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A New Amazonian Species of the Diminutive Frog Genus *Adelophryne* (Anura: Brachycephaloidea: Eleutherodactylidae) from the State of Amapá, Northern Brazil

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During recent field expeditions to an Amazonian region in eastern Guiana Shield (Serra do Navio, state of Amapá, northern Brazil), we collected and recorded calls of a species of *Adelophryne*, a diminutive leaf-litter-dwelling, direct-developing frog genus. After a careful integrative taxonomic evaluation using morphological, molecular, and bioacoustic data, we concluded that the series of specimens collected represent a new taxon, which we describe herein. The new species of *Adelophryne* is distinguished from all ten congeners by the following combination of character states: (1) male SVL = 12.5 mm; female SVL = 13.0–14.4 mm; (2) tympanic membrane present; (3) tympanic annulus present, incomplete; (4) vomerine teeth absent; (5) finger terminal discs absent; (6) tips of Fingers I–IV mucronate; (7) finger pads present (formula 1–1–2–1); (8) three phalanges in Finger IV; (9) dorsum smooth; (10) cloacal flap absent; (11) multi-note advertisement call composed of non-pulsed notes; (12) the call dominant frequency (4,802–5,706 Hz) coincides with the fundamental harmonic. Our study describes the eleventh species of *Adelophryne*, and, despite the increase in taxonomic knowledge within the past few years, there are still some species in the genus lacking a formal taxonomic description.

Recentemente, durante trabalhos de campo em uma região da floresta Amazônica na parte leste do Escudo das Guianas, município de Serra do Navio, estado do Amapá, região norte do Brasil, nos foi possível coletar alguns exemplares de *Adelophryne*, um gênero composto de diminutas espécies de rãs de desenvolvimento direto que habitam a serapilheira. Após um cuidadoso estudo de taxonomia integrativa utilizando dados morfológicos, moleculares e bioacústicos, chegamos à conclusão de que os espécimes coletados representam um novo táxon, que descrevemos no presente trabalho. A nova espécie pode ser distinguida de todas as outras espécies de *Adelophryne* pela seguinte combinação de caracteres: (1) CRC em machos 12,5 mm; fêmeas 13,0–14,4 mm; (2) membrana do tímpano presente; (3) anel do tímpano presente, incompleto; (4) dentes vomerianos ausentes; (5) discos terminais dos dedos ausentes; (6) pontas dos dedos I–IV mucronadas; (7) almofadas dos dedos presentes (fórmula 1–1–2–1); (8) três falanges no dedo IV; (9) dorso liso; (10) flap cloacal ausente; (11) canto de anúncio multi-nota, composto por notas sem pulso; (12) frequência dominante do canto (4,802–5,706 Hz) coincidente com o harmônico fundamental. Nosso estudo descreve a décima primeira espécie de *Adelophryne* e, apesar do enorme aumento no conhecimento taxonômico sobre o gênero nos últimos anos, ainda existem espécies do gênero que carecem de descrição taxonômica formal.

ADELOPHRYNE, one of the four genera in the Eleutherodactylidae, has a discontinuous distribution, with some species inhabiting the Guiana Shield and others inhabiting the northern and central portions of the Brazilian Atlantic rainforest (Frost, 2019). These frogs are very small (snout–vent length [SVL] varying from 7.6 to 23 mm; MacCulloch et al., 2008; Lourenço-de-Moraes et al., 2018) and are secretive leaf-litter dwellers (Fouquet et al., 2012), which are very difficult to collect (MacCulloch et al., 2008; Lourenço-de-Moraes et al., 2012), making the number of specimens of *Adelophryne* available in scientific collections very low (Lourenço-de-Moraes et al., 2014). Fouquet et al. (2012) assessed the phylogenetic relationships of *Adelophryne* using molecular data and recovered it as monophyletic and as the sister genus of *Phyzelaphryne*, also confirming the phyzelaphrynine subfamily as monophyletic, in accordance with previous studies (Hedges et al., 2008; Pyron and Wiens, 2011), but with a more comprehensive taxon sampling. The authors also concluded that the diversity of the *Adelophryne* was deeply underestimated and proposed that there were seven unnamed candidate species besides the six species already described at that time, namely *A. adiastola*, *A. baturitensis*, *A. gutturosa*, *A. maranguapensis*, *A. pachydactyla*, and *A. patamona*. Since then, taxonomists have published four additional species: *A. glandulata*, *A. meridionalis*, *A. michelin,* and *A. mucronata*, totaling ten formally named species in the genus.

During recent field expeditions to an Amazonian region of the eastern Guiana Shield in the municipality of Serra do Navio, state of Amapá, northern Brazil, we collected additional material from one of the candidate species of Fouquet et al. (2012). Here we provide an integrative approach to the newly collected data, combining three lines of evidence—molecular, morphological, and bioacoustic—to formally describe this Amazonian candidate species of *Adelophryne*.

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MATERIALS AND METHODS

Study area.—Fieldwork was conducted from 2014–2018 at Parque Natural Municipal do Cancão (0.91141°N, 52.00364°W, 100 m above sea level [a.s.l.]), in the municipality of Serra do Navio, Brazilian state of Amapá. The study area is characterized in detail in a recently published amphibian species survey conducted at the municipal park (Silva-e-Silva and Costa-Campos, 2018).

Molecular analyses.—We extracted genomic DNA from 99.5% ethanol-fixed muscle tissue using a standard ammonium precipitation method (Lyra et al., 2017) of five specimens of *Adelophryne*. We chose a fragment of the *16S ribosomal RNA* gene (*16S*), a molecular marker which is commonly used in anuran taxonomy (Vences et al., 2005; Fouquet et al., 2007; Lyra et al., 2017) to perform our analyses. We used the primers 16S-AR (5′–CGCCTGTTTATCAAAAACAT–3′) and 16S-BR (5′–GACCTGGATTACTCCGGTCTGA–3′) to amplify our fragment (Palumbi et al., 1991). We then performed PCR amplification using Taq DNA Polymerase Master Mix (Ampliqon S/A, Denmark) and Axygene Maxygene thermocyclers. The PCR program was a 3 min initial denaturing step at 95°C, followed by 35 cycles of 20 s at 95°C, 20 s at 50°C, and 1 min at 72°C, followed by a final extension step of 3 min at 72°C. We purified PCR product following Lyra et al. (2017) and sequenced it in both directions with a BigDye Terminator Cycle Sequencing Kit (version 3.0, Applied Biosystems) in an ABI 3730 automated DNA sequencer (Applied Biosystems) at Macrogen Inc. (Seoul, South Korea). Sequences were deposited in GenBank (MT472512–MT472516).

In order to allocate our newly collected material within the genus *Adelophryne*, we sampled all sequences available in GenBank belonging to the genus and sharing our chosen molecular fragment. From all candidate and named species of *Adelophryne*, we were not able to sample *A. meridionalis* because there are no available sequences from this species, and one of the candidate species of Fouquet et al. (2012), *Adelophryne* sp. 3, because there was no *16S* sequence available for this species. Nevertheless, the two species occur in the Brazilian Atlantic forest and the impact of not sampling them to our study should be small since the clades within *Adelophryne* have well circumscribed geographic patterns (Fouquet et al., 2012; Dominato et al., 2018; Lourenço-de-Moraes et al., 2018). As outgroups, we also included all *16S* available sequences in GenBank from the genus *Phyzelaphryne* plus one sequence each of *Eleutherodactylus* and *Diasporus*.

We used MAFFT v7.273 (Katoh and Standley, 2013) performing the E-INS-i algorithm to align our sequences. We used PartitionFinder v2.1.1 (Lanfear et al., 2017) to conduct the search for the best-fitting nucleotide substitution model considering our fragment as a single partition and using the corrected Akaike information criterion (AICc; Hurvich and Tsai, 1989). By default, PartitionFinder uses the software PhyML 3.0 (Guindon et al., 2010) to reconstruct a maximum likelihood tree to start the analysis.

We chose two optimality criteria to infer phylogenetic relationships. We performed a maximum-likelihood analysis with the software RAxML 8.2.10 (Stamatakis, 2014) searching for the most likely tree 100 times, and for assessing support we conducted 1,000 non-parametric bootstrap replicates. We also performed a Bayesian-inference analysis with the software MrBayes 3.2.6 (Ronquist et al., 2012) using two independent runs of 20,000,000 generations, starting with random trees and four Markov chains (one cold), sampled every 2,000 generations and discarding 25% of generations and trees as burn-in. To assess convergence we checked the standard deviation of split frequencies (should be less than 0.01) and effective sample size (should be larger than 200).

We performed uncorrected pairwise distances using R v3.3.3 (R Core Team, 2017) using the packages APE v5.1 (Paradis et al., 2004) and SPIDER v1.4.2 (Brown et al., 2012). Because alignments of the *16S* mitochondrial fragment are usually rich in gaps, we deleted the sites with gaps in a pairwise way (using *pairwise.deletion=T* in the *dist.dna* command) in order to reduce the effect of the alignment on the genetic distances.

Morphological analyses.—The following measurements were taken by PPGT to the nearest 0.05 mm and reported to the nearest 0.1 mm with a digital caliper under a stereomicroscope: snout–vent length (SVL), head length (HL), head width (HW), eye diameter (ED), distance between the anterior margins of the eyes (AMD), internarial distance (IND), eye to nostril distance (END), tympanum diameter (TD), forearm length (FAL), hand length (HAL), thigh length (THL), tibia length (TL), tarsal length (TAL), and foot length (FL). SVL, HL, HW, FAL, TL, FL, ED, TD, END, and IND follow Duellman (1970); HAL, THL, and TAL follow Heyer et al. (1990); and AMD follows Garcia et al. (2003). Nomenclature of the morphologic structures follows most literature on the *Adelophryne* (Hoogmoed et al., 1994; Lourenço-de-Moraes, 2012). We determined sex by the observation of secondary sexual characters of male specimens (presence of vocal sac and vocal slits). For female specimens, when it was not possible to observe eggs through the skin, we only regarded as females those specimens larger than the individuals identified as males but lacking male secondary sexual characters. Institutional abbreviations follow Sabaj (2020), with the addition of MTR (field number of Miguel Trefaut Rodrigues, housed at the herpetological collection of Laboratório de Herpetologia da Universidade de São Paulo).

We took X-ray images from all specimens of the type series using a Faxitron imaging system (model LX-60, DC 12, V. 1.0) at Laboratório de Ictiologia de Ribeirão Preto (LIRP) of the University of São Paulo (USP), Brazil. The exposition parameters were set as11 s at 31 kV. In order to clarify what we saw on the X-ray images, we cleared and double-stained bone and cartilage of one specimen with alizarin red and alcian blue following procedures modified from Taylor and Van Dyke (1985).

Call analysis.—We recorded calls in the study area (see above) using digital recorders (Marantz PMD 670 and 671; sampling rate: 44.1 kHz; bit depth: 16) and Sennheiser ME67/K6 unidirectional microphones. Recordings were stored as uncompressed wave files. Sound recordings are deposited in the acoustic repositories of AAG-UFU and CFBH collections and voucher specimen (CFBH 43257) is housed at CFBH; information on the files is provided in the Appendix 1. We analyzed calls using SoundRuler (Gridi-Papp, 2007) implemented in MATLAB scripts (MATLAB, 2004, The language of technical computing, version 6.5.2., The MathWorks, Inc., Natick, MA). We applied a high-pass filter up to 200 Hz to sound files in SoundRuler prior to conducting the acoustic analysis to reduce background noise. Acoustic traits were quantified through automated analysis in the time domain (durations and rates, and the rise time) and in the frequency domain (dominant frequency and frequency modulation). Spectrogram parameters were set as follows: FFT size = 1024 points, FFT overlap = 90%, window type = Hanning, contrast = 70%. Specific parameters for automated recognition in SoundRuler were set as: detection (smoothing = 120, resolution = 12); delineation (smooth factor = 1, smoothing = 25, and resolution = 1); critical amplitude ratio = –1 (disabled). Acoustic definitions and terminology follow those in Carvalho et al. (2019). We produced oscillograms and spectrograms using seewave (Sueur et al., 2008) and tuneR (Ligges et al., 2017) in R 3.5.0 (R Core Team, 2018), under the following parameters: window type = Hanning, FFT size = 256 points, FFT overlap = 90%, and amplitude scale = –40 dB relative to the signal level of the entire call; –60 dB relative to the signal level of one call note). The amplitude spectrum was produced in SoundRuler at an FFT size of 2048.

RESULTS

We obtained a final alignment of the *16S* fragment with 603 base pairs and the best-fitting substitution model was general timerReversible with γ-distribution and proportion of invariable sites (GTR + Γ + I). The standard deviation of split frequencies was 0.00357 and all ESS values were above 375, showing the Bayesian analysis converged as expected. *Adelophryne* was recovered as monophyletic with a posterior probability of 1.0 and a maximum-likelihood bootstrap of 98% (Fig. 1). All nominal and candidate species of *Adelophryne* were also recovered as monophyletic with a posterior probability of 1.0 and a maximum-likelihood bootstrap of 98–100%, and our new species grouped with *Adelophryne* sp. 7 of Fouquet et al. (2012). The new species was not recovered in the clade containing the other three Amazonian species, but as the sister group of the South Atlantic forest clade that is composed of *A. glandulata*, *A. mucronata*, and two candidate species with high support in the Bayesian analysis (1.0), but low support in the maximum-likelihood analysis (49%). Our uncorrected pairwise distance also indicated that all species of *Adelophryne* are highly divergent from each other and the distances ranged from 10.4% (minimum distance between *A. patamona* and *Adelophryne* sp. 7; Table 1) to 25.5% (maximum distance between *A. mucronata* and *A. pachydactyla*). The genetic distances among *Adelophryne* sp. 7 and the other species of *Adelophryne* ranged from 10.4% to 24.2% (maximum distance from *A. pachydactyla*).

The molecular evidence, together with the morphological and bioacoustic data (both presented hereafter), lead us to conclude that *Adelophryne* sp. 7 is a distinct evolving lineage and deserve to be named. So, herein we describe this species.

***Adelophryne amapaensis*, new species**

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Figures 2, 3, 4; Table 2

Adelophryne sp. 7: Fouquet et al., 2012 (550: table 1; 553; table 3; 555: fig. 1; 556: fig. 2; 557: fig. 3; 558: fig. 4; 559: table 4); Santana et al., 2012 (190; 191: fig. 4); Dominato et al., 2018 (580: table 2; 581: fig. 2); Lourenço-de-Moraes et al., 2018 (10: fig. 5; 11: table 2).

Holotype.—CFBH 43257 (field number TRC 180), adult male, Parque Natural Municipal do Cancão, municipality of Serra do Navio, state of Amapá, Brazil, 0.91141°N, 52.00364°W, 100 m a.s.l., T. R. de Carvalho, C. E. Costa-Campos, J. C. Souza, P. Sanches, and M. R. D. Souza, 30 March 2018.

Paratypes.—CFBH 43467–43470, 1 juvenile, 1 adult male, 2 adult females, respectively, Parque Natural Municipal do Cancão, municipality of Serra do Navio, state of Amapá, Brazil, C. E. Costa-Campos, 29 April 2018; CFBH 44897, cleared and double-stained specimen, adult female, collected at the same site, C. E. Costa-Campos, 17 August 2019.

Diagnosis.—*Adelophryne amapaensis* is distinguished from all other species of *Adelophryne* by the following combination of character states: (1) SVL in males 12.5 mm; females 13.0–14.4 mm; (2) tympanic membrane present; (3) tympanic annulus present, incomplete; (4) vomerine teeth absent; (5) finger terminal discs absent; (6) tips of Fingers I–IV mucronate (Fig. 2); (7) finger pads present (formula 1–1–2–1); (8) three phalanges in Finger IV (Fig. 3); (9) dorsum smooth; (10) cloacal flap (=anal flap in previous studies) absent; (11) multinote advertisement call composed of non-pulsed notes; (12) the call dominant frequency (4,802–5,706 Hz) coincides with the fundamental harmonic (Fig. 5).

Comparison with other species.—We based the comparisons on both collection specimens (see Material Examined) and literature data (references in parentheses). By its smaller size, *Adelophryne amapaensis* (male SVL = 12.5 mm, female SVL = 13.0–14.4 mm) differs from *A. patamona* (male SVL = 17.0–18.4 mm, female SVL = 20.7–23.0 mm; MacCulloch et al., 2008); by its larger size differs from *A. meridionalis* (SVL 9.0–10.9 mm, there is no sex differentiation in the original description; Santana et al., 2012), *A. michelin* (male SVL = 7.0–9.1 mm, female SVL = 10.0–11.4 mm; Lourenço-de-Moraes et al., 2018), and *A. pachydactyla* (male SVL = 11.1 mm; Hoogmoed et al., 1994). The presence of a tympanic membrane and an incomplete tympanic annulus distinguishes *A. amapaensis* from *A. glandulata*, *A. meridionalis*, and *A. michelin* (tympanic membrane absent; Santana et al., 2012; Lourenço-de-Moraes et al., 2014, 2018). The incomplete tympanic annulus also distinguishes *A. amapaensis* from *A. mucronata* (complete tympanic annulus; Lourenço-de-Moraes et al., 2012). The lack of vomerine teeth distinguishes *A. amapaensis* from *A. adiastola*, *A. baturitensis*, *A. gutturosa*, *A. maranguapensis*, *A. meridionalis*, *A. michelin*, *A. mucronata*, and *A. patamona* (vomerine teeth present; Hoogmoed and Lescure, 1984; Hoogmoed et al., 1994; MacCulloch et al., 2008; Santana et al., 2012; Lourenço-de-Moraes et al., 2012, 2018). The absence of finger terminal discs distinguishes *A. amapaensis* from *A. baturitensis*, *A. maranguapensis*, and *A. patamona* (present; Hoogmoed et al., 1994; MacCulloch et al., 2008). The mucronate tips of Fingers I–IV distinguish *A. amapaensis* from *A. baturitensis* (tips mucronate in Fingers II and IV only; Hoogmoed et al., 1994), *A. glandulata* (tips slightly mucronate; Lourenço-de-Moraes et al., 2014), and *A. meridionalis* (tip mucronate in Finger III; Santana et al., 2012). The presence of finger pads with formula 1–2–3–2 distinguishes *A. amapaensis* from *A. adiastola, A. gutturosa*, and *A. patamona* (formula 1–1–2–1; MacCulloch et al., 2008), *A. baturitensis* (subarticular tubercles instead of finger pads; Hoogmoed et al., 1994), *A. glandulata* and *A. michelin*

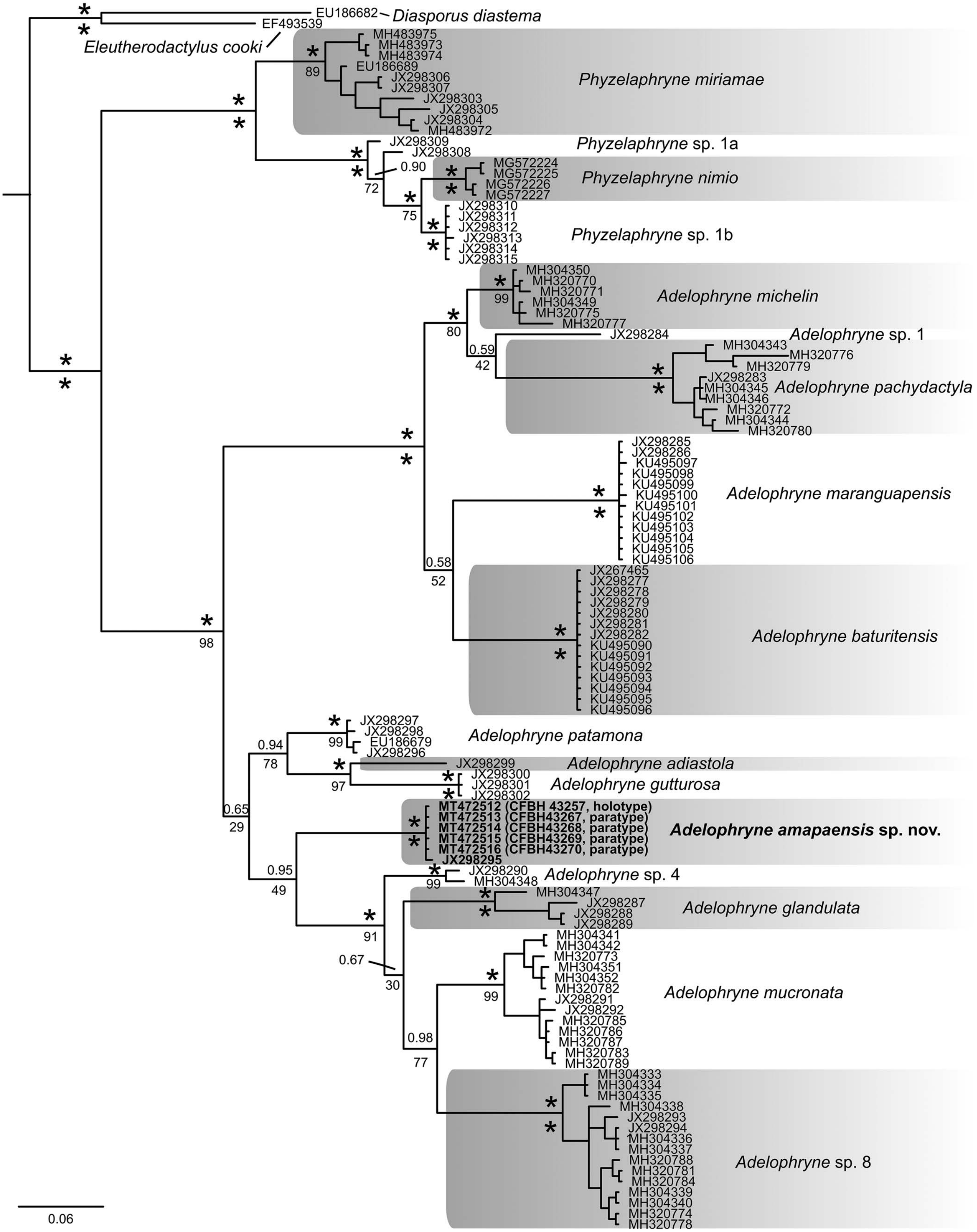

Fig. 1. The 50% majority rule consensus tree from Bayesian inference of mitochondrial 16S rRNA gene (*16S*) showing the relationships within the species of *Adelophryne*. Numbers above branches indicate posterior probabilities and numbers below branches indicate maximum likelihood non-parametric bootstrap values. Asterisks stand for fully supported clades, and we only show support of species level and above. Branch lengths are given in substitutions per site. See Data Accessibility for tree file.

Table 1. Uncorrected pairwise genetic distances of partial 16S rDNA (595 bp) within (bold font) and between species of *Adelophryne*. Sites with gaps were ignored (*pairwise.deletion* = *TRUE*). Data are shown as min–max and NA = non-applicable.

	Species	*n*	1	2	3	4	5	6	7	8	9	10	11	12	13
1	*A. amapaensis*	6	**0.0–0.4**												
2	*A. adiastola*	1	15.7–16.0	NA											
3	*A. baturitensis*	14	17.8–21.7	19.1–22.6	**0.0**										
4	*A. glandulata*	4	14.3–18.0	15.9–22.1	18.6–24.7	**0.0–6.0**									
5	*A. gutturosa*	3	13.8–20.1	9.8–13.5	18.5–22.6	16.4–23.8	**0.0**								
6	*A. maranguapensis*	12	19.7–22.4	23.0–24.5	12.7–15.3	19.5–23.4	21.8–26.2	**0.0–0.5**							
7	*A. michelin*	6	17.9–19.4	16.5–18.9	9.9–12.3	17.2–22.5	16.6–24.2	13.9–16.3	**0.2–2.5**						
8	*A. mucronata*	13	12.9–16.3	14.9–20.4	18.1–23.7	9.7–16.8	15.1–21.8	19.6–22.7	16.4–20.7	**0.0–6.1**					
9	*A. patamona*	4	10.4–15.0	10.7–13.3	17.1–22.0	13.9–18.4	10.2–15.2	18.5–21.8	15.3–19.9	12.9–18.2	**0.2–0.8**				
10	*A.* sp. 1	1	18.5–18.8	19.6–19.6	12.0–13.2	17.3–20.0	18.1–21.3	13.9–16.0	8.7–10.6	17.2–19.4	16.9–19.9	NA			
11	*A. pachydactyla*	9	19.4–24.2	19.0–21.6	12.7–18.5	19.8–26.6	17.1–24.1	16.8–23.3	11.1–15.3	20.0–25.5	16.7–22.9	13.7–16.4	**0.2–7.6**		
12	*A.* sp. 4	2	11.3–11.5	16.4–16.6	17.1–22.0	9.5–14.8	14.4–20.3	20.9–23.7	15.7–17.4	8.7–12.6	12.5–16.4	17.0–17.3	17.8–21.5	**1.3–1.3**	
13	*A.* sp. 8	15	13.2–16.3	17.6–18.5	19.4–25.3	11.5–17.9	14.4–20.6	19.6–23.1	19.5–21.5	9.4–13.6	13.1–17.6	20.9–22.8	20.0–24.3	9.9–12.3	**0.0–3.3**

(formula 1–2–2–1; Lourenço-de-Moraes et al., 2014, 2018), *A. maranguapensis* (formula 1–1–2–2; Hoogmoed et al., 1994); *A. mucronata* (formula 1–2–3–1; Lourenço-de-Moraes et al., 2012), and *A. pachydactyla* (formula 2–3–4–2, MacCulloch et al., 2008). *Adelophryne amapaensis* has Finger IV with three phalanges, which distinguishes it from *A. adiastola, A. glandulata, A. meridionalis*, *A. michelin*, and *A. pachydactyla* (Finger IV with two phalanges; Hoogmoed and Lescure, 1984; Hoogmoed et al., 1994; Santana et al., 2012; Lourenço-de-Moraes et al., 2014, 2018). The smooth dorsum distinguishes *A. amapaensis* from *A. adiastola* (granular dorsum; Hoogmoed and Lescure, 1984), *A. glandulata* (shagreened dorsum; Lourenço-de-Moraes et al., 2014), *A. maranguapensis* (pustulous dorsum; Hoogmoed et al., 1994), and *A. mucronata* (smooth dorsum with small, round granules; Lourenço-de-Moraes et al., 2012). The absence of a cloacal flap distinguishes *A. amapaensis* from *A. maranguapensis* and *A. mucronata* (present; Hoogmoed et al., 1994; Lourenço-de-Moraes et al., 2012).

The multi-note advertisement call of *Adelophryne amapaensis* differs from the single-note calls of *A. mucronata* (Lourenço-de-Moraes et al., 2012). The call of *A. amapaensis* has the dominant frequency coinciding with the fundamental harmonic, whereas the call of *A. adiastola* (referred to as *Phyzelaphryne miriamae* by Heyer, 1977) has the dominant frequency coinciding with the second harmonic. Call notes of *A. amapaensis* are non-pulsed and other patterns of amplitude modulation are also absent, whereas those of *A. gutturosa* have incomplete amplitude modulations, classified as pulses by MacCulloch et al. (2008), and call notes of *A. maranguapensis* have three to six pulses (Lima et al., 2014). *Adelophryne amapaensis* is further distinguished from *A. maranguapensis* (16–59 ms; Lima et al., 2014) in note length (5–16 ms), and from *A. adiastola* (3,200–3,700 Hz; Heyer, 1977) and *A. patamona* (3,251–3,269 Hz; MacCulloch et al., 2008) by its higher dominant frequency (4,802–5,706 Hz). The new species also differs by the higher dominant frequency (4,802–5,706 Hz) and by the lower note rate (3.0–6.6 notes/s) from *A. gutturosa* (3,896–4,979 Hz and 6.1–22.2 notes/s, respectively; MacCulloch et al., 2008), despite some overlap in both acoustic parameters. Calls of the remainder congeners (*A. baturitensis*, *A. glandulata*, *A. meridionalis*, *A. michelin*, and *A. pachydactyla*) remain unknown to date.

Description of the holotype.—Adult male with subgular vocal sac and vocal slits. Head length 39% of SVL; head slightly longer than wide (head width 88% of head length), as wide as immediately posterior region of body; snout rounded in dorsal and lateral views; canthus rostralis indistinct, straight; loreal region flat; nostril barely protuberant, elliptical, oriented laterally; tympanum diameter 42% of eye diameter; tympanic membrane present, slightly distinct; tympanic annulus present, indistinct dorsally. Choanae small, laterally placed; vomerine dentigerous processes absent; tongue narrow, attached anteriorly; vocal sac subgular, vocal slits placed posterolaterally, its size about half of jaw length.

Skin of dorsum, venter, and limbs smooth; cloacal opening above midpoint of thigh, directed posteriorly, dorsal flap absent. Hand with large flat oval palmar and thenar tubercles, palmar almost twice as large as thenar tubercle; fingers with large, flat subdigital pads (1–2–3–2), no pads under distal phalanges; fingers flattened and unwebbed; tips

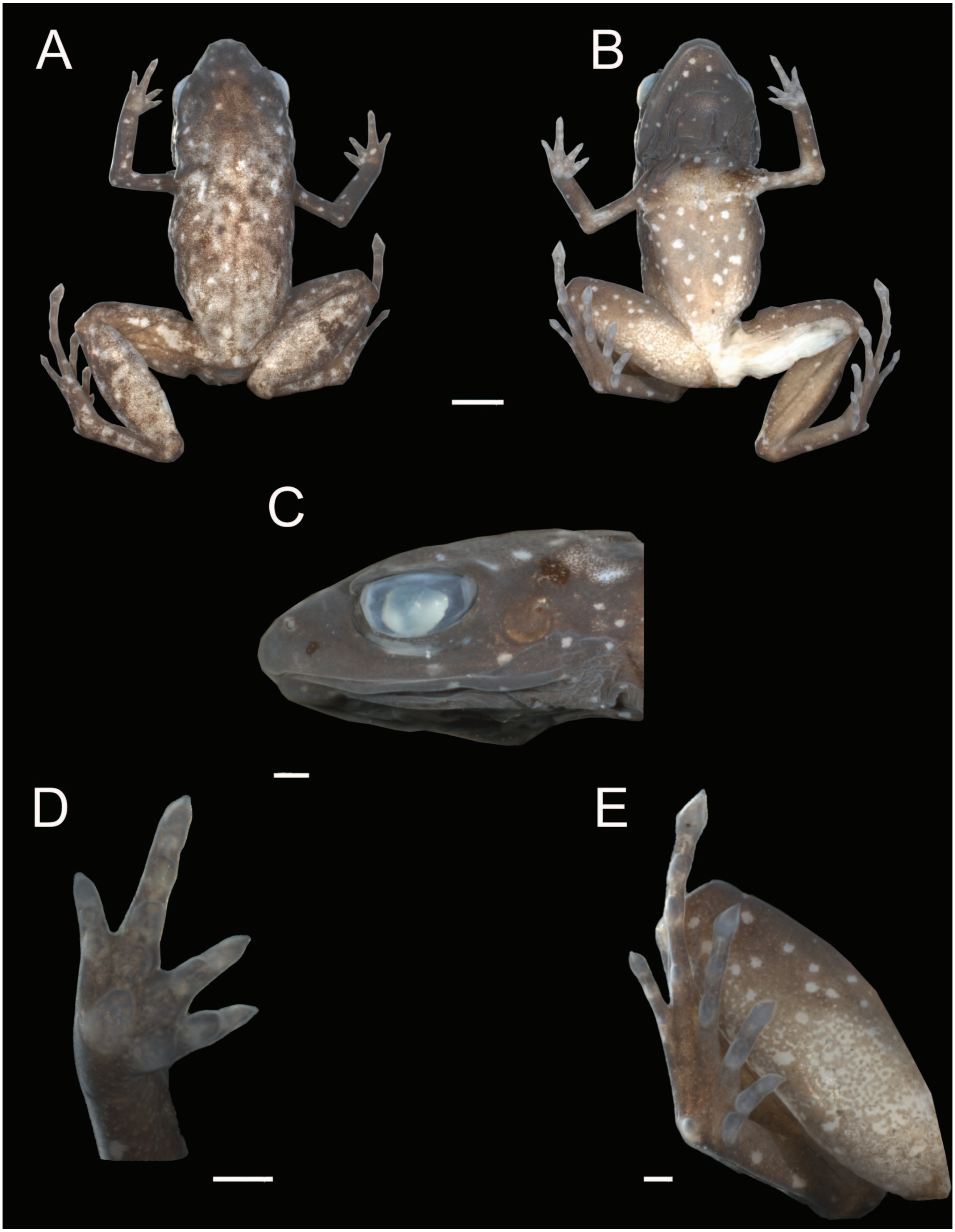

Fig. 2. Preserved holotype of *Adelophryne amapaensis* (adult male, CFBH 43257). (A) Dorsal and (B) ventral views of the body; (C) lateral view of the head; (D) ventral view of the hand; and (E) ventral view of the foot. Scale bars equal to 2 mm (A–B) and 0.5 mm (C–E).

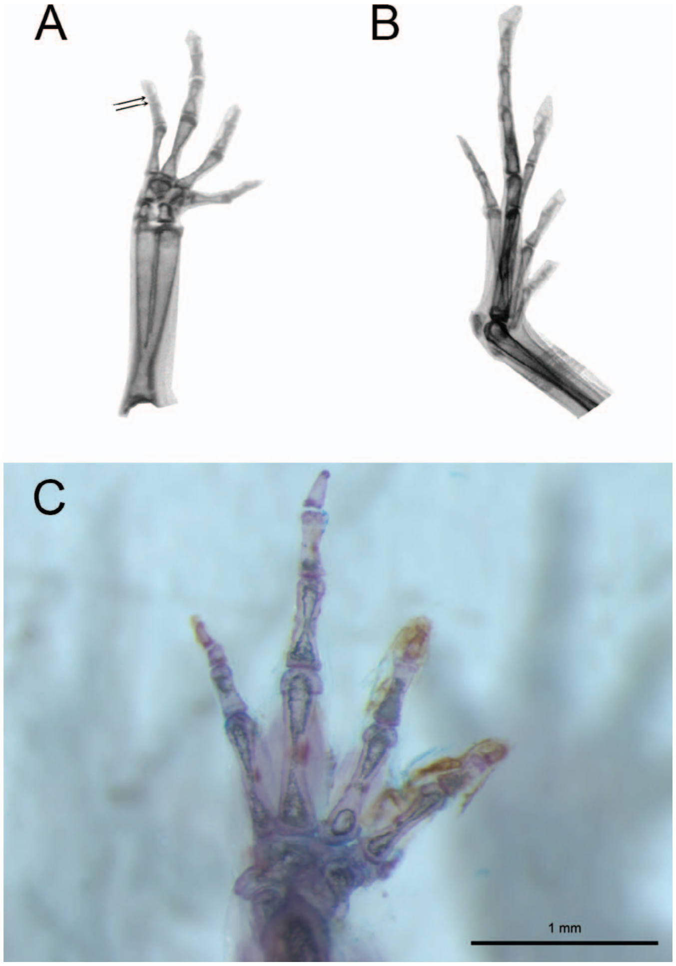

Fig. 3. X-ray images of the (A) hand (CFBH 43267) and (B) foot (CFBH 43270) and (C) dorsal view of the cleared and double-stained left hand of *Adelophryne amapaensis* (CFBH 44897). Note the three phalanges in Finger IV. Arrows indicate the separation between phalanges.

of fingers mucronate, without circumferential grooves; finger length formula I $<$ IV $\approx$ II $<$ III; distal portion of fingers surrounded by a transparent rim of skin; phalangeal formula 2–2–3–3.

Leg short, robust; tibia as long as thigh (tibia length and thigh length 52% of SVL); tarsus smooth, no ridge or tubercle; large, distinct, oval, and flat inner metatarsal tubercle; smaller, round, and conical outer metatarsal tubercle; subdigital pads (1–1–2–3–2) large, prominent, located just distal to digital articulations; no supernumerary pads; toes flattened, unwebbed; Toe V more slender than the others; tips of toes bluntly pointed, without circumferential grooves; tips of Toes II, III, and IV with small discs, ending in asymmetrically pointed tips; toe length formula I $<$ II $<$ V $<$ III $<$ IV; distal portions of toes surrounded by a transparent rim of skin; phalangeal formula 2–2–3–4–3.

Coloration of the holotype.—(Fig. 4) In life, dorsum dark brown with some sparse white spots, several metallic dots shading from yellowish light brown to reddish brown; dots forming blotches; flank, lateral portion of the head, and forelimb dark brown with some sparse white spots; dorsal surfaces of hind limb same as dorsum; venter, ventral surfaces of forelimb and hind limb dark brown with some sparse white spots; inguinal region and groin pale beige with several small brown blotches; gular region dark brown, almost black, with some sparse white spots. In preservative, colors become lighter and the metallic hues fade.

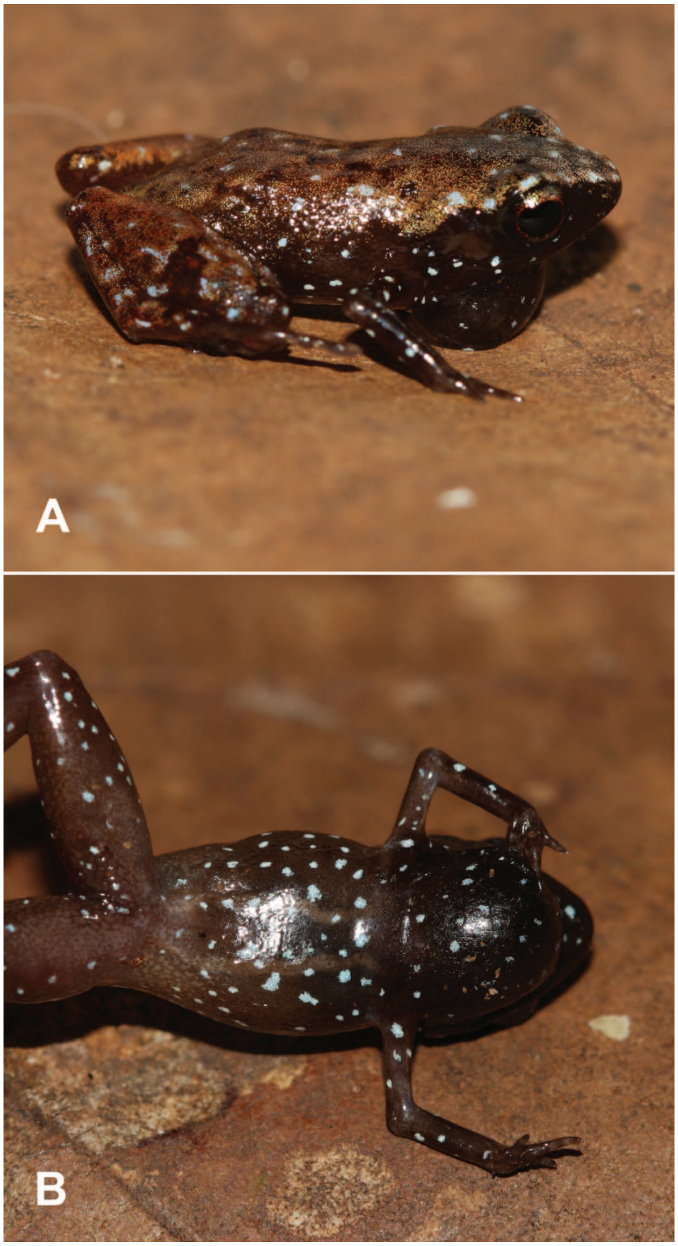

Fig. 4. Dorsolateral (A) and ventral (B) views of the holotype of *Adelophryne amapaensis* (CFBH 43257; SVL = 12.5 mm) in life.

Variation.—(Table 2) The three females are a bit larger than the two males (female SVL 13.0–14.4 mm; male 12.5 mm), and the gular region in males is darker than it is in females.

Distribution.—*Adelophryne amapaensis* is only known from its type locality: Parque Natural Municipal do Cancão, municipality of Serra do Navio, state of Amapá, Brazil (Fig. 6).

Natural history notes.—Calling males of *Adelophryne amapaensis* were heard during March–April both at the forest border from steep terrain and inside the forest. Individuals were calling from deep under the dense leaf litter. Calling

Table 2. Measurements (in millimeters) and body proportions of the type series of *Adelophryne amapaensis*. See text for abbreviations.

Specimen	CFBH 43257 adult male holotype	CFBH 43467 juvenile	CFBH 43468 adult male	CFBH 43469 adult female	CFBH 43470 adult female	CFBH 44897 adult female
SVL	12.5	12.0	12.5	13.0	13.0	14.4
HL	4.8	4.7	4.6	4.9	4.9	5.4
HW	4.2	4.4	4.5	4.4	4.6	4.9
ED	1.8	1.6	1.7	1.7	1.8	1.7
AMD	2.2	2.4	2.5	2.4	2.5	2.5
IND	1.4	1.5	1.5	1.5	1.4	1.5
END	0.8	0.9	1.0	0.9	1.1	1.0
TD	0.8	0.9	0.8	0.7	0.8	0.7
FAL	2.9	2.7	2.7	2.8	2.6	3.5
HAL	2.3	2.6	2.3	2.1	2.2	2.6
THL	6.6	6.7	6.0	6.3	6.0	6.8
TL	6.6	6.1	6.1	6.3	5.9	6.8
TAL	3.9	3.5	3.6	3.4	3.4	4.1
FL	5.8	5.9	6.1	5.3	5.3	6.2
HL/SVL	0.39	0.39	0.36	0.38	0.38	0.38
HW/HL	0.88	0.95	0.99	0.89	0.93	0.91
TD/ED	0.42	0.55	0.50	0.43	0.46	0.41
THL/SVL	0.52	0.56	0.48	0.48	0.46	0.48
TL/SVL	0.52	0.51	0.49	0.49	0.46	0.47

activity was recorded from mid-afternoon to the early hours of night. Syntopic frog species were *Adenomera andreae*, *Adenomera hylaedactyla*, and *Ameerega pulchripecta*.

Advertisement call.—The description is based on calls of five males ($n = 15$ calls and 143 notes; voucher specimen CFBH 43257). Values are given as range (x±SD). *Adelophryne amapaensis* produces the advertisement call as calling bouts given a few times (1–3) per minute. Call length varies from 1.0–3.5 (1.8±0.4) s, and rise time is 27–99% (69.7±22.4) of call length. Calls are formed by 4–24 (9.7±3.4) non-pulsed notes with length varying from 5–16 (8.0±1.3) ms, and given at a rate of 3.0–6.6 (4.4±0.6) notes per second. The dominant frequency coincides with the fundamental harmonic, ranging from 4,802–5,706 (5,147.3±277.5) Hz. Frequency modulation is negligible (<50 Hz) or modest, ranging from –215 to 474 (63.7±77.2) Hz.

Etymology.—The species is named after the Brazilian state of Amapá, from which all known specimens come. The specific epithet is used here as a noun in apposition.

Remarks.—*Adelophryne amapaensis* corresponds to *Adelophryne* sp. 7 of Fouquet et al. (2012). The type series has five specimens and the only other known specimen belonging to *A. amapaensis* is MTR 13808, collected at the same locality as the type specimens (A. Fouquet, pers. comm.), the single terminal assigned to the lineage in Amapá in their phylogeny. The paper was the first one assessing the molecular phylogenetic relationships of the genus, and, at that time, Fouquet et al. (2012) indicated seven candidate species as *Adelophryne* spp. 1–7. More recently, relevant taxonomic changes occurred. In 2018, Dominato et al. and Lourenço-de-Moraes et al. published two important papers dealing with the phylogenetic relationships and the taxonomy of *Adelophryne*. The two groups of authors used different names for the clades. Lourenço-de-Moraes et al. (2018) followed Fouquet et al. (2012) and used a candidate species approach. On the other hand, Dominato et al. (2018), although following Fouquet et al. (2012) regarding some of the candidate species names, used operational taxonomic units (OTUs). The two papers were published concomitantly, so the authors did not have access to each other's data during the production and review process of their manuscripts. Because we use all *16S* sequences available for *Adelophryne* in GenBank, we summarized the names of the species and candidate species used in these papers in order to help clarify the taxonomy of the genus (see Table 3).

DISCUSSION

Fouquet et al. (2012), in a phylogenetic reconstruction using four mitochondrial and three nuclear gene fragments, recognized four main biogeographic clades of Phyzelaphryninae, three of them within *Adelophryne*: the North Amazonia Clade (NAMC), North Atlantic forest Clade (NAFC), and South Atlantic forest Clade (SAFC), all of them recovered with high support in both Bayesian-inference and maximum-likelihood analyses. However, the authors recovered the relationships among the three clades with poor resolution, and the relationships were different between the two chosen optimality criteria. Lourenço-de-Moraes et al. (2018), using an alignment of 798 bp of the *16S* mitochondrial gene fragment recovered the same three biogeographic clades within *Adelophryne*, but only the SAFC and the NAFC were highly supported in both Bayesian-inference and maximum-likelihood analyses. The NAMC was poorly supported in the maximum-likelihood analysis, appearing in only 69% of the bootstrap replicates. In this study, the NAMC was composed of four species: *A. adiastola*, *A. gutturosa*, *A. patamona*, and *A. amapaensis* (formerly referred to as *Adelophryne* sp. 7), with the latter species as the sister group of the remaining three species. In our phylogenetic analyses of the *16S* gene fragment, we did not recover *A. amapaensis* within the NAMC, but as the sister species of the remaining taxa within the SAFC, even though this relationship was poorly supported in the maximum likelihood analysis (49%). These differences could be explained by differences in the number

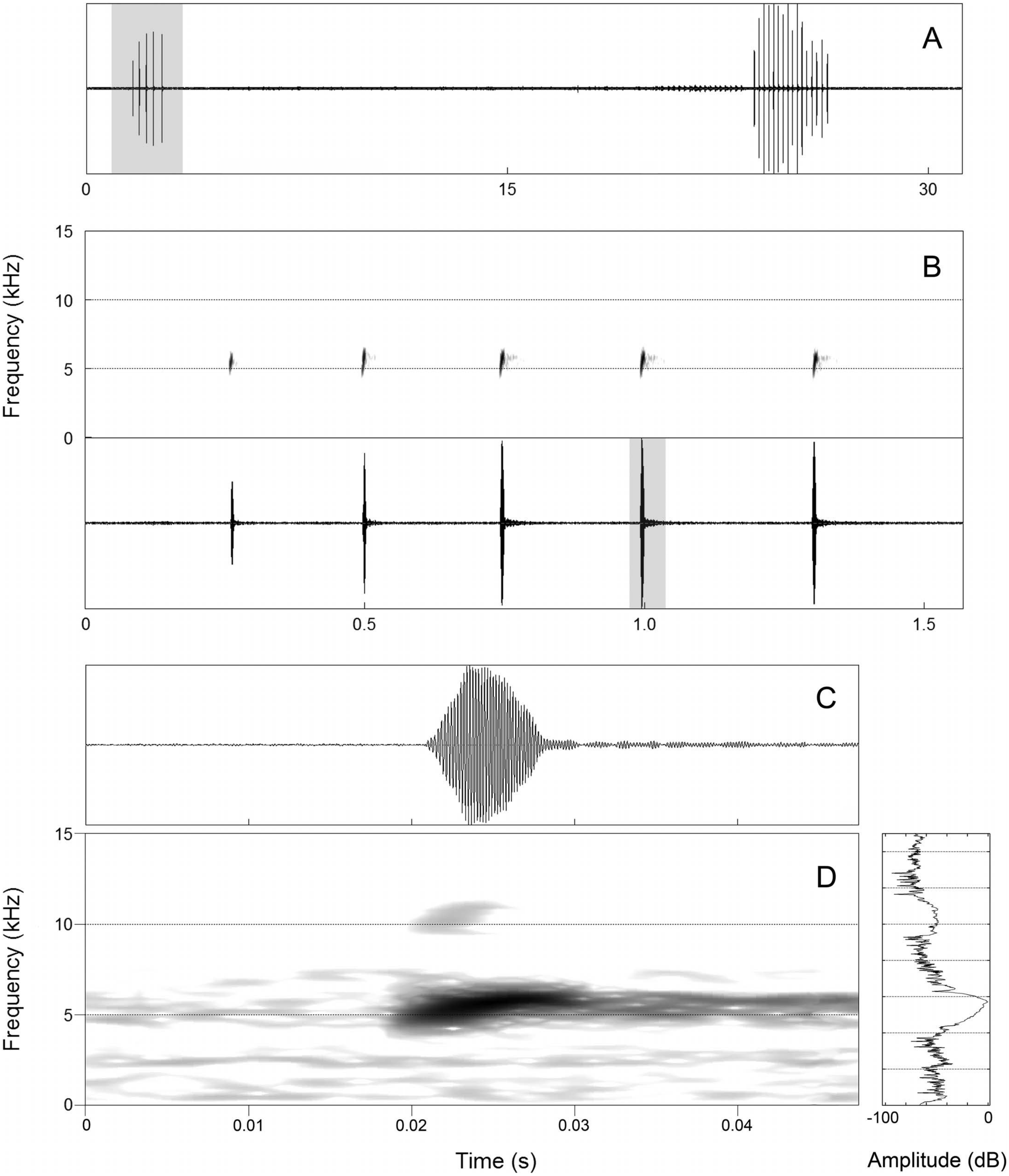

Fig. 5. Advertisement call of *Adelophryne amapaensis* from the type locality (Serra do Navio, Amapá, Brazil). (A) Time-domain section containing two calls; (B) spectrogram and oscillogram of the multi-note call highlighted in A, formed by five non-pulsed notes; (C–D) waveform and spectrogram with corresponding amplitude spectrum, respectively, of the fourth note (=the amplitude peak in the call) highlighted in B. Some notes of the second call in A are clipped and thus excluded from the acoustic analysis.

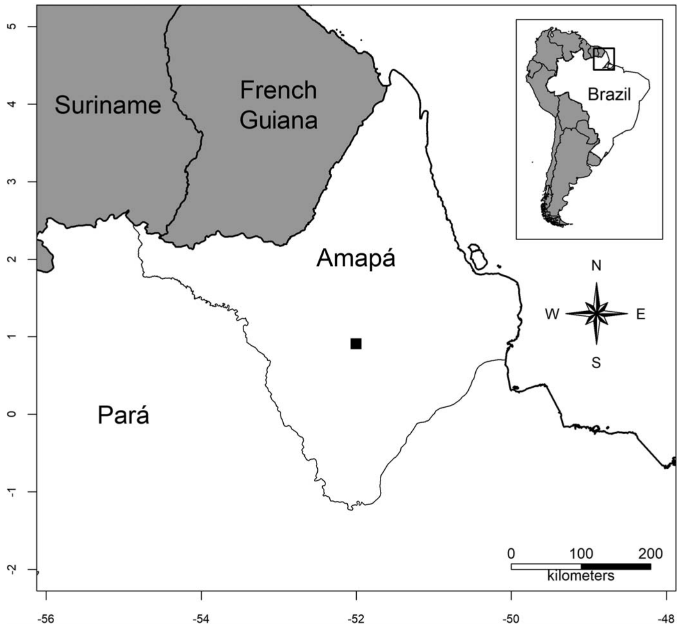

Fig. 6. Type locality of *Adelophryne amapaensis* (black square): Parque Natural Municipal do Cancão, municipality of Serra do Navio, state of Amapá, Brazil. Elevation 100 m above sea level.

of molecular markers used among the studies, as well as the addition of three terminals belonging to *A. amapaensis* in our study.

Since the first molecular study assessing the diversity within *Adelophryne* (Fouquet et al., 2012) seven years ago, five species have been described (Lourenço-de-Moraes et al., 2012, 2014, 2018; Santana et al., 2012; and the one herein described), raising the number of species in the genus by more than 80%. Among these, *A. amapaensis*, *A. mucronata*, and *A. glandulata* had already been treated as candidate species (*Adelophryne* sp. 7, sp. 6, and sp. 5, respectively; Fouquet et al., 2012), whereas *A. meridionalis* and *A. michelin* were presented only at the time of their original descriptions—although *A. michelin* appeared, almost simultaneously, as OTU 2 in Dominato et al. (2018). This fact demonstrates that studies focusing on a first molecular approach of a poorly known group, as is the case in the genus *Adelophryne*, even though not describing species *per se*, strongly contribute to uncover species diversity, aiding and guiding future taxonomic studies. Moreover, the true diversity within *Adelophryne* remains unknown. Despite the huge increase in taxonomic knowledge during the past few years, four candidate species of *Adelophryne* still lack formal descriptions and a more thorough phenotypic approach: *Adelophryne* sp. 1, sp. 3, sp. 4 (Fouquet et al., 2012), and sp. 8 (Lourenço-de-Moraes et al., 2018). More comprehensive taxonomic studies involving these species will contribute both to the knowledge of species diversity in *Adelophryne* and insights into the evolution of important morphological traits (e.g., number of distal phalanges in Finger IV, presence of tympanic annulus and membrane) within the genus.

MATERIAL EXAMINED

Adelophryne adiastola: INPAH 38291, Brazil, Amazonas, Japurá; KU 20564, 220475 Peru, Loreto, Quebrada Vasquez (all examined by pictures).

Adelophryne baturitensis: CFBH 20469–24576, Brazil, Ceará, Guaramiranga; CFBH 24554–24567, Brazil, Ceará, Tianguá; CFBH 24579–24585, Ceará, Viçosa do Ceará.

Adelophryne glandulata: MZUESC 12178–12180, UFMG 13750–13752, 13754–13756, 13758, Brazil, Espírito Santo, Santa Teresa, Reserva Biológica Augusto Ruschi; UFMG 17572–17579, Brazil, Minas Gerais, Itabira; UFMG 19693, 19695, Brazil, Minas Gerais, Mariana.

Adelophryne gutturosa: MZUSP 150814–150820, Guyana, Potaro-Siparuni, vicinity of Kuribrong River.

Adelophryne maranguapensis: CFBH 24515–24527, Brazil, Ceará, Maranguape.

Adelophryne meridionalis: UFMG 611, 614, 615, 617–624, Brazil, Minas Gerais, Bom Jardim de Minas.

Adelophryne mucronata: MZUESC 9091–9096, 18758, 18759, 18761–18763, Brazil, Bahia, Una; MZUESC 18754, 18757, Brazil, Bahia, Igrapiúna.

Adelophryne pachydactyla: MZUESC 15094, Brazil, Bahia, Itarantim; MZUESC 18725, 18728, Brazil, Bahia, Igrapiúna; MZUESC 18726, Brazil, Bahia, Una.

DATA ACCESSIBILITY

Supplemental material associated with this work is available at https://www.copeiajournal.org/ch-19-254.

Table 3. Summary of the candidate species of *Adelophryne* and conflicting species names within the main papers assessing the phylogenetic relationships of the genus.

Fouquet et al., 2012	Lourenço-de-Moraes et al., 2018	Dominato et al., 2018	Present work
Adelophryne sp. 1	*Adelophryne* sp. 1	*Adelophryne* sp. 1	*Adelophryne* sp. 1
Adelophryne sp. 2	*Adelophryne* sp. 2	OTU 1/*A. pachydactyla*	*A. pachydactyla*
Adelophryne sp. 3	not sampled	*Adelophryne* sp. 3	not sampled
Adelophryne sp. 4	*Adelophryne* sp. 4	*Adelophryne* sp. 4	*Adelophryne* sp. 4
Adelophryne sp. 5	*A. glandulata*	*Adelophryne* sp. 5	*A. glandulata*
Adelophryne sp. 6	*A. mucronata*	OTU 3a/*A. mucronata*	*A. mucronata*
Adelophryne sp. 7	*Adelophryne* sp. 7	*Adelophryne* sp. 7	*A. amapaensis*
A. pachydactyla	*Adelophryne* sp. 8	OTU 3b	*Adelophryne* sp. 8
not sampled	*A. michelin*	OTU2	*A. michelin*

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APPENDIX 1

Sound recordings of five males of *Adelophryne amapaensis* from the type locality (Serra do Navio, Amapá, Brazil): (1) Voucher CFBH 43257 (holotype); sound file: TRC102a–b; 30 March 2018, 1837–1842 h, 25.5°C. (2) Voucher CECCAMPOS 2592; sound file: Adelophryne_gutturosa_5ind; 19 April 2014, 0912 h, air 26.6°C. (3) Voucher CECCAMPOS 2593; sound file: Adelophryne_gutturosa_1063; 22 April 2017, 1014 h, air 26.4°C. (4) Voucher CECCAMPOS 2594; sound file: Adelophryne_gutturosa_1064; 22 April 2017, 1032 h, air 26.8°C. (5) CECCAMPOS 2595; sound file: Adelophryne_gutturosa_1066; 22 April 2017, 1128 h, air 26.8°C.