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A New Species of the Genus *Adelophryne* (Anura: Eleutherodactylidae: Phyzelaphryninae) From the Atlantic Forest of Northeastern Brazil

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Abstract: Species in the *Adelophryne* genus consist of diminutive, agile, and secretive frogs that inhabit forest leaf litter and have direct development. Species richness within *Adelophryne* was previously underestimated, and several new species have recently been described. Here, we describe a new species of *Adelophryne* from the Atlantic Forest in the Brazilian states of Alagoas, Pernambuco, and Paraíba. The new species is characterized by its small body size, small and distinct tympanum, toes with subarticular tubercles, fingers mucronate, three phalanges in the Finger IV, and an advertisement call composed of one single pulsed note with a high dominant frequency. The new species is phylogenetically related to other *Adelophryne* species of the Northern Atlantic Forest Clade and is endemic to the "Pernambuco Endemism Center" in the Atlantic Forest biodiversity hotspot.

Key words: Alagoas; Amphibia; Biodiversity; Hotspot; Miniature; Paraíba; Pernambuco

The genus *Adelophryne* Hoogmoed and Lescure 1984 currently contains 10 species of diminutive frogs that inhabit forest leaf litter (Lourenço-de-Moraes et al. 2018), are agile and secretive (MacCulloch et al. 2008; Lourenço-de-Moraes et al. 2012), have direct development (Hedges et al. 2008; Cassiano-Lima et al. 2011), and use periphytotelmata (i.e., bromeligen periphytotelm eggs; Cassiano-Lima et al. 2020). The species of this genus have a discontinuous distribution in eastern Brazil, the Guiana Shield, and the upper Amazon Basin (Frost 2020). The currently described species are *Adelophryne adiastola* Hoogmoed and Lescure (1984); *Adelophryne baturitensis* Hoogmoed, Borges and Cascon (1994); *Adelophryne glandulata* Lourenço-de-Moraes, Ferreira, Fouquet, and Bastos (2014); *Adelophryne gutturosa* Hoogmoed and Lescure (1984); *Adelophryne maranguapensis* Hoogmoed, Borges and Cascon (1994); *Adelophryne meridionalis* Santana, Fonseca, Neves and Carvalho (2012); *Adelophryne michelin* Lourenço-de-Moraes, Dias, Mira-Mendes, Oliveira, Barth, Ruas, Vences, Solé, and Bastos (2018); *Adelophryne mucronata* Lourenço-de-Moraes, Solé and Toledo (2012); *Adelophryne pachydactyla* Hoogmoed, Borges and Cascon (1994); and *Adelophryne patamona* MacCulloch, Lathrop, Kok, Minter, Khan, and Barrio-Amorós (2008).

The species of the genus are clustered in three deeply divergent and well-supported clades that correspond to geography and are referred to as the Northern Amazonia Clade (NAMC; from northern Brazil to eastern Colombia and western Guyana), Southern Atlantic Forest Clade (SAFC; from southern Bahia to Minas Gerais and Espírito Santo states), and Northern Atlantic Forest Clade (NAFC; from Ceará to northern Bahia states; Fouquet et al. 2012). However, species belonging to both SAFC and NAFC occur sympatrically in some areas of Bahia State (Lourenço-de-Moraes et al. 2018). Within these Atlantic Forest clades, Fouquet et al. (2012) recognized six unnamed candidate species, and afterward, the species *A. pachydactyla* included in their analysis was recognized as another candidate species (*Adelophryne* sp.8; see Lourenço-de-Moraes et al. 2018). Of these seven candidate species occurring in the Atlantic Forest, two have been described (*Adelophryne* sp.5 as *A. glandulata* and *Adelophryne* sp.6 as *A. mucronata*) and five (*Adelophryne* sp.1–4, 8) remain unnamed.

Among the candidate species pointed out by Fouquet et al. (2012), *Adelophryne* sp.1 is one of the lineages that is still unnamed. This candidate species occurs in the northeastern region of the Atlantic Forest hotspot (Myers et al. 2000)

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north of the São Francisco River, an area known as the "Pernambuco Endemism Center" (Prance 1982), an important region of diversification and climatic refugia for amphibians (Carnaval et al. 2009; Lourenço-de-Moraes et al. 2019; Santos et al. 2020). *Adelophryne* sp.1 (from Caruaru, Pernambuco State, sensu Fouquet et al. 2012) was identified as *A. baturitensis* by Loebmann et al. (2011) due to similar morphology and geographic proximity. On the other hand, Mesquita et al. (2018) did not refer the *Adelophryne* species of Paraíba State to any described or candidate species. Here, we analyze the populations of Alagoas, Pernambuco, and Paraíba states and, based on three lines of evidence (molecular, morphological, and bioacoustic), describe them as a new species of the genus *Adelophryne* from the Atlantic Forest of northeastern Brazil. We also provide information on its natural history, phylogenetic relationships, advertisement call, and conservation status.

Materials and Methods

We analyzed populations of *Adelophryne* found in fragments of the Atlantic Forest in the municipalities of Murici, Alagoas State, Brazil (09°12′S, 35°52′W; 597 m a.s.l.; datum = WGS84 in all cases); Cabo de Santo Agostinho, Pernambuco State, Brazil (08°17′S, 35°2′W; 29 m a.s.l.); Caruaru, Pernambuco State, Brazil (08°15′17.928″S, 35°54′15.869″W; 544 m a.s.l.), and Mamanguape, Paraíba State, Brazil (06°43′S, 35°10′W; 118 m a.s.l.). According to Köppen–Geiger's climate classification, the region is of type AS (Peel et al. 2007).

Collected specimens are putative new species and therefore not listed in the Brazilian Red List of Threatened Species, Red List of Threatened Species of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), or the appendices of the International Union for Conservation of Nature (IUCN). This study was conducted with appropriate permissions and guidelines from the responsible authority (license 33507-1/33597-2) issued by "Instituto Chico Mendes de Conservação da Biodiversidade" (ICMBio) that also evaluated protocols for our collection and research. Geographic coordinates and elevation (m a.s.l.) were taken using a handheld Garmin GPS unit 78s and the WGS84 datum. Relative humidity and temperature were measured using an Incoterm digital thermo hygrometer. Specimens were collected by hand, euthanized with a lidocaine solution, fixed in 10% formalin, and preserved in 70% ethyl alcohol. Muscle samples were taken from thighs before specimen fixation and stored in absolute ethanol for subsequent DNA extraction and sequencing. Voucher specimens were deposited in the Coleção Herpetológica do Museu de História Natural da Universidade Federal do Alagoas (MUFAL), Coleção Herpetológica e Paleoherpetológica da Universidade Federal Rural de Pernambuco (CHP-UFRPE), and Coleção Zoológica da Universidade Federal do Mato Grosso do Sul (ZUFMS-AMP).

Terminology of the snout shape in lateral view follows that of Cei (1980); of the dorsal view follows Heyer et al. (1990); of the tympanum, fingers, toes, and pads follows Hoogmoed and Lescure (1984); of terminal tips follows Lourenço-de-Moraes et al. (2012); and of skin texture follows Kok and Kalamandeen (2008). Measurements were taken under a stereomicroscope with digital calipers. The following measurements follow Lourenço-de-Moraes et al. (2012, 2014): snout–vent length (SVL), head length (HL), head width (HW), eye diameter (ED), upper eyelid width (UEW), tympanum diameter (TD), interorbital distance (IOD), internarial distance (IND), eye–nostril distance (END), nostril to tip of snout distance (NSD), eye to tip of snout distance (ETSD), foot length (FL), thigh length (THL), and tibia length (TL). The phalanges were counted using a stereomicroscope with adequate illumination, and skeletal characters were confirmed and determined from two (CHP UFRPE 3007 and MUFAL 11770) cleared and stained individuals following the protocol of Taylor and van Dyke (1985).

Data for comparative species were taken from original species descriptions (Hoogmoed and Lescure 1984; Hoogmoed et al. 1994; MacCulloch et al. 2008; Santana et al. 2012; Lourenço-de-Moraes et al. 2012, 2014, 2018) and direct examination of specimens from the following collections (institutional abbreviations follow Sabaj 2019): CFBH, DHMECN, MNRJ, MZUFV, UFMG, ZUEC, CHUFPB, MZUESC, MBML, ZUFG, and UFC (see Appendix for details).

Calls of four males of *Adelophryne* sp. nov. were recorded from May to September 2013 at Mata da Bananeira, Estação Ecológica de Murici, municipality of Murici (ESEC Murici), Alagoas State, Brazil (09°12′S, 35°52′W; 597 m a.s.l.; air temperature, 23.2–26.2°C; and relative humidity ,71–93%). All individuals were found calling during the day in the leaf litter (between 1100–1315 h) in areas of primary and secondary forest. Recordings were made with a Marantz PMD660 digital recorder at a sampling rate of 44.1 kHz and resolution of 16 bits, coupled to an external unidirectional Sennheiser ME66/K6 microphone, positioned at approximately 50 cm from the calling males. We analyzed a total of 80 calls.

Bioacoustic terminology and analyses followed Köhler et al. (2017) and were conducted using Raven Pro v1.5 (Bioacoustics Research Program 2014). Spectrograms were generated with window type Hamming, overlap 90%, and window size and DFT of 512 samples. Sound graphics were obtained using the packages Seewave (Sueur et al. 2008) and tuneR (Ligges et al. 2016) of R platform v3.5.1 (R Core Team 2017). The following call parameters were analyzed: number of notes per call, call duration (s), intercall interval (s), number of pulses per call, lower frequency (Hz), upper frequency (Hz), and dominant frequency (Hz). Values are presented as mean, standard deviation (±), and minimum–maximum. To analyze the spectral structure call more accurately, we produced a power spectrum of the call using a high FFT value (1024) with a log-transformed dB scale.

The genomic DNA was extracted from muscle tissue using the DNeasy Blood and Tissue Kit (QIAGEN). PCR amplification and sequencing were performed using the primers 16Sar-L (5-CGC CTG TTT ATC AAA AAC AT-3) and 16Sbr-H (5-CCG GTC TGA ACT CAG ATC ACG T-3) to amplify a section of the mitochondrial 16S ribosomal RNA gene (Palumbi et al. 2002). PCR conditions followed those described by Costa et al. (2016). The PCR products were purified with ethanol/sodium acetate and sequenced with an ABI 3730 XL DNA Analyzer (Applied Biosystems, LLC). Sequences were edited for quality, and forward and reverse reads were assembled using Geneious v9.1.8 (available at https://www.geneious.com/; Biomatters, Auckland, New

TABLE 1.—GenBank accession numbers for 16S mitochondrial rRNA fragments of specimens used in the phylogenetic analyses.

Species	Locality	16S rRNA accession number
Adelophryne nordestina	Pernambuco, Brazil	JX298284
Adelophryne nordestina	Paraíba, Brazil	MT731283/MT731282
Adelophryne nordestina	Alagoas, Brazil	MT731284
Adelophryne sp.2	Bahia, Brazil	JX298283/MH304343
Adelophryne sp.4	Bahia, Brazil	MH304348/JX298290
Adelophryne sp.7	Amapá, Brazil	JX298295
Adelophryne sp.8	Bahia, Brazil	JX298294/MH304337
Adelophryne adiastola	Colombia	JX298299
Adelophryne baturitensis	Ceará, Brazil	JX298279/JX298280
Adelophryne glandulata	Espirito Santo, Brazil	MH304347
Adelophryne glandulata	Minas Gerais, Brazil	JX298288
Adelophryne gutturosa	Venezuela	JX298300
Adelophryne gutturosa	Guyana	JX298301
Adelophryne maranguapensis	Ceará, Brazil	JX298285/JX298286
Adelophryne michelin	Bahia, Brazil	MH304349/MH304350
Adelophryne mucronata	Bahia, Brazil	MH304342/JX298291
Adelophryne patamona	Guyana	JX298297/JX298298
Phyzelaphryne sp.1a	Amazonas, Brazil	JX298308
Phyzelaphryne sp.1b	Colombia	JX298310
Phyzelaphryne miriamae	Amazonas, Brazil	JX298303
Phyzelaphryne nimio	Amazonas, Brazil	MG572225/MG572224
Brachycephalus ephippium[a]	Brazil	AY326008
Diasporus diastema[a]	Costa Rica	EU186682
Eleutherodactylus coqui[a]	Puerto Rico	EF493550
Euparkerella brasiliensis[a]	Rio de Janeiro, Brazil	JX298316
Haddadus binotatus[a]	São Paulo, Brazil	EF493361

[a] Indicates the out-group species.

Zealand). Final sequences were deposited in GenBank under accession numbers MT731282–MT731284.

Phylogenetic analyses were performed to confirm that all populations studied here are associated with *Adelophryne* sp.1 (sensu Fouquet et al. 2012). Homologous sequences of species of the genera *Phyzelaphryne* and *Adelophryne* used by Fouquet et al. (2012), Lourenço-de-Moraes et al. (2018), and Simões et al. (2018) and representative species of the families Brachycephalidae, Craugastoridae, and Eleutherodactylidae as outgroups were downloaded from GenBank (Table 1). One candidate species, *Adelophryne* sp.3 (sensu Fouquet et al. 2012), was not included because 16S sequences were not available for it.

We aligned the newly generated sequences and the downloaded sequences using MAFFT v7 (Katoh 2013) with the L-INS-i strategy. We conducted phylogenetic analyses using two optimality criteria, namely, Bayesian inference (BI) and maximum likelihood (ML). We performed BI analysis using MrBayes v3.2.7 (Huelsenbeck and Ronquist 2001). The model of molecular evolution was selected using Partition Finder v2.1.1 (Lanfear et al. 2012). The substitution model GTR+I+G was selected as the optimal nucleotide substitution model. Two independent runs of 75 million generations were performed, with sampling every 1000 generations, and 25% of generations and trees discarded as burn-in. To determine MCMC mixing and convergence, we examined trace plots and effective sample size (estimated sample size [ESS], >200) in Tracer v1.7.1 (available at http://tree.bio.ed.ac.uk/software/tracer/). The consensus of the post burn-in trees was visualized in FigTree v1.4.4 (available at http://tree.bio.ed.ac.uk/software/figtree/). We performed ML analysis using RAxML-HPC BlackBox v8.2.10 (Stamatakis 2014) under the GTRCAT model with 1000 bootstrap replicates. All phylogenetic analyses were performed using the CIPRES Science gateway portal (Miller et al. 2010). Uncorrected p-distances were calculated for *Adelophryne* sp. nov. and species of the genus *Adelophryne* using MEGA X v10.0.5 (Kumar et al. 2018) with gap positions removed for each sequence pair (pairwise deletion option).

SPECIES DESCRIPTION

Adelophryne ***nordestina*** sp. nov.
(Table 2; Figs. 1–3, 5)

Adelophryne baturitensis Loebmann et al. (2011):75.
Adelophryne sp.1 Fouquet et al. (2012):550.
Adelophryne sp. Mesquita et al. (2018):459.

Holotype.—Adult male (MUFAL 11771; Fig. 1) collected by B.S. Lisboa on 29 September 2013 in a forested area at

TABLE 2.—Measurements of the type series of *Adelophryne* ***nordestina*** sp. nov. Values are presented in millimeters as mean and (±) standard deviation (minimum–maximum values).

	Holotype MUFAL 11771	Paratypes	
Measurement	Male	Males (n = 9)	Females (n = 5)
SVL	11.6	11.3 ± 0.55 (10.3–11.8)	12.5 ± 0.32 (12.2–13.0)
HL	4.7	4.2 ± 0.38 (3.4–4.9)	4.2 ± 0.35 (3.8–4.8)
HW	4.0	4.1 ± 0.15 (3.7–4.2)	4.1 ± 0.16 (3.8–4.3)
ED	1.4	1.5 ± 0.14 (1.3–1.7)	1.6 ± 0.12 (1.5–1.8)
UEW	0.9	0.9 ± 0.13 (0.6–1.1)	1.0 ± 0.12 (0.9–1.2)
IOD	1.4	1.6 ± 0.14 (1.4–1.8)	1.83 ± 0.20 (1.5–2.1)
TD	0.5	0.5 ± 0.05 (0.4–0.6)	0.6 ± 0.08 (0.6–0.7)
IND	1.5	1.4 ± 0.19 (1.0–1.75)	1.4 ± 0.19 (1.0–1.6)
END	0.9	0.9 ± 0.05 (0.8–1.0)	1.0 ± 0.06 (0.9–1.1)
NSD	0.7	0.7 ± 0.07 (0.6–0.8)	0.7 ± 0.05 (0.7–0.8)
ETSD	1.7	1.6 ± 0.12 (1.5–1.9)	1.8 ± 0.17 (1.6–2.0)
FL	8.1	9.0 ± 0.21 (8.4–9.3)	9.6 ± 0.30 (9.4–9.9)
THL	5.0	5.3 ± 0.14 (5.1–5.6)	5.7 ± 0.12 (5.5–5.9)
TL	5.3	5.5 ± 0.25 (4.9–6.0)	5.7 ± 0.25 (5.3–6.0)

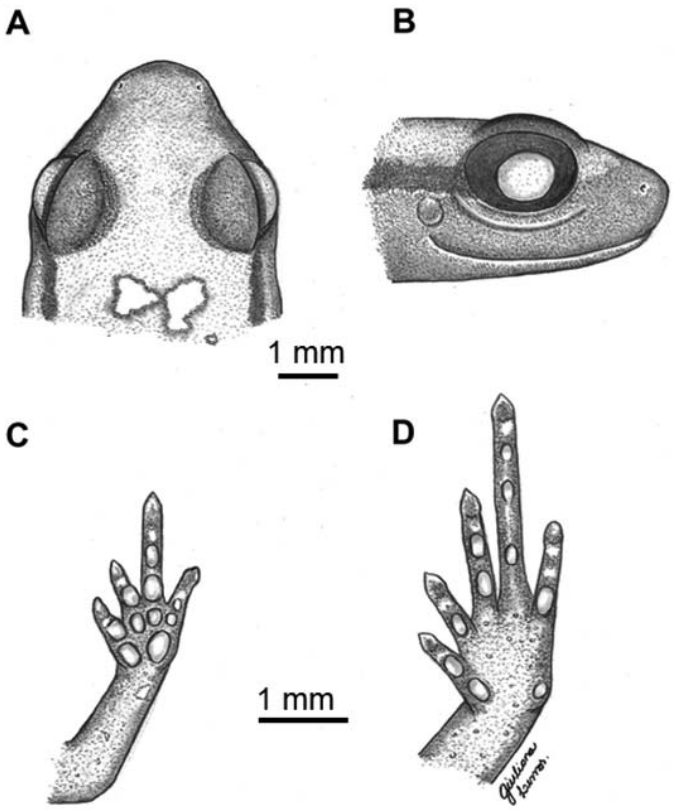

Fig. 1.—Adult holotype of *Adelophryne* ***nordestina*** sp. nov. (MUFAL 11771). Dorsal (A) and lateral (B) view of head; ventral view of hand (C) and foot (D).

the Mata da Bananeira, Estação Ecológica de Murici (09°12′45.72″S, 35°52′21.75″W; 597 m a.s.l.), municipality of Murici (ESEC Murici), Alagoas State, Brazil.

Paratypes.—Three adult females (CHP UFRPE 3005, 3007, 3009) and two adult males (CHP UFRPE 3006, 3008) collected by C.C.M. Moura and E.B.F. Lisboa from the municipality of Cabo de Santo Agostinho (08°17′S, 35°2′W; 29 m a.s.l.), Pernambuco State, Brazil; six adult males (MUFAL 10850, 11064, 11767–11770) and one adult female (MUFAL 10851) from the holotype locality collected by B.S. Lisboa; and one adult male (ZUFMS-AMP13680), one adult female (ZUFMS-AMP13679), and two juveniles (ZUFMS-AMP1367 and AMP1368) collected by L.O. Drummond, M. Wachlevski, M. Almeida-Gomes, M. Almeida-Santos, and P. Nogueira-Costa from the Reserva Biológica Guaribas (REBIO Guaribas; 06°43′S, 35°10′W; 118 m a.s.l.) in the municipality of Mamanguape, Paraíba State, Brazil.

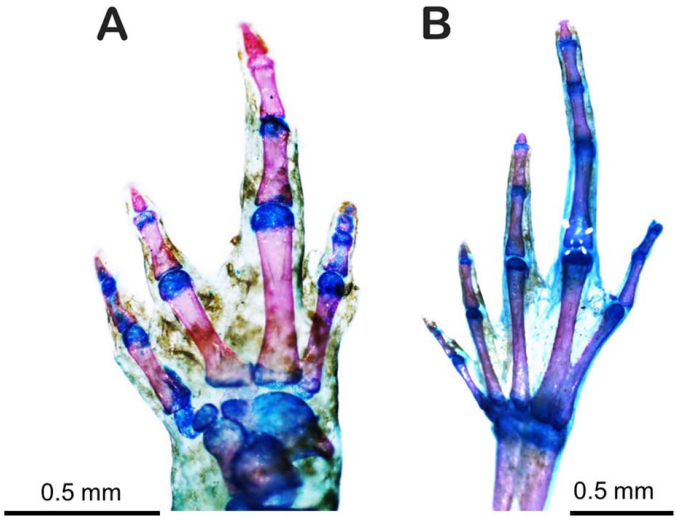

Fig. 2.—Ventral views of the hand (A) and foot (B) of a cleared and stained paratype (CHPUFRPE 3007) of *Adelophryne* ***nordestina*** sp. nov. A color version of this figure is available online.

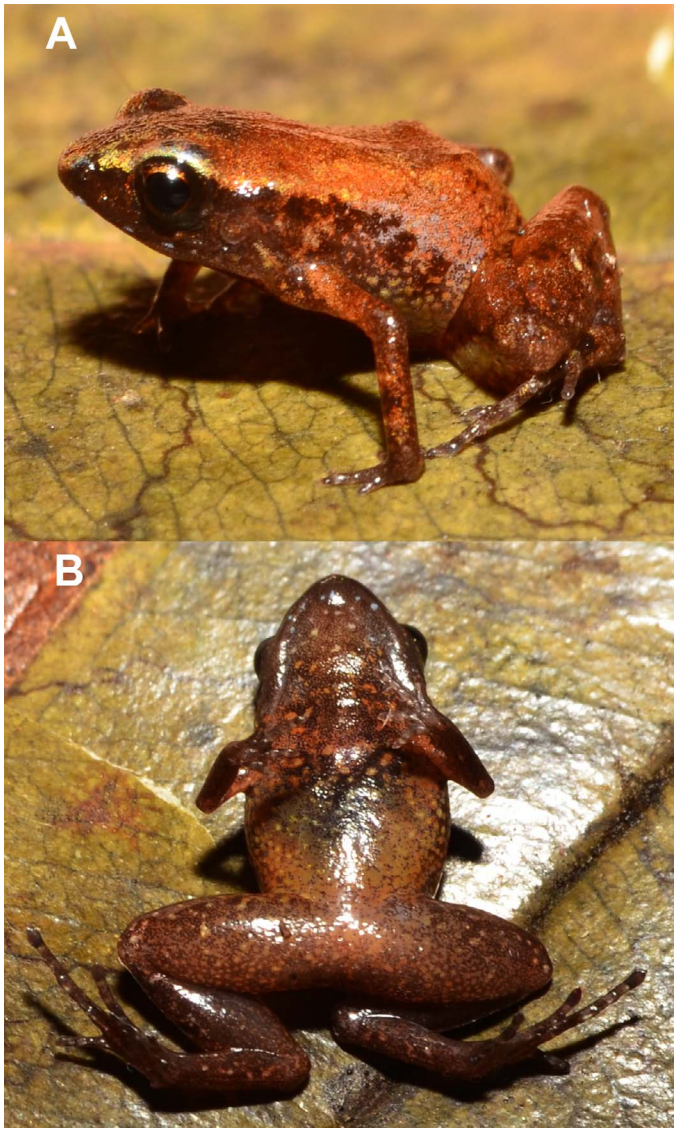

Fig. 3.—Holotype of *Adelophryne* ***nordestina*** sp. nov. in life (SVL = 11.6 mm; MUFAL 11771) in dorsal (A) and ventral (B) views. A color version of this figure is available online.

Diagnosis.—The new species is included in the subfamily Phyzelaphryninae due to the combination of the presence of apically pointed digits, terminal digits either barely or not expanded (Hedges et al. 2008), and SVL not exceeding 23.0 mm (MacCulloch et al. 2008). The new species can be distinguished from species in the genus *Phyzelaphryne* by not having subarticular tubercles under the fingers, which are usually present in *Phyzelaphryne* (Hoogmoed and

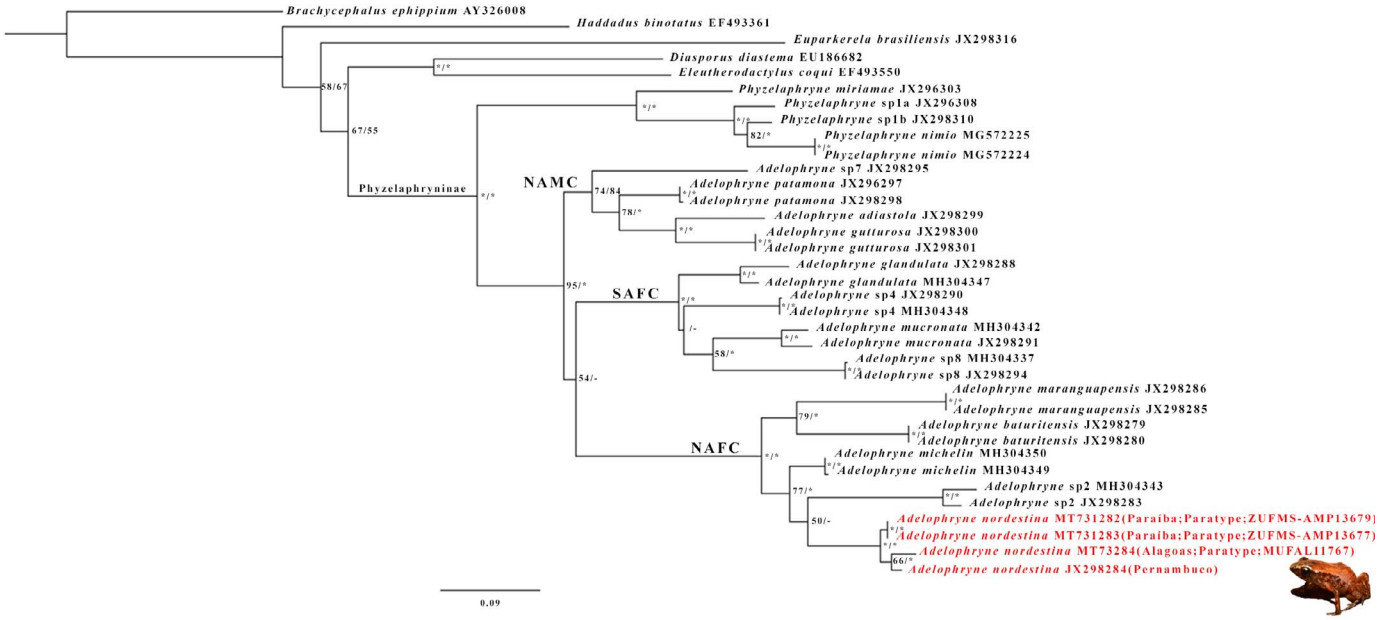

FIG. 4.—Maximum likelihood phylogeny illustrating relationships of the genus *Adelophryne* in the mitochondrial 16S rRNA gene. Maximum likelihood bootstrap and Bayesian posterior probabilities values are indicated at nodes. Values of ≥99% are shown with an asterisk (*) and values below 50% are not shown (-). Branches in red indicate *Adelophryne* ***nordestina*** sp. nov. Numbers next to species names are GenBank accession numbers. Clades names follow Fouquet et al. (2012), namely, NAFC, Northern Atlantic Forest Clade; NAMC, Northern Amazonia Clade; and SAFC, Southern Atlantic Forest Clade. Scale indicates the number of substitutions per site. A color version of this figure is available online.

Lescure 1984; Hedges et al. 2008). The generic assignment of *Adelophryne* ***nordestina*** sp. nov. is based on the combination of possession of a head narrower than the body; small size; cranial crests absent; with subdigital pads and mucronate tip on the fingers I, II, III, and IV; toes I, II, III, IV, and V with subarticular tubercles, discs, and mucronate tips; terminal phalanges of toes and fingers T-shaped (Hoogmoed and Lescure 1984); and its molecular phylogenetic position (Fig. 4).

The new taxon is differentiated by the following combination of character states: (1) SVL less than 13.0 mm (males, 10.3–11.8 mm; females, 12.2–13.0 mm); (2) tympanum small, distinct with a visible membrane (Fig. 1B); (3) tympanic annulus present (Fig. 1B); (4) absence of a distinct glandular ridge that runs from the posterior part of the eye to the insertion of the forelimb (Fig. 1B); (5) dentigerous processes of vomers absent; (6) fingers without terminal discs or circumferential grooves, and fingers with mucronate tips (Fig. 1C); (7) toes with terminal discs or circumferential grooves and mucronate tips (Fig. 1D); (8) Finger IV with three phalanges (Fig. 2A); (9) subdigital pads present under fingers; (10) subarticular tubercles present under toes (Fig. 1D and 3A); (11) skin of dorsum smooth (Fig. 3A); (12) skin of belly smooth (Fig. 3B); (13) anal flap absent; (14) advertisement call composed of one note; (15) call note pulsed with side bands; and (16) high dominant frequency.

Phylogenetic analyses.—The consensus of the BI trees and best ML tree resulted in a similar topology supporting the monophyly of genus *Adelophryne* (ML bootstrap = 95% and BI posterior probability = 100%; Fig. 4). All populations of *A.* ***nordestina*** sp. nov. clustered together in a well-supported clade (ML bootstrap and BI posterior probability = 100%) inserted in the North Atlantic Forest Clade (NAFC; sensu Fouquet et al. 2012) with high support values (ML bootstrap and BI posterior probability = 100%). The relationships among species within the NAFC were not always strongly supported. *Adelophryne* ***nordestina*** sp. nov. was recovered as sister to *A.* sp.2. (sensu Fouquet et al. 2012), but with nonsignificant support values in both inferences, and to form a clade with *A. michelin* (MI bootstrap = 77% and BI posterior probability = 99%). *Adelophryne baturitensis* and *A. maranguapensis* were recovered as sister species (MI bootstrap = 79% and BI posterior probability = 100) and sister to a clade containing *A.* ***nordestina*** sp. nov. + *A.* sp2 + *A. michelin* (Fig. 4). The uncorrected pairwise distances for the 16S rRNA gene within *A.* ***nordestina*** sp. nov. ranged between 3.5 and 3.6% (Table 3). The results of uncorrected pairwise distances of *A.* ***nordestina*** sp. nov. compared to other species of the genus ranged between 9.6 and 25.9%, being lowest between *Adelophryne* ***nordestina*** sp. nov. from the municipality of Caruaru, Pernambuco State, and *A. michelin* from the municipality of Igrapiuna, Bahia State (Table 3).

Description of the holotype.—Adult male, SVL 11.6 mm (Fig. 1). Snout rounded, slightly triangular in dorsal view (Fig. 1A) and pointed, slightly rounded in lateral view (Fig. 1B). ETSD slightly larger than ED. END smaller than IND. Nostrils ovoid, not protruding. IND slightly smaller than IOD. Canthus rostralis indistinct, loreal region slightly concave. Choanae small, round, located laterally. Dentigerous processes absent. Tongue ovoid, free except for anterior margin. Pupil horizontally oval. Upper eyelid slightly convex. Temporal region vertical. Tympanum small, 35% of ED, annulus distinct and complete. Skin texture of venter, dorsum, and limbs smooth; flanks and ventral region of thighs areolate. Anal flap absent, cloacal opening horizontally positioned at slightly below the level of dorsal surface of thigh. Fingers without disks and with mucronate tips.

TABLE 3.—Uncorrected p-distances among specimens of *Adelophryne nordestina* and other species of genus *Adelophryne*, calculated for the mitochondrial 16S rRNA gene fragment (790 aligned bp). Values are presented in percents. AL = Alagoas State; PE = Pernambuco State; PB = Paraíba State.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. *A. nordestina* JX298284 (PE)	—															
2. *A. nordestina* MT731282 (PB)	3.5	—														
3. *A. nordestina* MT731283 (PB)	3.5	0.0	—													
4. *A. nordestina* MT731284 (AL)	3.6	3.5	3.5	—												
5. *A. michelin* MH304349	9.6	10.5	10.5	10.8	—											
6. *A.* sp.2 MH304343	14.0	15.9	15.9	18.5	12.2	—										
7. *A. baturitensis* JX298279	12.1	15.6	15.6	15.9	12.3	14.6	—									
8. *A. maranguapensis* JX298286	13.8	17.4	17.4	18.0	14.5	18.7	13.7	—								
9. *A.* sp.8 JX298294	21.2	25.2	25.2	25.9	19.7	24.0	21.5	20.5	—							
10. *A. mucronata* MH304342	19.2	19.4	19.4	20.7	17.2	20.8	21.2	20.3	12.3	—						
11. *A. glandulata* MH304347	18.1	20.1	20.1	20.7	17.8	21.8	20.0	19.5	13.2	10.9	—					
12. *A.* sp.4 MH304348	17.9	19.7	19.7	21.0	16.4	20.5	19.1	21.0	13.4	10.2	10.2	—				
13. *A.* sp.7 JX298295	18.2	22.4	22.4	22.8	17.1	20.7	17.8	19.4	16.3	14.8	15.9	15.8	—			
14. *A. patamona* JX298298	18.4	21.2	21.2	22.3	19.5	19.2	17.7	19.6	16.7	16.4	17.3	17.0	15.8	—		
15. *A. gutturosa* JX298301	19.5	22.1	22.1	22.5	22.7	21.8	20.9	24.5	18.4	19.9	21.3	19.6	18.6	13.3	—	
16. *A. adiastola* JX298299	18.9	20.9	20.9	21.9	17.0	21.1	20.5	22.9	18.8	15.1	17.8	19.4	16.8	12.7	12.6	—

Fingers thin, short, and without webbing. Relative finger lengths I<IV<II<III (Fig. 1C). Phalangeal formula 2–2–3–3 (Fig. 2B). Fingers and palm surrounded by narrow strip of transparent skin. Subarticular tubercles absent, with round subdigital pads, formula 1–2–3–2, pads absent under ultimate phalanges and no supernumerary tubercles. Inner metacarpal tubercle ovoid. Outer metacarpal tubercle round, slightly larger than inner. Toes without webbing, cylindrical, slightly flattened. Relative toe lengths I<II<V<III<IV. Toes I, II, III, and IV with discs and mucronate tips; Toe V with circumferential grooves and without mucronate tips (Fig. 1D). Phalangeal formula 2–2–3–4–3 (Fig. 2A). Skin transparent on distal portion of toes. Subarticular tubercles present, formula 1–1–2–3–1, absent under ultimate phalanges. No supernumerary tubercles. Inner metatarsal tubercle oval. Outer metatarsal tubercle rounded, smaller than inner. Measurements are summarized in Table 2.

Color of the holotype.—In life, venter dark with many small orange and red dots; gular region with yellow and blue dots. Throat and underside of thighs and shanks orange. Dorsum orange with some dots. Upper eyelid and canthus rostralis gold. Loreal region dark brown, with a dark brown stripe extending along flank and reaching groin, with many small orange, red, and yellow dots. Thigh and tibia with numerous dark brown dots of various sizes. Iris reddish with black reticulations (Fig. 3). In preservative, color pattern remains visible, but colors darken.

Variation.—The type series varies in dorsal coloration in life (Fig. 5), ranging from black, brown, gold, to red. Some individuals have a dark brown interorbital V-shaped marking. Forelimbs reddish in some individuals. The coloration of the venter is dark and shows in some individuals scattered white or blue spots. Males are smaller than females (SVL of males, 10.3–11.8 mm; females, 12.2–13.0 mm). Morphometric variation is summarized in Table 2.

Etymology.—The name of the new species *nordestina* is a Portuguese feminine adjective meaning "from the northeast," referring to its distribution in northeastern Brazil.

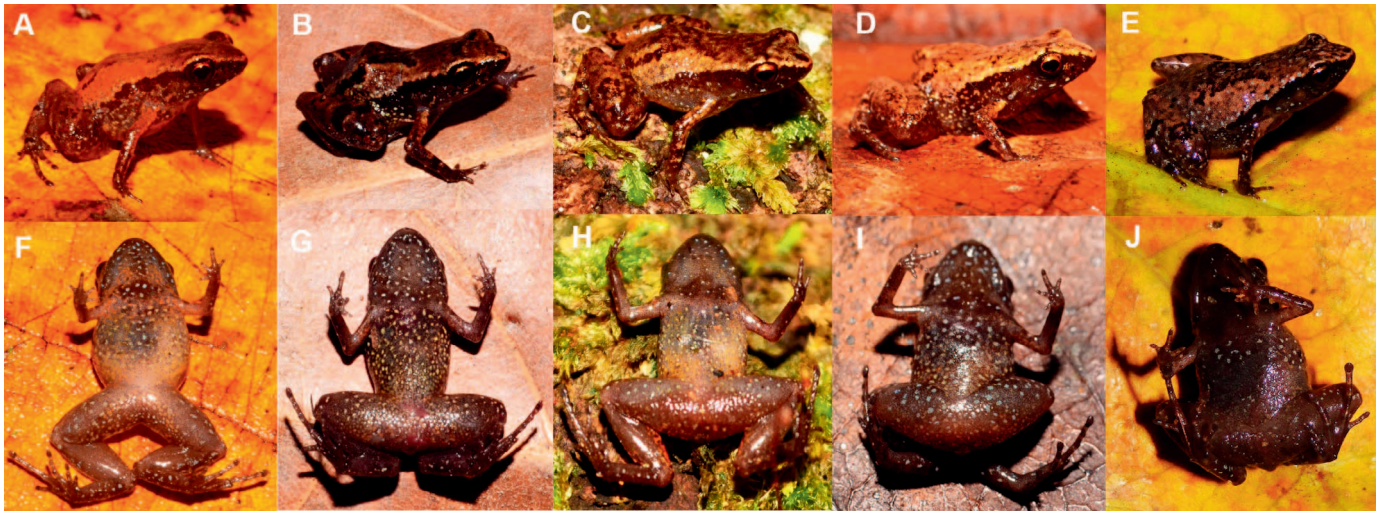

FIG. 5.—Paratypes of *Adelophryne* ***nordestina*** sp. nov. in life (MUFAL 11767, SVL = 11.5 mm; MUFAL 10851, SVL = 12.5 mm; MUFAL 11770, SVL = 10.5 mm; MUFAL 11769, SVL = 11.5 mm; ZUFMS-AMP13680, SVL = 12.2 mm). Specimens showing dorsal coloration (A–E) and showing ventral coloration (F–J).

TABLE 4.—Advertisement call of *Adelophryne* ***nordestina*** sp. nov. Values are presented as mean and (±) standard deviation (minimum–maximum values).

Parameters	MUFAL 11767	MUFAL 11769	MUFAL 11770	MUFAL 11771 (holotype)	Mean
Call duration (s)	0.052 ± 0.007 (0.041–0.065)	0.045 ± 0.004 (0.037–0.053)	0.035 ± 0.004 (0.030–0.044)	0.034 ± 0.005 (0.025–0.045)	0.042 ± 0.009 (0.025–0.065)
Intercall interval (s)	3.488 ± 1.253 (2.1–7.5)	1.889 ± 0.315 (1.4–2.6)	3.144 ± 1.027 (0.3–4.9)	3.559 ± 3.376 (1.8–17.5)	3.020 ± 1.96 (0.3–17.5)
Note number per call	1	1	1	1	1
Number pulses per call (pulse/note)	4.2 ± 0.6 3–6	3.9 ± 0.97 2–5	4.35 ± 0.59 3–5	3.45 ± 0.94 2–5	3.98 ± 0.86 2–6
Lower frequency (Hz)	4978.5 ± 149.4 (4737–5254)	5124.9 ± 102.7 (4823–5254)	5258.4 ± 19.3 (5254–5340)	4935.4 ± 112.1 (4823–5168)	5074.3 ± 165.7 (4737–5340)
Upper frequency (Hz)	6063.7 ± 51.5 (5943–6115)	6193 ± 346.7 (5943–6891)	5956.1 ± 31.5 (5943–6029)	6313.5 ± 63.1 (6202–6374)	6131.6 ± 221.4 (5943–6891)
Dominant frequency (Hz)	5714.9 ± 57.8 (5599–5857)	5598.6 ± 48.4 (5512–5685)	5671.9 ± 50.6 (5599–5771)	5770.9 ± 27.9 (5685–5857)	5689.1 ± 78.6 (5512–5857)
Sample	20 calls	20 calls	20 calls	20 calls	80 calls
Air temperature (°C)	23.5	23.2	23.2	26.2	24 ± 1.5 (23.2–26.2)
Air relative humidity (%)	78	76	93	71	79.5 ± 9.5 (71–93)

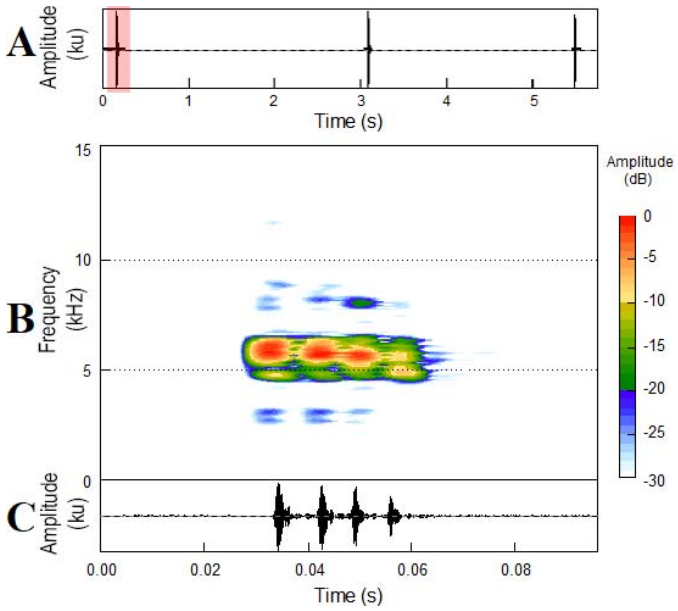

FIG. 6.—Advertisement call of the holotype of *Adelophryne* ***nordestina*** sp. nov. (MUFAL 11771) recorded at Mata da Bananeira, ESEC Murici, Municipality of Murici, Alagoas State, Brazil, on 29 September 2013 (time of day = 1315 h, air temperature = 26.2°C, relative humidity = 71%). (A) The oscillogram of three consecutive calls, with a single call highlighted in red; (B) spectrogram and (C) oscillogram of the call highlighted. A color version of this figure is available online.

Advertisement call.—The advertisement call of *A. **nordestina*** sp. nov. (Table 4 and Fig. 6) is composed of one single note formed by 2–6 pulses (3.98 ± 0.86). Call duration has a mean of 0.042 ± 0.009 s (0.025–0.065 s). The calls are emitted with intervals of 3.020 ± 1.96 s (0.342–17.552 s). The lower frequency has a mean of 5074.3 ± 165.7 Hz (4737–5340 Hz), the upper frequency has a mean of 6136.6 ± 221.4 Hz (5943–6891 Hz), and the dominant frequency has a mean of 5689.1 ± 78.6 Hz (5512–5857 Hz). The apparent harmonics observed in the spectrogram are sidebands (Fig. 6B), an artifact generated by the pulsatile nature of the call (Vielliard 1993). Sidebands were also reported for the advertisement calls of *A. mucronata* (Lourenço-de-Moraes et al. 2012) and *A. maranguapensis* (Cassiano-Lima et al. 2014).

Distribution and natural history.—*Adelophryne **nordestina*** sp. nov. is known from interior Atlantic Forest fragments in Alagoas, Pernambuco, and Paraíba states, Brazil (Fig. 7). These fragments of forest are located in the Pernambuco Endemism Center (Prance 1982), a region very threatened by deforestation and the least studied part of the Atlantic Forest domain, located near the northern portion of the São Francisco River (Tabarelli et al. 2006). *Adelophryne **nordestina*** sp. nov. is a cryptic species associated with leaf litter. In the type locality (ESEC Murici), individuals of *A. **nordestina*** sp. nov. were generally found in humid sites with abundant leaf litter having a depth of 10–30 cm. In Pernambuco and Paraíba states, the new species was found in similar habitat conditions as in the type locality. This species is common and active during the day, and its reproduction period appears to be continuous because males were found calling continuously from January to December at temperatures between 21 and 26°C. Calling males were

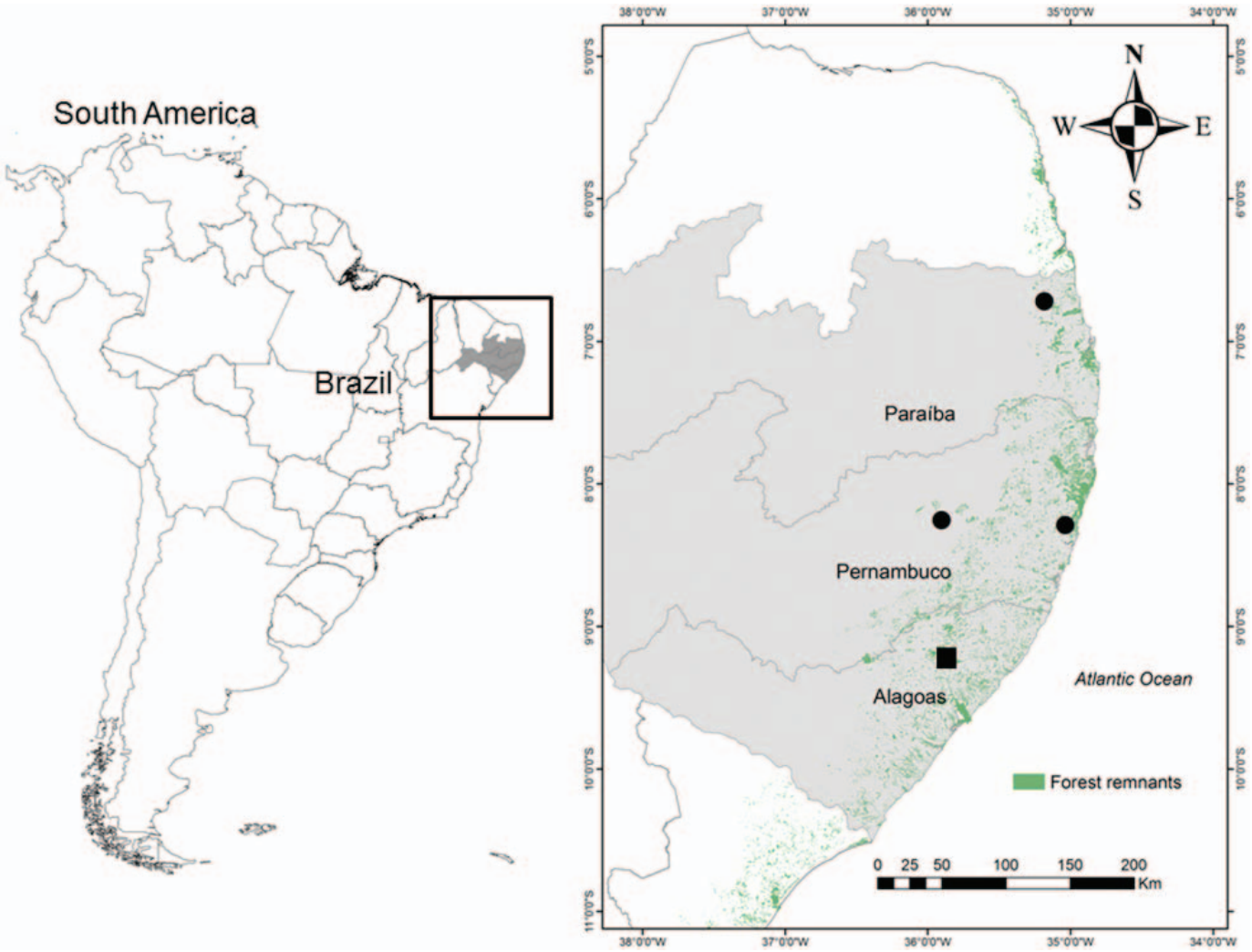

Fig. 7.—Geographic distribution of *Adelophryne* ***nordestina*** sp. nov. in eastern Brazil. The square indicates the type locality of *Adelophryne* ***nordestina*** sp. nov. in the municipality of Murici, Alagoas State, Brazil. Circles indicate additional localities. A color version of this figure is available online.

found under leaves. The daily vocalization period began around 0830 h, with activity reaching its peak between 1130 h and 1330 h, and usually ending around 1730 h (sunset). Some individuals were observed calling until 2200 h during rain events. The vocalization of one male would often stimulate neighboring males to call, resulting in synchronized alternation in the emission of vocalizations. Egg clutches or females with oocytes were not found.

Comparisons with other species.—*Adelophryne nordestina* sp. nov. is distinguished from all other *Adelophryne* species, except *A. baturitensis*, by the presence of subarticular tubercles under the toes (subdigital pads in other species). *Adelophryne* ***nordestina*** sp. nov. is distinguished from *A. glandulata, A. meridionalis*, and *A. michelin* by having a distinct tympanum and tympanic annulus present (indistinct in the other species). *Adelophryne* ***nordestina*** sp. nov. is distinguished by having smaller body size (maximum SVL, 13.0 mm) than *A. adiastola* (maximum SVL, 14.0 mm), *A. mucronata* (maximum SVL, 14.9 mm), *A. baturitensis* (maximum SVL, 16.3 mm), *A. gutturosa* (maximum SVL, 16.0 mm), *A. maranguapensis* (maximum SVL, 17.4 mm), and *A. patamona* (maximum SVL, 23.0 mm). *Adelophryne* ***nordestina*** sp. nov. is distinguished from *A. baturitensis, A. maranguapensis* and *A. patamona* by the absence of discs or circumferential grooves on fingers (present in these species). The new species is further distinguished from *A. glandulata* and *A. gutturosa* by the absence of a distinct glandular ridge that runs from the posterior part of the eye to the insertion of the forelimb (present in these species). *Adelophryne* ***nordestina*** sp. nov. is distinguished from *A. adiastola*, *A. baturitensis*, *A. maranguapensis*, *A. meridionalis*, *A. michelin*, *A. mucronata*, *A. pachydactyla*, and *A. patamona* by lacking dentigerous processes of vomers (present in these species). *Adelophryne* ***nordestina*** sp. nov. is distinguished from *A. adiastola*, *A. glandulata*, *A. meridionalis*, *A. michelin*, and *A. pachydactyla* by having three phalanges in Finger IV (two phalanges in these species). *Adelophryne* ***nordestina*** sp. nov. is distinguished from *A. adiastola*, *A. glandulata*, *A. mucronata*, and *A. patamona* by having the dorsum with smooth skin (vs. shagreened to granular in *A. adiastola*, shagreened with small and rounded granules in *A. glandulata*, smooth with scattered small granules in *A. mucronata*, and tuberculated in *A. patamona*). *Adelophryne* ***nordestina*** sp. nov. is distinguished from *A. meridionalis* by

Table 5.—Comparisons of call parameters among *Adelophryne* species. Values are presented as mean and (±) standard deviation (minimum–maximum values).

Parameters	*Adelophryne adiastola*[a]	*Adelophryne gutturosa*	*Adelophryne maranguapensis*	*Adelophryne mucronata*	*Adelophryne **nordestina*** sp. nov.	*Adelophryne patamona*
Call structure	Notes multipulsed, with visible harmonics	Notes multipulsed with visible harmonics	Notes multipulsed, with side-bands	One note, nonpulsed, with side-bands	One note, pulsed, with side-bands	Notes unpulsed, with visible harmonics
Call duration (s)	0.24 —	1.27 (0.058–2.392)	0.798 ± 0.159 (0.663–1.075)	0.029.5 ± 0.004 (0.022–0.034)	0.042 ± 0.009 (0.025–0.065)	0.63 (0.584–0.712)
Number of notes per call	— (1–10)	6.8 (2–15)	6 ± 1.3 (5–8)	1	1	3
Inter-call interval (s)	—	15.08 (1.8–110.7)	—	9.29 ± 1.22 7.2–10.5	3.020 ± 1.96 (0.342–17.552)	3.85 (3.365–4.255)
Number of pulses per note	2 —	(11–12)	4 ± 0.588 (3–6)	Nonpulsed	3.98 ± 0.86 (2–6)	—
Dominant frequency (Hz)	— (3200–3700)	4242 3896–4979	4824 ± 187.23 (4484–4831)	5290 ± 0.05 (5240–5370)	5689 ± 78.6 (5512–5857)	3255 (3251–3269)
Sample	1 male	12 males and 29 calls	4 males and 5 calls	1 male and 6 calls	4 males and 80 calls	1 male and 6 calls
Source	Heyer (1977)	MacCulloch et al. (2008)	Cassiano-Lima et al. (2014)	Lourenço-de-Moraes et al. (2012)	Present study	MacCulloch et al. (2008)

[a] Call erroneously reported as from *Phyzelaphryne miriamae* in Heyer (1977).

having Toes II, III, and IV with circumferential grooves and discs (vs. only Toe IV with circumferential grooves in *A. meridionalis*). *Adelophryne* ***nordestina*** sp. nov. is distinguished from *A. mucronata* and *A. maranguapensis* by the absence of an anal flap (present in these species).

The advertisement call of 5 of the 10 recognized species in the genus *Adelophryne* has been described, namely, *A. adiastola* (erroneously reported as *Phyzelaphryne miriamae* in Heyer 1977), *A. gutturosa* (MacCulloch et al. 2008), *A. maranguapensis* (Cassiano-Lima et al. 2014), *A. mucronata* (Lourenço-de-Moraes et al. 2012), and *A. patamona* (MacCulloch et al. 2008). The advertisement call of *A.* ***nordestina*** sp. nov. differs from these other *Adelophryne* species by having the highest mean dominant frequency, with 5551 Hz (3200–5290 Hz in other species; Table 5). The advertisement call of *A.* ***nordestina*** sp. nov. differs from *A. gutturosa*, *A. maranguapensis*, and *A. patamona* by being formed of one single pulsed note (more than one note per call in these species). The call duration in *A.* ***nordestina*** sp. nov. (mean = 0.042 ± 0.009 s) is shorter than that of *A. adiastola* (mean = 0.24 s), *A. gutturosa* (mean = 1.27 s), *A. maranguapensis* (mean = 0.798 ± 0.159 s), and *A. patamona* (mean = 0.63 s) but is longer than that of *A. mucronata* (mean = 0.029.5 ± 0.004 s). The advertisement call of *A.* ***nordestina*** sp. nov. is most similar to that of *A. mucronata* because both are composed of one single note per call and have the dominant frequency above 5200 Hz, but the call of *A.* ***nordestina*** sp. nov. differs by having a pulsed call structure (nonpulsed in *A. mucronata*).

Discussion

Adelophryne ***nordestina*** sp. nov. was recovered as belonging to the NAFC of *Adelophryne* with high support values. *Adelophryne* ***nordestina*** sp. nov. has intermediate morphological features between the species of the NAFC. This species showed a maximum SVL of 13.0 mm, three phalanges in Finger IV, a distinct tympanum, and subarticular tubercles under the toes. Nevertheless, it showed a reduced phalange in Finger IV (Supplemental Fig. SA1B, available online) and a reduced tympanum. *Adelophryne baturitensis* and *A. maranguapensis* are similar to *A.* ***nordestina*** sp. nov. However, they are larger, with a maximum SVL between 16.3 and 17.4 mm; have a large and distinct tympanum; and have three well visible phalanges in the Finger IV (see Supplemental Fig. SA1.A). Some species of the NAFC showed a total reduction of a phalange in Finger IV (e.g., *Adelophryne michelin* and *Adelophryne* sp.2; see Supplemental Fig. SA1.C), and a reduction or complete loss of the external tympanum occurs in *A. michelin*. This new evidence of feature reduction (i.e., phalange) supports the adaptive evolution of the genus *Adelophryne* toward miniaturization with extensive homoplasy because this reduction is common for species in different clades (e.g., *A. adiastola* in NAMC, *A. glandulata* in SAFC, and *A. michelin* in NAFC; see Lourenço-de-Moraes et al. 2018 and Fig. 4).

Adelophryne species possibly dispersed to the Atlantic Forest across northern Amazonia approximately 23–16 Ma (Fouquet et al. 2012), and new species found in the Atlantic Forest (e.g., *A. michelin*) have high values of interspecific mitochondrial DNA (mtDNA) sequences of the mitochon-

drial 16S rRNA divergence (>20%). Diminutive species are more susceptible to desiccation due to having a high surface-to-volume ratio (MacLean 1985). As a result, *Adelophryne* species are sensitive to changes in the environment (Lourenço-de-Moraes et al. 2014) and restricted to intact, humid forests (Rodríguez et al. 2015; Ferreira et al. 2016). Thus, the restricted gene flow among populations with accelerated genetic differentiation can lead to species diversification (Pabijan et al. 2012) and can have significant consequences on the number of species with small ranges in *Adelophryne* (Lourenço-de-Moraes et al. 2018).

Adelophryne species could be threatened by climate change (Lourenço-de-Moraes et al. 2019) and be susceptible to negative effects of chytridiomycosis (Mesquita et al. 2017) and direct anthropogenic actions (Lourenço-de-Moraes et al. 2014; Ferreira et al. 2016). Most of the described species have a restricted distribution (<5000 km^2); *A.* ***nordestina*** sp. nov. occurs in three states in northeastern Brazil, and its distribution does not exceed 240 km^2 of fragmented forests (see Fig. 7). *Adelophryne* ***nordestina*** sp. nov. occurs in the Pernambuco Endemism Center (Prance 1982) in the Atlantic Forest biodiversity hotspot (Myers et al. 2000), a region threatened by anthropogenic actions (Tabarelli et al. 2006). Amphibians with direct development have a low resistance to *Batrachochytrium dendrobatidis* (Mesquita et al. 2017), and the occurrence of this fungus in the region has been documented (Lisboa et al. 2013; Valencia-Aguilar et al. 2015). The four specimens collected in the municipality of Cabo de Santo Agostinho, in Pernambuco State, were not found in protected areas and could be locally threatened. The other specimens were found in protected areas, reinforcing the importance of these areas for the conservation of the Atlantic Forest biodiversity hotspot. Based on the forest remnant size, the area of occupancy of *A.* ***nordestina*** sp. nov. is less than 500 km^2; *Adelophryne* ***nordestina*** sp. nov. can be included as Near Threatened (NT) under criterion B2a of IUCN (2001), i.e., restricted geographic distribution with declines in ecosystem distribution. Further studies are necessary to verify their vulnerability to environmental factors and their relation with anthropogenic changes.

The genus *Adelophryne* contains diminutive species with some of the smallest anurans in the world, and its diversity is still underestimated (Fouquet et al. 2012; Lourenço-de-Moraes et al. 2018). Currently, there are 11 (including *A.* ***nordestina*** sp. nov.) described *Adelophryne* species, and according to Fouquet et al. (2012) and Lourenço-de-Moraes et al. (2018), there are still 5 candidate species to be described, of which 4 are from the Brazilian Atlantic Forest. Their diminutive size and secretive and agile behavior (Ferreira et al. 2019) helps them elude notice and capture, and we suspect that more species may be found in the future.

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Supplemental Material

Supplemental material associated with this article can be found online at https://doi.org/10.1655/Herpetologica-D-20-00022.S1

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Note added in proof. While this manuscript was in the proof stage, Taucce, Costa-Campos, Haddad, and Carvalho (2020, Copeia. 108(4):746–757) described the species *Adelophryne amapaensis* from Amapá, Northern Brazil. This species was reported by Fouquet et al. (2012) as *Adelophryne* sp. 7, and considered to be a member of the North Amazonia Clade.

Appendix

Specimens Examined

Adelophryne adiastola.—Ecuador: Pastaza: Kurintza DHMECN 4378;

Adelophryne baturitensis.—Brazil: Ceará: Guaramiranga, CFBH 20469–76; Tiangua CFBH 24554–67; Viçosa do Ceará CFBH 24579–85, -UFC 3912, 3696;

Adelophryne glandulata.—Brazil: Espírito Santo: Santa Teresa (Reserva Biológica Santa Lúcia), MNRJ 28344–34932, (Reserva Biológica Augusto Ruschii) MBML 7636–7, MNRJ 87081, MZUESC 12178 and 12180, ZUFG 7961–2. Minas Gerais: Mariana (Mata estrada Mariana-Catas Altas), UFMG 4108, 4115, 4117–8, (Mina de Fabrica Nova Vale S.A) UFMG 11643–4, 11646, 11751, 11755; Catas Altas (ADA PDE Trevo. Margem estrada Catas Altas-Mariana) UFMG 8110, 8127, 8140, 8157–60; 8167–68;

Adelophryne maranguapensis.—Brazil: Ceará: Maranguape: CFBH 24515–27, UFC 3810, 3745;

Adelophryne meridionalis.—Brazil: Minas Gerais: Juiz de Fora (Parque Municipal de Lajinha), MZUFV 12625;

Adelophryne michelin.—Brazil: Bahia: Igrapiuna (Reserva Ecológica Michelin) MBML 10495–10499, MZUESC 17506–17510, 17513 and ZUFG 10693–10698;

Adelophryne mucronata.—Brazil: Bahia: Una CFBH 23672, ZUEC 6169, 12139, 16626, MZUESC 9091–96; Igrapiúna MZUESC 19140–41; Wenceslau Guimarães MZUESC 19149–50;

Adelophryne pachydactyla.—Brazil: Bahia: Itacaré (RPPN Capitão) ZUEC 18212–13, and Una (RPPN Nova Angélica) ZUEC 17825.

Institutional abbreviations follow Sabaj (2019): DHMECN, División de Herpetología, Museo Ecuatoriano de Ciencias Naturales, Quito, Ecuador; CFBH, Célio F.B. Haddad Collection, Universidade Estadual Paulista; UFC, NUROF-UFC Núcleo Regional de Ofiologia, Laboratório de Herpetologia da Universidade Federal do Ceará; MNRJ, Museu Nacional do Rio de Janeiro MBML, Museu de Biologia Mello Leitão; MZUESC, Museu de Zoologia da Universidade Estadual de Santa Cruz; ZUFG, Coleção Zoológica da Universidade Federal de Goiás; UFMG, Centro de Coleções Taxonômicas, Universidade Federal de Minas Gerais; MZUFV, Museu de Zoologia João Moojen, Universidade Federal de Viçosa; and ZUEC, Museu de Zoologia da Universidade Estadual de Campinas "Adão José Cardoso."