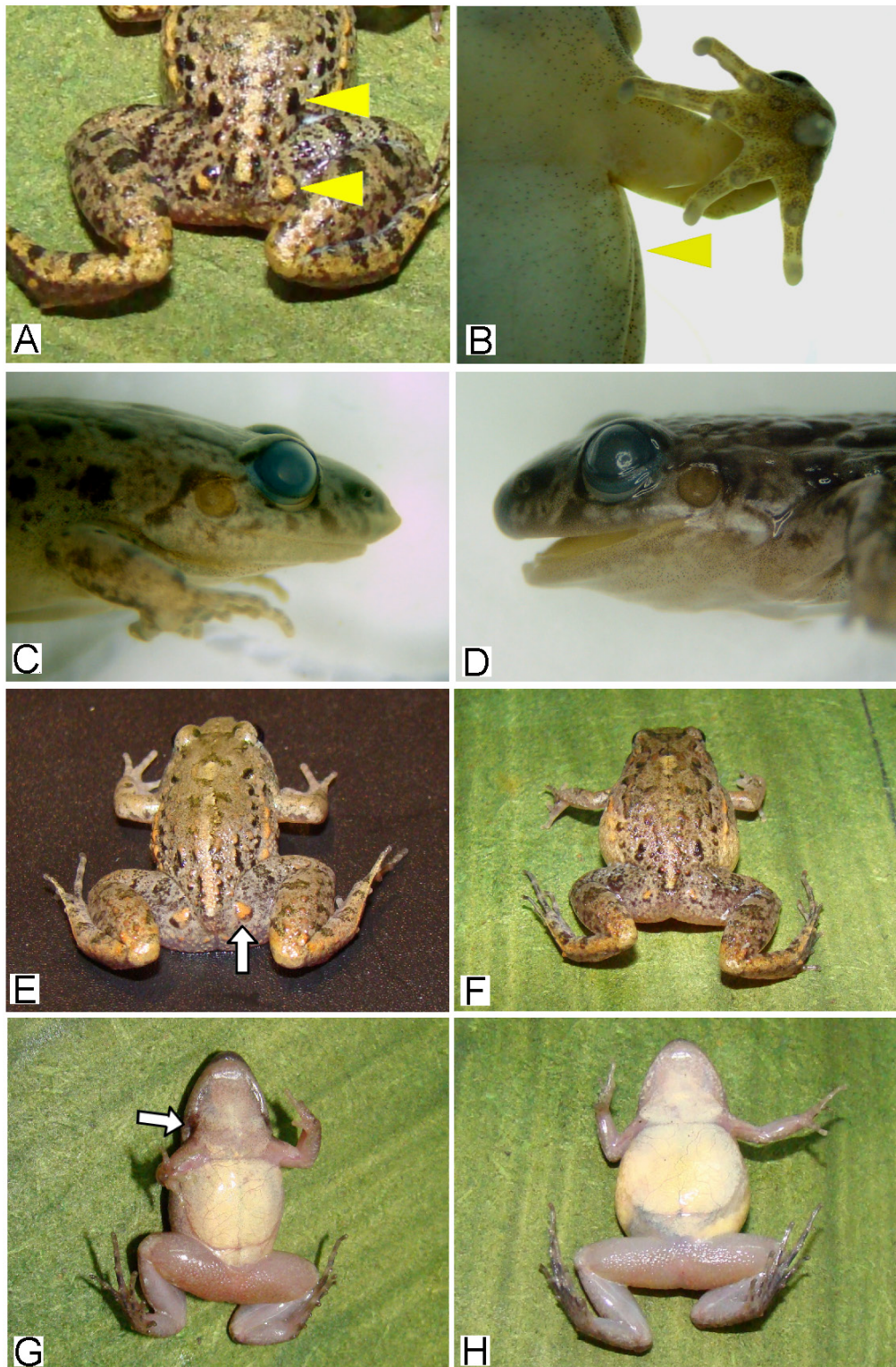
Fig. 12. Variation and sexual dimorphism in *Adenomera guarani* sp. nov. **A**. Lumbar and paracloacal glands. **B**. Axillary gland. **C–H**. Males at left and females at right. **C–D**. Lateral view of the head (MNHNP 4398, ♂ and 0784, paratype, ♀). **E–F**. Development of the paracloacal glands (arrow) (UNNEC 11965, ♂ and 11967, ♀, note the poor development in females). **G–H**. Ventral view (UNNEC 11970, ♂ and 11967, note the pigmented lateral sides of vocal sac in male indicated by arrow).

Dark marks on the posterior surface of the dorsum variable in shape and number. The coloration of the glandular rows is homogeneous (dorsal row dark and lateral row light). The medial region of the paracloacal gland is always light-colored, but its borders bear dispersed dark-colored marks of variable size and shape. Color pattern on legs is variable from well-defined cross-bands to a splash of small and dark marks. In life, the ventral surface is usually whitish, but one individual (UNNEC 8357) showed a ventral coloration with black stains.

Sexual dimorphism is evident. The snout of males in lateral view is shovel-shaped, with a well-developed, fleshy white ridge; in females, the snout is rounded and without ridges (Fig. 12C–D). Aside from the snout shape, nostrils are closer to the tip of the snout in females than in males. Only males have axillary glands. Paracloacal glands are more well-developed in males than in females (smaller or represented only by a pair of pale, small spots (Fig. 12E–F). Vocal sac in males with dark lateral sides (Fig. 12E). In life, the gular region of females is white (Fig. 12H).

Advertisement call (Fig. 7, Table 3)

We characterized the advertisement call of *A. guarani* sp. nov. based on 23 males from Argentina and Brazil (N = 399 quantified calls and 2423 quantified pulses; see Appendix 2 for locality data and voucher specimens). The call consists of single notes given at a repetition rate of 73 to 147 per minute (102 ± 18). Note duration varies from 45 to 98 (66 ± 11) ms, and the rise time is 1 to 59 % of note duration (10 ± 12). Notes are formed by 4 to 9 (6 ± 1) incomplete pulses (i.e., not separated by silent gaps) emitted at a repetition rate of 60 to 146 per second (106 ± 16). The dominant frequency coincides with the second harmonic, ranging from 3984 to 4974 (4329 ± 165) Hz. Notes can have a modest or pronounced frequency modulation, either positive or negative, ranging from -345 to 603 (150 ± 112) Hz.

Natural history

Data about reproductive aspects from Brazilian and Argentinean populations were published by Zaracho & Kokubum (2017) under the name *A. diptyx*. In summary, *Adenomera guarani* sp. nov. lay eggs in a foam nest inside an underground chamber with the entrance partially closed, constructed by males near temporary pools and seasonally flooded areas. When the breeding microhabitat is flooded, embryos/tadpoles are washed out of the underground chamber and complete their development in the water. The larvae have functional mouthparts and a labial tooth row LTRF: 2(2)/3(1) (Zaracho & Kokubum 2017: fig. 2C). Diet and parasitism from Argentinean populations were provided also by Zaracho & Lamas (2008) and Zaracho *et al.* (2012a), both under the name *A. diptyx*.

Chromosome number

The chromosome number 2n = 26 of *A. guarani* sp. nov. from Argentinean populations was reported by Zaracho & Hernando (2011) under the name of *Adenomera diptyx*.

Geographic distribution and habitat (Fig. 1)

Adenomera guarani sp. nov. occurs in Argentina, Paraguay, and Brazil, associated mainly with the Paraguay and Paraná river basins, and the Pantanal wetlands. All known populations of *Adenomera* from the Argentinean Humid Chaco belong to *A. guarani*.

Adenomera guarani sp. nov. inhabits open areas that can easily be flooded by rainwater during the wet season. The species is abundant in partially urbanized areas, such as roadsides or gardens. Males call amidst grasses, outside water, during the dusk and early in the night. They can easily be detected by their vocalizations, but due to their small size and secretive habits, are very difficult to observe.

At Parque Nacional Cerro Corá (Amambay Department, Paraguay), populations of *A. guarani* sp. nov. and *A. diptyx* were found at two sites less than 1 km from each other. Two males of *A. guarani* were found calling at dusk at the margins of a pond inside the forest, near Aquidabán stream, and *A. diptyx* males were calling on roadsides amidst grasses and at the margin of a stream inside the forest. Other species calling syntopically with *A. guarani* were *Scinax fuscovarius* (Lutz, 1925), *S. berthae* (Barrio, 1962), *Pithecopus azureus* (Cope, 1862), *Dendropsophus nanus* (Boulenger, 1889), *D. minutus* (Peters, 1872), *Leptodactylus elenae* Heyer, 1978, *Rhinella diptycha* (Cope, 1862), and *Physalaemus nattereri* (Steindachner, 1863). In Argentina, *A. guarani* was also found on roadsides associated with *Copernicia alba* Morong palms and *Ludwigia* sp. grassland, syntopically with *S. acuminatus* (Cope, 1862), *P. azureus*, *D. nanus* and *Boana raniceps* (Cope, 1862), and in banana plantations, under fallen leaves.

Conservation

In Paraguay, *Adenomera guarani* sp. nov. occurs in two national parks: Cerro Corá and Ybicuy. In Argentina, it was found in the Parque Nacional Mburucuyá (Corrientes Province), Parque Nacional Chaco (Chaco Province), and on the outskirts of Parque Nacional Río Pilcomayo (Formosa). Recently, Argentinean populations, under the name of *Leptodactylus diptyx*, were categorized at national level as "No Amenazada" (= Least Concern according to IUCN) since the finding of several new populations. Apparently, *A. guarani* reproduces in urbanized areas in the Argentinean Humid Chaco (Zaracho *et al.* 2012b).

Discussion

Fouquet *et al.* (2014) found that *Adenomera diptyx* contains four lineages based on deep genetic structuration. However, phenotypic traits for each of these four lineages were not assessed by those authors. Two lineages (1 and 2) have a sympatric occurrence in Paraguay. This is particularly relevant for the assignment of one of these two lineages to nominal *A. diptyx*, especially because its type locality is imprecise, referred to only as "Paraguay, Amer. Merid.". In our study, we provided morphological, acoustic, and molecular data that supported these two lineages as distinct evolutionary units. We also addressed the taxonomic identity of nominal *A. diptyx* (lineage #2), which also led us to confirm that lineage #1 represented an unnamed species, described herein as *A. guarani* sp. nov. The redefinition of *A. diptyx* and naming of *A. guarani* will contribute to future studies focusing on the taxonomic identity of the other two genetic lineages (3 and 4) that are closely related to *A. guarani* and *A. diptyx.*

In this context, it is also pertinent to discuss the taxonomic status of *Leptodactylus glandulosus* Cope, 1887, currently considered a junior synonym of *A. diptyx.* The type specimen (lectotype ANSP 11275) from Chapada dos Guimarães (Mato Grosso State, Brazil) also has the vertebral pinstripe of nominal *A. diptyx*. Complementary, the specimen MZUSP 82281 (WCAB 15550 field number) from the same locality exhibits the same pattern, and the high note repetition rate is also similar to that of *A. diptyx* (Heyer 1973: fig. 26; Table 3). However, Fouquet *et al.* (2014) found that specimens from the type locality of *L. glandulosus* correspond to a one of the eleven lineages of *A. hylaedactyla* (lineage #9; see Fouquet *et al.* 2014: appendix s3c). Therefore, *Leptodactylus glandulosus* Cope, 1887 should possibly be a junior synonym of *A. hylaedactyla* or one of the candidate new species within this species complex outside Amazonia (see Carvalho *et al.* 2019a). The taxonomic status of this junior synonym should be addressed as part of a taxonomic review of the *A. hylaedactyla* complex (T.R. Carvalho *et al.*, in prep.).

Adenomera diptyx (Boettger, 1885) was removed from the synonymy of *A. hylaedactyla* and applied to populations of *Adenomera* from Bolivia with exotrophic tadpoles, reproductive mode 21 of Duellman & Trueb (1994), by De la Riva (1996). Moreover, the same author suggested that the populations from Paraguay and those from Argentina mentioned by Heyer (1973), Yanosky *et al.* (1993), and Mercolli *et al.* (1995) also corresponded to *A. diptyx*. However, the lectotype of *A. diptyx* had not been examined

when that taxonomic act was proposed (Angulo & Reichle 2008). The reproductive mode with freely feeding larvae after the flooding of the foam nest was observed in populations from Buenavista and Amboró national parks, both in Bolivia (De la Riva 1995). These two populations were later assigned to subclade 3 of Fouquet *et al.* (2014). More recently, Zaracho & Kokubum (2017) described the same reproductive mode and larval morphology in populations from the Pantanal wetlands of Corumbá (Mato Grosso do Sul, Brazil) and two Argentinean localities: Laguna Naick Neck (Formosa Province) and Corrientes (Corrientes Province). These populations belong to subclade 1, now *A. guarani* sp. nov. The reproductive mode of nominal *A. diptyx* (subclade 2) and subclade 4 remains unknown. *Adenomera diptyx* most likely has the same reproductive mode with exotrophic larvae based on its calling habitat, which is susceptible to flooding. Field observations of *A. diptyx* in Três Lagoas, Brazil (the easternmost record of the species; see Fig. 1), indicate that the species has an aquatic reproductive mode. The recorded male was calling in a completely flooded area next to a river, where there were a few spots above the water's surface. Species with a terrestrial reproductive mode, such as *A. hylaedactyla*, would never use such a type of habitat as a calling and breeding site (T.R. Carvalho, pers. obs.). On the other hand, species of *Adenomera* with endotrophic larvae occupy areas completely isolated from water bodies and that are not prone to flooding. The available data on the distribution of subclade 4 suggest that this lineage is restricted to Bolivia's Chiquitano region. Additional data on morphological, acoustic, and reproductive behavior data of both subclades 3 and 4 in Bolivia need to be assessed for the evaluation of their taxonomic status relative to *A. diptyx* and *A. guarani*.

The reproductive mode of *A. guarani* sp. nov. (i.e., tadpoles that require an aquatic phase to complete their development) is unusual in *Adenomera*. Exotrophic tadpoles have been reported only for two other species: *A. saci* and *A. thomei* (Almeida & Angulo 2006; Carvalho & Giaretta 2013b), and assumed for *A. martinezi* (see Carvalho & Giaretta 2013b) and *A. diptyx* (see the previous paragraph). *Adenomera guarani* and *A. saci* share an LTRF: 2(2)/3(1), different from that of *A. thomei* larvae (LTRF: 2(2)/3). Heyer (1973) described exotrophic tadpoles from Campos do Jordão, in the Mantiqueira Mountain Range of the State of São Paulo (southeastern Brazil), and assigned them to *A. bokermanni*. However, the distribution range of this species was subsequently restricted to the coastal Atlantic Forest of the State of Paraná, southern Brazil (Fouquet *et al.* 2014). Moreover, tadpoles from the type locality of *A. bokermanni* remain undocumented for direct comparison. Based on the known distribution range of *Adenomera*, the only two species that could occur in the Campos do Jordão area are *A. marmorata* and *A. thomei* (Fouquet *et al.* 2014; Cassini *et al.* 2020). The latter species has an exotrophic tadpole (Almeida & Angulo 2006), which renders *A. thomei* the most likely identification of the tadpole lot examined and described by Heyer (1973). A review of the populations from southeastern Brazil assigned to *A. bokermanni* and its tadpole characterization from the type locality is still needed.

The acoustic and morphological similarities among several populations of *A. diptyx* and *A. hylaedactyla* have led to historical misidentifications of these species. With the redescription of *A. diptyx*, some characters can be used to discriminate the two species. *Adenomera hylaedactyla* has a terrestrial developmental mode, with lecithotrophic tadpoles (Heyer & Silverstone 1969; Reproductive Mode 22 according to Duellman & Trueb 1994; assumedly exotrophic in *A. diptyx*). Although *A. hylaedactyla* could constitute a species complex (Carvalho *et al.* 2019a), individuals have a robust body shape and a larger body size (male SVL = 22.2–26.5 mm) in comparison with *A. diptyx*, which has a slender body and a smaller body size (male SVL = 18.2–23.1 mm).

Calls of *A. diptyx* described by Márquez *et al.* (1995) from Buenavista (Santa Cruz, Bolivia), previously assigned to *A. hylaedactyla* (see further discussion in Carvalho *et al.* 2019a), should correspond to at least one of the two candidate new species closely related to *A. diptyx* in Bolivia (lineages 3 and 4 of Fouquet *et al.* 2014). Both lineages were genetically sampled from Santa Cruz, but those sequences are not linked to a recorded male. By looking at the calling rate of four recordings (Fonozoo #10574,

10577, 10586, and 10629), we found that the note repetition rate (up to 130 per minute) falls within the value range of *A. guarani* sp. nov. (73–147 per minute) and differs from that of *A. diptyx* (176–299 per minute). Lineages 3 and 4 form a clade together with *A. guarani*, with *A. diptyx* being sister to the other three lineages (Figs 1–2; Fouquet *et al.* 2014: fig. 1, appendix s3c). It seems reasonable that Bolivian populations are more acoustically similar to *A. guarani* because of their phylogenetic proximity to each other. Therefore, lineages 3 and 4 could correspond to one or two additional unnamed species or, alternatively, could be conspecific with *A. guarani*. However, additional morphological and acoustic data need to be acquired for an appropriate assessment of the taxonomic identity of Bolivian populations closely related to *A. guarani*.

Biogeographically, new records of *Adenomera guarani* sp. nov. and *A. diptyx* will allow a better understanding of the distribution of both species. *Adenomera guarani* inhabits mainly the Humid Chaco ecoregion of Argentina and Paraguay and the Pantanal wetlands ecoregion of Brazil and Paraguay (Dinerstein *et al.* 2017), and a few populations were also found in transitional areas with the Atlantic Forest. Instead, *A. diptyx* inhabits the ecotonal area between the Humid Chaco or Cerrado and the Atlantic Forest, except for the northernmost occurrence records in the Chiquitano dry forest, in the boundary region between Brazil and Bolivia.

The population from the Argentinean Atlantic Forest reported by Heyer (1973) needs a deeper review since the available data are insufficient to determine its identity. To date, this population is known only from a few individuals housed at MACN, collected by A. Barrio between 1971 and 1972. Ever since, and despite numerous search efforts at the locality where those individuals had been collected, additional sampling has been unsuccessful. This population was suggested to correspond to *A. hylaedactyla* (Heyer op. cit.), but taking into consideration the new data provided in our study, it is likely that this population belongs to one of the taxa (named or unnamed) comprising the open-habitat *Adenomera* clade that occurs in the Atlantic Forest domain. For the moment, we prefer not to assign a specific name to this population until we have advertisement calls and/or tissue samples to confirm its identity.

Our study highlights the need for the use of multiple sources of information (i.e., morphology, acoustics, cytogenetics, molecular phylogenetics, and reproductive mode data) for the resolution of taxonomic problems in *Adenomera*. The historical reevaluation of species of *Adenomera*, whose original descriptions are brief or ambiguous, including imprecise type localities, is also needed, as is the case of *A. diptyx* and related lineages that remain without a definite taxonomic status. The novel data will contribute to addressing the taxonomy of several other lineages of *Adenomera* broadly distributed in open habitats in South America.

Acknowledgments

References

Airaldi Wood K., Lavilla E.O. & Garcete-Barrett B.R. 2013. Anuros de la Reserva de Recursos Manejados Ybyturuzú. Un enfoque sobre su estado de conservación. *Reportes Científicos de la FACEN* 4: 21–33.

Aljanabi S.M. & Martinez I. 1997. Universal and rapid salt-extraction of high quality genomic DNA for PCR based techniques. *Nucleic Acids Research* 25: 4692–4693. https://doi.org/10.1093/nar/25.22.4692

Almeida A.P. & 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

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

Angulo A., Cocroft R.B. & Reichle S. 2003. Species identity in the genus *Adenomera* (Anura: Leptodactylidae) in Southeastern Peru. *Herpetologica* 59 (4): 490–504. https://doi.org/10.1655/20-104

Audacity Team. 2017. *Audacity(R): Free Audio Editor and Recorder*. Ver. 2.1.1. Available from https://audacityteam.org [accessed 5 May 2018].

Berneck B.V.M., Costa C.O.R. & Garcia P.C.A. 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

Boettger O. 1885. Liste von Reptilien und Batrachiern aus Paraguay. *Zeitschrift für Naturwissenschaften* 58: 213–248. Available from https://www.biodiversitylibrary.org/page/31401231 [accessed 31 May. 2023].

Boettger O. 1892. Kriechthiere der Kaukasusländer, gesammelt durch die Radde-Valentin'sche Expedition nach dem Karabagh und durch die Herren Dr. J. Valentin un P. Reibisch. *Bericht über die Senckenbergische Naturforschende Gesellschaft* 1892: 131–150.

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

Brusquetti F. & Lavilla E. 2006. Lista comentada de los anfibios de Paraguay. *Cuadernos de Herpetología* 20 (2): 3–79.

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

Carvalho T.R. & Giaretta A.A. 2013b. 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 T.R., Giaretta A.A., Maciel N.M., Barrera D.A., Aguilar-Puntriano C., Haddad C.F.B., Kokubum M.N.C., Menin M. & Angulo A. 2019a. 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 T.R., Angulo A., Kokubum M.N.C., Barrera D.A., Souza M.B., Haddad C.F.B. & Giaretta A.A. 2019b. 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 T.R., Giaretta A.A., Angulo A., Haddad C.F.B. & Peloso P.L.V. 2019c. A new Amazonian species of *Adenomera* (Anura: Leptodactylidae) from the Brazilian state of Para: a tody-tyrant voice in a frog. *American Museum Novitates* 3919:1–21 https://doi.org/10.1206/3919.1

Carvalho T.R., Cassini C.S., Taucce P.P.G. & Haddad C.F.B. 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 T.R., Angulo A., Barrera D.A., Aguilar-Puntriano C. & Haddad C.F.B. 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 T.R., Moraes L.J.C.L., Angulo A., Werneck F.P., Icochea J. & Lima A.P. 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: 1–18. https://doi.org/10.5852/ejt.2020.682

Carvalho T.R., Simões P.I., Gagliardi-Urrutia G., Rojas-Runjaic F.J.M., Haddad C.F.B. & 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 T.R., Moraes Leandro J.C.L, Lima A.P., Fouquet A., Peloso P.L.V., Pavan D., Drummond L.O., Rodrigues M.T., Giaretta A.A., Gordo M., Neckel-Oliveira S. & Haddad C.F.B. 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* 191 (2): 395-433. https://doi.org/10.1093/zoolinnean/zlaa051

Cassini C.S., Taucce P.P.G., Carvalho T.R., Fouquet A., Solé M., Haddad C.F.B. & Garcia P.C.A. 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

Cei J.M. 1956. Nueva lista sistemática de los batracios de Argentina y breves notas sobre su biología y ecología. *Investigaciones Zoológicas Chilenas* 3: 35–68.

Cei J. M. 1980. *Amphibians of Argentina*. Monitore Zoologico Italiano (NS), Monografia 2.

Cei, J.M. 1987. Additional notes to "Amphibians of Argentina": an update, 1980-1986. *Monitore Zoologico Italiano* (NS) 21: 209–272.

Cochran D.M. 1955. Frogs of southeastern Brazil. *Bulletin of the United States National Museum* 206: 1–423. https://doi.org/10.5479/si.03629236.206.1

Cope E.D. 1868. An examination of the Reptilia and Batrachia obtained by the Orton Expedition to Equador and the Upper Amazon, with notes on other species. *Proceedings of the Academy of Natural Sciences of Philadelphia* 1868: 96–140.
Available from https://www.biodiversitylibrary.org/page/9768245 [accessed 31 May 2023].

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 la Riva I. 1996. The specific name of *Adenomera* (Anura: Leptodactylidae) in the Paraguay River basin. *Journal of Herpetology* 30 (4): 556–558. https://doi.org/10.2307/1565706

Dinerstein, E., Olson, D., Joshi A., Vynne C., Burgess N.D., Wikramanayake E., Hahn N., Palminteri S., Hedao P., Noss R., Hansen M., Locke H., Ellis E.C, Jones B., Barber C.V., Hayes R., Kormos C., Martin V., Crist E., Sechrest W., Price L., Baillie J.E.M., Weeden D., Suckling K., Davis C., Sizer N., Moore R., Thau D., Birch T., Potapov P., Turubanova S., Tyukavina A., De Souza N., Pintea L., Brito J.C., Llewellyn O.A., Miller A.G., Patzelt A., Ghazanfar S.A., Timberlake J., Klöser H., Shennan-Farpón Y., Kindt R., Barnekow Lillesø J.-P., Van Breugel P., Graudal L., Voge M., Al-Shammari K.F. & Saleem M. 2017. An ecoregion-based approach to protecting half the terrestrial realm. *BioScience* 67 (6): 534–545. https://doi.org/10.1093/biosci/bix014

Duellman W.E. & Trueb L. 1994. *Biology of Amphibians*. The John Hopkins University Press, Baltimore, Maryland.

Faivovich J., Haddad C.F.B., Garcia P.C.A., Frost D.R., Campbell J.A. & Wheeler W.C. 2005. Systematic review of the frog family Hylidae, with special reference to Hylinae: phylogenetic analysis and taxonomic revision. *Bulletin American Museum Natural History* 294: 1–240. https://doi.org/10.1206/0003-0090(2005)294[0001:SROTFF]2.0.CO;2

Fouquet A., Cassini C.S., Haddad C.F.B., Pech N. & Trefaut Rodrigues M. 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

Freiberg M.A. 1942. Enumeración sistemática y distribución geográfica de los batracios argentinos. *Physis* 19 (52): 219–240.

Gallardo J.M. 1966. Zoogeografía de los anfibios chaqueños. *Physis* 26 (71): 67–81.

Gallardo J.M. 1986. La diversidad de la herpetofauna en la selva subtropical misionera. Actas X Congreso Latinoamericano de Zoología. *Anales del Museo de Historia Natural de Valparaíso* 17: 153–159.

Gallardo J.M. 1987a. *Anfibios Argentinos. Guía para su Identificación*. Biblioteca Mosaico, Buenos Aires.

Gallardo J.M. 1987b. Anfibios argentinos. Parte 1. *Amphibia & Reptilia (Conservación)* 1 (3): 60–66.

Gallardo J.M. & Varela de Olmedo E. 1992. *Anfibios de la República Argentina: Ecología y Comportamiento*. Fauna de agua dulce de la República Argentina 41 (1): 1–116.

Gavetti E. & Andreone F. 1993. *Revised Catalogue of the Herpetological Collection in Turin University. I. Amphibia.* Museo Regionale di Scienze Naturali, Cataloghi X, Torino.

Gelman A. & Rubin D.B. 1992. Inference from iterative simulation using multiple sequences. *Statistical Sciences* 7: 457–472.

Gorham S.W. 1966. Liste der rezenten Amphibien and Reptilien. *Das Tierreich* 85: 1–222.

Gorham S.W. 1974. *Check-list of World Amphibians up to January 1, 1970*. The New Brunswick Museum, St. John, Canadá.

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

Heyer W.R. 1975. *Adenomera lutzi* (Amphibia: Leptodactylidae), a new species of frog from Guyana. *Proceedings of the Biological Society of Washington* 88: 315–318.
Available from https://www.biodiversitylibrary.org/page/34561319 [accessed 31 May 2023].

Heyer W.R & Silverstone P.A. 1969. The larva of the frog *Leptodactylus hylaedactylus* (Leptodactrylidae). *Fieldiana Zoology* 51 (11): 141–145. https://doi.org/10.5962/bhl.title.3047

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

Howell W.M. & Black D.A. 1980. Controlled silver-staining of nucleolus organizer regions with a protective colloidal developer: a 1- step method. *Experientia* 36: 1014–1015.
https://doi.org/10.1007/BF01953855

Hurvich C. & Tsai C. 1989. Regression and time series model selection in small samples. *Biometrika* 76 (2): 297–307. https://doi.org/10.1093/biomet/76.2.297

Kok P.J.R., Kokubum M.N.C., MacCulloch R.D. & 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 M.N.C. & Sousa M.B. 2008. Reproductive ecology of *Leptodactylus* aff. *hylaedactylus* (Anura, Leptodactylidae) from an open area in Northern Brazil. *South American Journal of Herpetology* 3: 15–21. https://doi.org/10.2994/1808-9798(2008)3[15:REOLAH]2.0.CO;2

Kumar S., Stecher G., Li M., Knyaz C. & Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35: 1547–1549. https://doi.org/10.1093%2Fmolbev%2Fmsy096

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

Lanfear, R., Calcott, B., Ho, S. Y. & Guindon S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29 (6): 1695–1701. https://doi.org/10.1093/molbev/mss020

Lanfear, R., Frandsen, P.B., Wright, A.M., 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 (3): 772–773. https://doi.org/10.1093/molbev/msw260

Lavilla E.O. 1992. Tipos portadores de nombre y localidades tipo de anfibios de Argentina. *Acta Zoológica Lilloana* 42 (1): 61–100.

Lavilla E.O. & Cei J. M. 2001. *Amphibians of Argentina, a Second Update, 1987-2000*. Monitore Zoologico Italiano (NS) Monografie 28, Torino, Italia.

Ligges U., Krey S., Mersmann O. & Schnackenberg S. 2017. tuneR: Analysis of music and speech. Available from https://CRAN.R-project.org/package=tuneR [accessed 11 Jan. 2019].

Lyra M.L., Haddad C.F.B. & Azeredo-Espin A.M.L. 2017. Meeting the challenge of DNA barcoding Neotropical amphibians: Polymerase chain reaction optimization and new COI primers. *Molecular Ecology Resources* 17: 966–980. https://doi.org/10.1111/1755-0998.12648

Lutz A. 1930. Segunda memoria sobre especies brasileiras do genero *Leptodactylus*, incluindo outras alliadas. *Memórias do Instituto Oswaldo Cruz* 23 (1): 1–34. https://doi.org/10.1590/S0074-02761930000100001

Mcdiarmid R.W. & Foster M.S. 1987. Additions to the reptile fauna of Paraguay with notes on a small herpetological collection from Amambay. *Studies on Neotropical Fauna and Environment* 22 (1): 1–9. https://doi.org/10.1080/01650528709360714

Márquez R., De la Riva I. & Bosch J. 1995. Advertisement calls of Bolivian Leptodactylidae (Amphibia, Anura). *Journal of Zoology* 237: 313–336. https://doi.org/10.1111/j.1469-7998.1995.tb02765.x

Mercolli C., Yanosky A.A. & Dixon J.R. 1995. Natural history of *Adenomera hylaedactyla* (Anura: Leptodactylinae) in the eastern Chaco of Argentina. *Bulletin of the Maryland Herpetological Society* 31: 117–129.

Miller M.A., Pfeiffer W. & Schwartz T. 2011. The CIPRES science gateway: a community resource for phylogenetic analyses. *Proceedings of the 2011 TeraGrid Conference: extreme digital discovery* 41: 1–8. https://doi.org/10.1145/2016741.2016785

Milto K.D. 2010. On the identity of *Bufo levicristatus* Boettger, 1885. *Russian Journal of Herpetology* 17 (4): 257–258.

Moritz C., Schneider C.J. & Wake D.B. 1992. Evolutionary relationships within the *Ensatina eschscholtzii* complex confirm the ring species interpretation. *Systematic Biology* 41: 273–291. https://doi.org/10.1093/sysbio/41.3.273

Nieden F. 1923. Anura I Subordo Aglossa und Phaneroglossa. Sectio 1. Arcifera. *Das Tierreich* 46: i–xxxii + 1–584.

R Core Team 2018. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. Available from https://www.R-project.org/ [accessed 11 Jan. 2019].

Ronquist F., Teslenko M., Van Der Mark P., Ayres D. L., Darling A., Höhna, S., Larget B., Liu L., Suchard M.A. & Huelsenbeck J. P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* 61 (3): 539–542. https://doi.org/10.1093/sysbio/sys029

Schmid M. 1978. Chromosome banding in Amphibia I. Constitutive heterochromatin and nucleolus organizer regions in *Bufo* and *Hyla*. *Chromosoma* 66: 361–388. https://doi.org/10.1007/BF00328536

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

Vaidya G., Lohman D.J. & Meier R. 2011. SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* 27 (2): 171–180. https://doi.org/10.1111/j.1096-0031.2010.00329.x

Yanosky A.A., Dixon J.R & Mercolli C. 1993. The herpetofauna of El Bagual Ecological Reserve (Formosa, Argentina) with comments on its herpetological collection. *Bulletin of the Maryland Herpetological Society* 29 (4): 160–171.

Zaracho V.H. & Hernando A.B. 2011. The karyotype of *Adenomera diptyx* (Boettger 1885) (Anura, Leptodactylidae) from northeastern Argentina. *Genetics and Molecular Biology* 34 (1): 84–87. https://doi.org/10.1590/S1415-47572010005000116

Zaracho V.H. & Kokubum M.N.C. 2017. Reproduction and larval morphology of *Adenomera diptyx* (Anura: Leptodactylidae) from the Argentinean humid Chaco and Brazilian pantanal. *Salamandra* 53: 1–9.

Zaracho V.H. & Lamas M.F. 2008. *Leptodactylus diptyx*. Endoparasites. Natural History Notes. *Herpetological Review* 39 (3): 337.

Zaracho V.H., Acosta J.L. & Lamas M.F. 2012a. Dieta y parasitismo de *Leptodactylus diptyx* (Anura: Leptodactylidae) del nordeste argentino. *Revista Mexicana de Biodiversidad* 83: 1180–1186. https://doi.org/10.22201/ib.20078706e.2012.4.1016

Zaracho V.H., Schaefer E. & Duré M. 2012b. *Leptodactylus diptyx* (Boettger, 1885). *In*: Categorización del Estado de Conservación de la Herpetofauna de la República Argentina. Ficha de los Taxones. Anfibios. *Cuadernos de Herpetología* 26 (S1): 204.

Supplementary material

Supp. file 1. Alignment file (.nex). https://doi.org/10.5852/ejt.2023.888.2205.9483

Supp. file 2. A 50% majority-rule consensus tree from Bayesian inference (.tre). https://doi.org/10.5852/ejt.2023.888.2205.9485

Appendices

Appendix 1. Other examined specimens. For *Adenomera diptyx* (Boettger, 1885) and *A. guarani* sp. nov., we provide geographic coordinates.

Adenomera guarani sp. nov.

ARGENTINA – **Corrientes** • 3 ♀♀; Corrientes city (type locality); 27°28′04″ S, 58°46′53″ W; UNNEC 9000, 9001, 11967 • 10 ♂♂; same collection data as for preceding; UNNEC 8974, 9606, 9704, 11961 to 11962,11965, 11968 to 11970, 13611 • 2 ♂♂; same collection data as for preceding; MNHNP 12907, 12908 • 1 spec. (sex no determined); same collection data as for preceding; UNNEC 13607 • 1 ♀; Corrientes city; 27°29′26″ S, 58°50′13″ W; UNNEC 8362 • 6 ♂♂; same collection data as for preceding; UNNEC 8293 to 8295, 8354, 8363, 8505 • 1 ♂; Ituzaingó; 27°29′50″ S, 56°42′12″ W; UNNEC 12331 • 2 ♂♂; San Cayetano; 27°33′04″ S, 58°40′37″ W; MNHNP 12909, 12910 • 6 ♀♀; same collection data as for preceding; UNNEC 11974, 11976, 11980 to 11982, 13608 • 2 ♂♂; same collection data as for preceding; UNNEC 13609, 13610 • 1 ♀; Parque Nacional Mburucuyá; 28°02′09″ S, 58°05′45″ W; UNNEC 5260 • 3 ♂♂; same collection data as for preceding; UNNEC 8356, 8357, 8506 • 1 ♀; Paso de la Patria; 27°19′27″ S, 58°34′05″ W; UNNEC 9076 • 2 ♂♂; same collection data as for preceding; UNNEC 9002, 9075. – **Chaco** • 1 ♀; San Martín; 26°08′28″ S, 59°35′43″ W; UNNEC 8261 • 1 ♂; La Leonesa; 27°05′48″ S, 58°44′42″ W; UNNEC 13612. – **Formosa** • 2 ♂♂; Comandante Fontana; 25°20′19″ S, 59°41′19″ W; UNNEC 8800, 9727 • 3 ♀♀; Laguna Naick Neck; 25°10′58″ S, 58°07′29″ W; UNNEC 9725,9726, 9863 • 11 ♂♂; same collection data as for preceding; UNNEC 9717 to 9724, 9859, 9865, 9870 • 3 juvs; same collection data as for preceding; UNNEC 9860 to 9862 • 5 sub-adult (sex no determined); same collection data as for preceding; UNNEC 9864, 9866 to 9868, 9871.

BRAZIL – **Mato Grosso do Sul** • 10 ♂♂; Nhecolândia, Passo do Lontra; 19°34′00″ S, 57°00′00″ W; LHUFCG 173 to 179, 205 to 207.

PARAGUAY – **San Pedro** • 6 ♀♀; Lima; 23°53′09″ S, 56°28′40″ W; MNHNP 0419, 0422, 0434, 5696, 5701, 5702 • 8 ♂♂; same collection data as for preceding; MNHNP 0411, 0431, 0433, 0435, 5695, 5698 to 5700 • 1 ♂; same collection data as for preceding; UNNEC 13939 • 5 ♀♀; Villa del Rosario, Compañía Mbocayá; 24°26′18″ S, 57°05′48″ W; MNHNP 0412, 0424, 0425, 0441, 0442 • 1 ♂; Río Aguaray Guazú, 2 km NW of Lima; 23°52′28″ S, 56°29′46″ W; MNHNP 5332. – **Concepción** • 1 ♀; Rancho Z; 22°58′24″ S, 57°00′38″ W; MNHNP 7045 • 2 ♂♂; 20 km NE of Concepción, about Route to Loreto; 23°17′45″ S, 57°22′18″ W; MNHNP 0432, 5703 • 1 ♀; 85 km NE of Concepción, Arroyo Capitigo; 22°46′30″ S, 56°47′56″ W; MNHNP 0430. – **Presidente Hayes** • 1 ♀; Ea. La Golondrina; 24°57′02″ S, 57°42′07″ W; MNHNP 0428 • 1 ♀; 44 km W of Concepción, Riacho Negro; 23°27′48″ S, 57°48′02″ W; MNHNP 0426 • 1 ♂; same collection data as for preceding; MNHNP 0420. – **Caaguazú** • 2 ♂♂; 24 km N of Coronel Oviedo; 25°17′19″ S, 56°24′08″ W; MNHNP 0414, 0440. – **Central** • 1 ♀; Villeta; 25°30′48″ S, 57°33′30″ W; MNHNP 1404. – **Cordillera** • 1 ♂; Saltos de Piraretá; 25°29′55″ S, 56°55′20″ W; MNHNP 0423. – **Paraguari** • 1 ♀; Parque Nacional Ybycuí, Fundición La Rosada; 26°01′05″ S, 57°02′53″ W; MNHNP 5391 • 15 ♂♂; same collection data as for preceding; MNHNP 0427, 0439, 4396, 4398, 4408, 4440, 4452, 4494, 4496, 5532, 5704 to 5708. – **Amambay** • 2 ♀♀; Parque Nacional Cerro Corá, Administración; 22°39′56″ S, 56°00′24″ W; MNHNP 0443, 5710. – **Alto Paraguay** • 1 ♀; Ea. Doña Julia, 5 km N of Bahía Negra; 20°15′34″ S, 58°12′02″ W; MNHNP 3699. – **Ñeembucú** • 1 ♂; 26 km NE of Pilar; 26°39′27″ S, 58°08′40″ W; MNHNP 10062.

Adenomera diptyx (Boettger, 1885)

BRAZIL – **Mato Grosso do Sul** • 1 ♂; Três Lagoas; 20°51′42″ S, 51°44′38″ W; 294 m a.s.l.; CFBH 45426. – **Mato Grosso** • 4 specs (not sexed); Vale de São Domingos, U.H.E. Guaporé; 15°19′59″ S, 58°50′60″ W; MCP 6994, 7015, 7024, 7032 • 1 ♂; same collection data as for preceding; MCP 6996.

PARAGUAY – **San Pedro** • 5 specs (not sexed); Laguna Blanca; 23°48′26″ S, 56°16′46″ W; MNHNP 11637, 11663, 116664, 11670, 11679.

Adenomera sp.

ARGENTINA – **Misiones** • 4 ♀♀; General Belgrano, Arroyo Moncholito; 26°03′57″ S, 53°49′54″ W; MACN-CENAI 6091, 6780, 6018, 5836 • 3 ♂♂; same collection data as for preceding; MACN-CENAI 6490, 5742, 5740.

Adenomera ajurauna (Berneck, Costa & Garcia, 2008)

BRAZIL – **São Paulo** • 1 ♂; Santo André, Paranapiacaba; AAG-UFU 5024 • 1 ♂; same collection data as for preceding: MCP13115.

Adenomera amicorum Carvalho, Moraes, Lima, Fouquet, Peloso, Pavan, Drummond, Rodrigues, Giaretta, Gordo, Neckel-Oliveira & Haddad, 2021

BRAZIL– **Pará** • 5 ♂♂; Belterra, Fazenda Treviso: CFBH 44465 to 44469.

Adenomera andreae (Müller, 1923)

BRAZIL– **Pará** • 1 ♂; Belém: AAG-UFU 2797; 1 ♀; same collection data as for preceding; AAG-UFU 2798 • 7 ♂♂; Nova Timboteua; AAG-UFU 2788 to 2794.

Adenomera araucaria Kwet & Angulo, 2002

BRAZIL – **Rio Grande do Sul** • 1 ♂; São Francisco de Paula; MCP 12055 • 3 ♀♀; same collection data as for preceding; MCP 9896, 11199, 11241.

Adenomera aurantiaca Carvalho, Moraes, Lima, Fouquet, Peloso, Pavan, Drummond, Rodrigues, Giaretta, Gordo, Neckel-Oliveira & Haddad, 2021

BRAZIL – **Pará** • 1 ♀; Trairão; INPA-H 40518 • 1 ♂; same collection data as for preceding; INPA-H 40520.

Adenomera bokermanni (Heyer, 1973)

BRAZIL – **Paraná** • 6 ♂♂; Morretes; LHUFCG 181 to 186.

Adenomera chicomendesi Carvalho, Angulo, Kokubum, Barrera, Souza, Haddad & Giaretta, 2019.

BRAZIL – **Acre** • 3 ♂♂; Rio Branco, Parque Zoobotânico; AAG-UFU 5862 to 5864 • 2 ♂♂; same collection data as for preceding; CFBH 43562, 43563.

PERU – **Madre de Dios** • 4 ♂♂; Tambopata; MUSM 39462, 39463, 39467, 39473 • 3 ♀♀; same collection data as for preceding; MUSM 39468, 39472, 39474.

Adenomera cotuba Carvalho & Giaretta, 2013

BRAZIL – **Goiás** • 9 ♂♂; Teresina de Goiás; AAG-UFU 808, 1397 to 1404.

Adenomera engelsi Kwet, Steiner & Zillikens, 2009

BRAZIL – **Santa Catarina** • 3 ♂♂; Santo Amaro da Imperatriz; CFBH 43213 to 43215.

Adenomera glauciae Carvalho, Simões, Gagliardi-Urrutia, Rojas-Runjaic, Haddad & Castroviejo-Fisher, 2020

BRAZIL – **Amazonas** • 2 ♂♂; Japurá, ESEC Juami-Japurá; CFBH 44854, 44855 • 8 ♂♂; same collection data as for preceding; MCP 13874, 13880, 13881, 13884, 13887, 13888, 13891, 13897 • 1 ♀; same collection data as for preceding; CFBH 44856 • 2 ♀♀; same collection data as for preceding; MCP 13882, 13889.

Adenomera gridipappi Carvalho, Moraes, Lima, Fouquet, Peloso, Pavan, Drummond, Rodrigues, Giaretta, Gordo, Neckel-Oliveira & Haddad, 2021

BRAZIL – **Rondônia** • 3 ♂♂; Porto Velho; CFBH 44470 to 44472.

Adenomera guarayo Carvalho, Angulo, Barrera, Aguilar-Puntriano & Haddad, 2020

PERU – **Madre de Dios** • 5 ♂♂; Tambopata; MUSM 39456 to 39458, 39465, 39466.

Adenomera heyeri Boistel, Massary & Angulo, 2006

BRAZIL – **Pará** • 1 ♀; Oriximiná, ESEC Grão-Pará; MPEG 30009.

Adenomera hylaedactyla (Cope, 1868)

BRAZIL – **Acre** • 2 ♂♂; Cruzeiro do Sul; AAG-UFU 5907, 5908 • 3 ♀♀; same collection data as for preceding; AAG-UFU 5909 to 5911. – **Amazonas** • 8 ♂♂; Manaus; INPA-H 22410 to 22413, 26606 to 26609 • 1 ♀; same collection data as for preceding; LHUFCG 190. – **Roraima** • 4 ♂♂; Cantá; AAG-UFU 5540 to 5543.

PERU – **Madre de Dios** • 3 ♂♂; Tambopata; MUSM 39459 to 39461.

Adenomera inopinata Carvalho, Moraes, Lima, Fouquet, Peloso, Pavan, Drummond, Rodrigues, Giaretta, Gordo, Neckel-Oliveira & Haddad, 2021

BRAZIL – **Pará** • 1 ♂; Itaituba; INPA-H 40517.

Adenomera juikitam Carvalho & Giaretta, 2013

BRAZIL – **Goiás** • 3 ♂♂; Teresina de Goiás; AAG-UFU 807, 1405, 1406.

Adenomera kayapo Carvalho, Moraes, Lima, Fouquet, Peloso, Pavan, Drummond, Rodrigues, Giaretta, Gordo, Neckel-Oliveira & Haddad, 2021

BRAZIL – **Pará** • 2 ♂♂; Palestina do Pará; AAG-UFU 6245, 6246 • 2 ♂♂; same collection data as for preceding; CFBH 43885, 43886.

Adenomera kweti Carvalho, Cassini, Taucce & Haddad, 2019

BRAZIL – **Santa Catarina** • 8 ♂♂; São Bonifácio; CFBH 43183 to 43190.

Adenomera lutzi Heyer, 1975

GUAYANA ESEQUIBA (territory) • 4 ♀♀; Potaro-Siparuni; MZUSP 149405, 149407, 150799, 150800 • 5 ♂♂; same collection data as for preceding; MZUSP 149409, 150801 to 150804.

Adenomera marmorata Steindachner, 1867

BRAZIL – **Rio de Janeiro** • 4 ♂♂; Rio de Janeiro, Floresta Nacional da Tijuca; CFBH 34401 to 34403, 34406 • 1 ♀; same collection data as for preceding; CFBH 34404.

Adenomera martinezi (Bokermann, 1956)

BRAZIL – **Pará** • 10 ♂♂; Novo Progresso, Cachimbo; AAG-UFU 1515 to 1520, 1522 to 1525 • 1 ♀; same collection data as for preceding; AAG-UFU 1521.

Adenomera nana (Müller, 1922)

BRAZIL – **Santa Catarina** • 1 ♀; Ilhota, Parque Botânico Morro do Baú; MCP 1345 • 2 ♂♂; Jaraguá do Sul; MCP 8149, 8150 • 1 ♂; Joinville; MCP 8633 • 3 ♀♀; Blumenau; MCP 8719, 8720, 12268 • 5 ♂♂; São Bento do Sul; MCP 8751 to 8755.

Adenomera phonotriccus Carvalho, Giaretta, Angulo, Haddad & Peloso, 2019

BRAZIL – **Pará** • 2 ♂♂; Palestina do Pará; CFBH 43130, 43131 • 2 ♂♂; same collection data as for peceding; MPEG 41155, 41156.

Adenomera saci Carvalho & Giaretta, 2013

BRAZIL – **Goiás** • 5 ♂♂; Alto Paraíso de Goiás; AAG-UFU 108, 109, 762, 763, 1339.

Adenomera simonstuarti (Angulo & Icochea, 2010)

PERU – **Cusco** • 2 ♂♂; La Convención, Echarate, Río Camisea; MUSM 18218, 18220 • 2 ♀♀; same collection data as for preceding; MUSM 18221, 18229.

Adenomera tapajonica Carvalho, Moraes, Lima, Fouquet, Peloso, Pavan, Drummond, Rodrigues, Giaretta, Gordo, Neckel-Oliveira & Haddad, 2021

BRAZIL – **Pará** • 1 ♀; Itaituba; INPA-H 40515 • 1 ♂; same collection data as for preceding; INPA-H 40516.

Adenomera thomei (Almeida & Angulo, 2006)

BRAZIL – **Espírito Santo** • 2 ♂♂; Linhares, Povoação; AAG-UFU 6185, 6186.

Appendix 2. Information about the analyzed sound files of *Adenomera diptyx* (Boettger, 1885) and *A. guarani* sp. nov. from Argentina, Brazil, and Paraguay. Call vouchers are provided between parentheses.

Adenomera guarani sp. nov.

BRAZIL **– Mato Grosso do Sul** • Nhecolândia, Passo do Lontra; 19°34′00″ S, 57°00′00″ W
    LHUFCG207_canto078_parte 1_BEP: 29 Nov 2006, 20:47 h, air 30.0°C (LHUFCG 207).

ARGENTINA **– Corrientes** • Corrientes city (type locality); 27°28′04″ S, 58°46′53″ W
    FZ-UNNE 0709: 08 Feb 2007, 18:55 h, air 26.8°C
    FZ-UNNE 0711: 08 Feb 2007, 19:15 h, air 26.8°C
    FZ-UNNE 0712: 08 Feb 2007, 19:38 h, air 25.4°C
    FZ-UNNE 0713: 08 Feb 2007, 19:45 h, air 25.5°C
    FZ-UNNE 0714: 08 Feb 2007, 19:56 h, air 24.5°C
    FZ-UNNE 0715: 08 Feb 2007, 20:00 h, air 24.8°C
    FZ-UNNE 0716: 08 Feb 2007, 20:05 h, air 25°C (UNNEC 9074)
    FZ-UNNE 0096: 22 Nov 2011, 18:20 h, air 27.7°C
    FZ-UNNE 0284: 14 Nov 2013, 20:28 h, air 24.5°C (UNNEC 13611)
    FZ-UNNE 1013: 13 Dec 2013, 21:25 h, air 20.6°C
    FZ-UNNE 1014: 16 Dec 2013, 21:53 h, air 27.9°C
    FZ-UNNE 1016: 28 Nov 2013, 21:34 h, air 17.4°C

• Paso de la Patria
    FZ-UNNE 0706: 10 Feb 2007, 21:42 h, air 29.1°C
    FZ-UNNE 0708: 11 Feb 2007, 21:18 h, air 22.2°C

– Formosa • Laguna Naick Neck
    FZ-UNNE 0010: 23 Nov 2007, 19:50 h, air 22.7°C (UNNEC 9722)
    FZ-UNNE 0011: 23 Nov 2007, 20:00 h, air 22.3°C(UNNEC 9723)
    FZ-UNNE 0013: 23 Nov 2007, 20:10 h, air 22°C (UNNEC 9724)
    FZ-UNNE 0020: 29 Feb 2008, 20:00 h, air 28.7°C (UNNEC 9856)
    FZ-UNNE 0023: 29 Feb 2008, 20:35 h, air 26°C (UNNEC 9859)

• Comandante Fontana
    FZ-UNNE 0014: 24 Nov 2007, 21:27 h, air 20.2°C (UNNEC 9727)
    FZ-UNNE 0019: 25 Nov 2007, 18:37 h, air 23.7°C

• Clorinda
    FZ-UNNE 0081: 15 Nov 2010; 18:30 h; 25°C

Adenomera diptyx (Boettger, 1885)

BRAZIL **– Mato Grosso do Sul** • Três Lagoas; 20°51′42″ S, 51°44′38″ W
    Adenomera_diptyxTresLagoasMS1cTRC_AAGm671: 18:53 h, 20 Dec 2020, air 27.1°C (CFBH 45426)

• Bela Vista; 22°04'27"S, 56°30'59"W
    Adenomera_diptyxBelaVistaMS1cTRC_AAGmt: 19:31 h, 17 Dec 2010, air 31.0°C
    Adenomera_diptyxBelaVistaMS2bTRC_AAGmt: 19:16 h, 18 Dec 2010, air 32.0°C

PARAGUAY – **Amambay** • Parque Nacional Cerro Corá; 22°39′56″ S, 56°00′24″ W
    FZ-UNNE 0039: 12 Oct 2009, 16:20 h, air 19.1°C
    FZ-UNNE 0042: 14 Oct 2009, 16:24 h, 31.4°C
    FZ-UNNE 0332: 13 Jan 2015, 20:07 h, temperature unknown, upper 25°C
    FZ-UNNE 0344: 15 Jan 2015, 18:32 h, temperature unknown, upper 25°C

– **Cordillera** • Piribebuy; 25°28′06″ S, 57°02′33″ W
    FZ-UNNE 0073: 13 Nov 2010, 19:10 h, air 23.6°C (CZCEN 0640)
    FZ-UNNE 0074: 13 Nov 2010, 19:20 h, air 23.6°C (CZCEN 0641)
    FZ-UNNE 0075: 13 Nov 2010, 19:35 h, air 22.3°C
    FZ-UNNE 0079: 13 Nov 2010, 20:45 h, air 23°C
    FZ-UNNE 0080: 13 Nov 2010, 20:55 h, air 23°C (CZCEN 0643)

Appendix 3 (continued on next three pages). Genbank accession numbers of specimens of *Adenomera* Steindachner, 1867 used in the phylogenetic analysis.

Terminal	Clade	Field# / Tissue#	Museum#	Cyt-b	COI	RAG1	POMC	Locality
Adenomera andreae	*A. andreae*	MTR18580		KF674837	KF674525	KF674205	KF673893	BRAZIL: Amazonas, Beruri, Lago Chaviana (margem direita do Purus)
Adenomera diptyx	«Open habitat»	RGA5254		KF674910	KF674601	KF674282	KF673969	BRAZIL: Mato Grosso, Pontes e Lacerda, UHE Guaporé
Adenomera diptyx	«Open habitat»		IIBPH1133	KF674909	KF674600	KF674281	KF673968	PARAGUAY: Cordillera, San Bernardino
Adenomera diptyx	«Open habitat»		UNNEC 13936	OR250447	OR248766	OR250457		PARAGUAY: Amambay, Parque Nacional Cerro Corá
Adenomera diptyx	«Open habitat»	TRC279	CFBH45426		OR248765			BRAZIL: Mato Grosso do Sul, Três Lagoas
Adenomera guarani sp. nov.	«Open habitat»		UNNEC9074	OR250445	OR248761	OR250453		ARGENTINA: Corrientes, Capital, Corrientes, Universidad Nacional del Nordeste
Adenomera guarani sp. nov.	«Open habitat»		UFMT3923	KF674907	KF674598	KF674279	KF673966	BRAZIL: Mato Grosso, Poconé
Adenomera guarani sp. nov.	«Open habitat»		UNNEC9724	KF674908	KF674599	KF674280	KF673967	ARGENTINA: Formosa, Laguna Naick Neck
Adenomera guarani sp. nov.	«Open habitat»		UNNEC9719	OR250449	OR248763	OR250455		ARGENTINA: Formosa, Laguna Naick Neck
Adenomera guarani sp. nov.	«Open habitat»		UNNEC9704	OR250446	OR248762	OR250454		ARGENTINA: Corrientes, Capital, Corrientes
Adenomera guarani sp. nov.	«Open habitat»		UNNEC8293	KF674904	KF674595	KF674276	KF673963	ARGENTINA: Corrientes, Capital, Corrientes
Adenomera guarani sp. nov.	«Open habitat»	CBHT7463 / FSFL968	UFMG-AMP3675	KF674905	KF674596	KF674277	KF673964	BRAZIL: Mato Grosso do Sul, Corumbá
Adenomera guarani sp. nov.	«Open habitat»		IIBPH905	KC603966	KC603994	KF674278	KC604062	PARAGUAY: Concepción
Adenomera guarani sp. nov.	«Open habitat»		UNNEC9721	OR250450	OR248764	OR250456		ARGENTINA: Formosa, Laguna Naick Neck
Adenomera guarani sp. nov.	«Open habitat»		UNNEC9002	OR250448	OR248760	OR250452		ARGENTINA: Corrientes, Paso de la Patria
Adenomera guarani sp. nov.	«Open habitat»		MNHNP11333	OR250444	OR248759	OR250451		PARAGUAY: Amambay, Parque Nacional Cerro Corá
Adenomera heyeri	*A. heyeri*	264CM		KF675008	KF674699	KF674382	KF674067	FRENCH GUIANA: Montagne des singes
Adenomera hylaedactyla 1	«Open habitat»	MJ1344		KF674920	KF674611	KF674292	KF673979	BOLIVIA: Beni
Adenomera hylaedactyla 1	«Open habitat»		LSU17566	KF674919	KF674610	KF674291	KF673978	BRAZIL: Rondônia, Guajará-Mirim

Appendix 3 (continued).

Terminal	Clade	Field# / Tissue#	Museum#	Cyt-b	COI	RAG1	POMC	Locality
Adenomera hylaedactyla 2	«Open habitat»	MCF40		KF674930	KF674621	KF674303	KF673989	BRAZIL: Alagoas, Passo do Camaragibe
Adenomera hylaedactyla 2	«Open habitat»	CFBHT8289	CFBH19195	KF674929	KF674620	KF674301	KF673988	BRAZIL: Maranhão, Alcântara
Adenomera hylaedactyla 3	«Open habitat»		MPEG28084	KF674934	KF674625	KF674307	KF673993	BRAZIL: Pará, Juruti (margem direita do Mamuru)
Adenomera hylaedactyla 3	«Open habitat»	MTR13820		KF674932	KF674623	KF674305	KF673991	BRAZIL: Amapá, Serra do Navio
Adenomera hylaedactyla 4	«Open habitat»		MHNC5450	KF674935	KF674626	KF674308	KF673994	PERU: Tingo Maria-Huánuco
Adenomera hylaedactyla 4	«Open habitat»		MHNC10126	KF674966	KF674657	KF674339	KF674025	PERU: Echarate, Cusco
Adenomera hylaedactyla 5	«Open habitat»	MRT7511		KF674971	KF674662	KF674344	KF674030	BRAZIL: Tocantins, Guaraí
Adenomera hylaedactyla 6	«Open habitat»	ESTR1626		KF674972	KF674663	KF674345	KF674031	BRAZIL: Tocantins, Palmeirante
Adenomera hylaedactyla 6	«Open habitat»	PV293		KF674973	KF674664	KF674346	KF674032	BRAZIL: Piauí, Amarante
Adenomera hylaedactyla 7	«Open habitat»	CTMZ05278		KF674976	KF674667	KF674349	KF674035	BRAZIL: Mato Grosso, Sapezal
Adenomera hylaedactyla 7	«Open habitat»	ARI414		KF674975	KF674666	KF674348	KF674034	BRAZIL: Mato Grosso, Aripuanã
Adenomera hylaedactyla 8	«Open habitat»	CN2196		KF674983	KF674674	KF674356	KF674042	BRAZIL: Pará, ESEC Grão-Pará
Adenomera hylaedactyla 8	«Open habitat»	CN1800		KF674984	KF674675	KF674357	KF674043	BRAZIL: Pará, Paru
Adenomera hylaedactyla 9	«Open habitat»	CG332		KF674986	KF674677	KF674359	KF674045	BRAZIL: Mato Grosso, Chapada dos Guimarães
Adenomera hylaedactyla 9	«Open habitat»	4996		KF674985	KF674676	KF674358	KF674044	BRAZIL: Mato Grosso, APM Manso
Adenomera hylaedactyla 10	«Open habitat»	MTR21603		KF674991	KF674682	KF674364	KF674050	BRAZIL: Goiás, Pirenópolis
Adenomera hylaedactyla 10	«Open habitat»	CTMZ05373		KF674989	KF674680	KF674362	KF674048	BRAZIL: Mato Grosso, Tesouro
Adenomera hylaedactyla 11	«Open habitat»	AJC2882		KF464992	KF674683	KF674363	KF674051	VENEZUELA: Amazonas, Pueto Ayacucho, Atures
Adenomera lutzi	*A. lutzi*		ROM43828	KF674993	KF674684	KF674367	KF674052	GUYANA: Potaro River

Appendix 3 (continued).

Terminal	Clade	Field# / Tissue#	Museum#	Cyt-b	COI	RAG1	POMC	Locality
Adenomera marmorata	*A. marmorata*		CFBH12850	KF675087	KF674778	KF674465	KF674146	BRAZIL: Rio de Janeiro, Parque Nacional da Tijuca
Adenomera martinezi	«Open habitat»		CHUNB40220	KF675005	KF674696	KF674379	KF674064	BRAZIL: Pará, Novo Progresso
Adenomera martinezi	«Open habitat»		CHUNB40218	KF675006	KF674679	KF674380	KF674065	BRAZIL: Pará, Novo Progresso
Adenomera saci	«Open habitat»		CHUNB49509	KF675004	KF674695	KF674378	KF674063	BRAZIL: Goiás, Alto Paraíso de Goiás
Adenomera saci	«Open habitat»	PV0591		KF674999	KF674690	KF674373	KF674058	BRAZIL: Tocantins, Ananás
Adenomera sp. B	«Open habitat»	ESTR307		KF675007	KF674698	KF674381	KF674066	BRAZIL: Maranhão, Carolina
Adenomera «lineage 3»	«Open habitat»	MJ1373		KF674912	KF674603	KF674284	KF673971	BOLIVIA: Santa Cruz
Adenomera «lineage 3»	«Open habitat»	AM1357		KF674913	KF674604	KF674285	KF673972	BOLIVIA: Santa Anita
Adenomera «lineage 3»	«Open habitat»		MNCN4005	KF674914	KF674605	KF674286	KF673973	BOLIVIA: Santa Cruz
Adenomera «lineage 3»	«Open habitat»	MJ1308		KF674916	KF674607	KF674288	KF673975	BOLIVIA: Santa Cruz
Adenomera «lineage 3»	«Open habitat»	AM1007		KF674915	KF674606	KF674287	KF673974	BOLIVIA: Paraparau
Adenomera «lineage 4»	«Open habitat»	AS0212		KF674911	KF674602	KF674283	KF673970	BOLIVIA: Santa Cruz
Lithodytes lineatus	Outgroup	55MC		JQ321833	KC604003	KC604025	KC604060	FRENCH GUIANA