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Unexplored Urban Diversity: A New Species of *Adenomera* (Anura, Leptodactylidae) Related to *Adenomera ajurauna* from the Atlantic Forest of Southeastern and Southern Brazil

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Abstract: The Atlantic Forest is recognized as a biodiversity hotspot because of the high species richness and the remaining natural areas comprising less than 30% relative to its primary vegetation. Even though many anuran species from this biome are ecologically restricted to pristine ecosystems, there are some examples of new species discovered from anthropized areas. *Adenomera* represents a widespread and abundant frog genus in Atlantic Forest ecosystems, with species occurring in areas with varying degrees of human disturbance. In this paper, we name and describe a new species of *Adenomera* endemic to the Atlantic Forest typically found in human-altered ecosystems, such as urban and rural sites. The new species was recovered as belonging to the *Adenomera marmorata* clade, and sister to *A. ajurauna*. These two species have allopatric distributions in southeastern and southern Brazil, with a single known sympatric occurrence. They display different calls and occupy distinct habitats. The newly described species of *Adenomera* is an additional case of new species discovered from urban sites in the Atlantic Forest hotspot.

Key words: Anthropized ecosystems; Biodiversity hotspot; Cryptic species; Species delimitation; Sympatry

Brazil comprises 1,186 native species of amphibians (Segalla et al. 2021), representing the most species-rich country in the world for this vertebrate group. However, scientists are still far from documenting the entire amphibian diversity throughout the country, which has held the first position in the rank of total number per year of described new species over the past 5 yr (Streicher et al. 2020). The Atlantic Forest harbors more than 50% of Brazil's amphibian species richness, whose cover area overlaps with the largest urban sites in the country (70% of Brazil's human population; Haddad et al. 2013). Interestingly, half of the new species described over the past 5 yr in Brazil occur in this biome (Streicher et al. 2020). Many endemic anuran species to the Atlantic Forest are typical of pristine ecosystems. These include representatives of pumpkin toadlets and flea toads of the genus *Brachycephalus* (Clemente-Carvalho et al. 2012; Almeida-Silva et al. 2021), marsupial frogs of the genus *Fritziana* (Walker et al. 2016; Folly et al. 2018), and highland terrestrial frogs of the genus *Holoaden* (Pombal et al. 2008; Martins and Zaher 2013). Nevertheless, some anuran species have also been discovered and formally described from anthropized areas. Some examples of species described from rural or urban sites are *Dendropsophus nekronastes* (Dias et al. 2017), *Scinax arduous* (Peixoto 2002), and *Physalaemus feioi* (Cassini et al. 2010).

The terrestrial foam-nesting frogs of the genus *Adenomera* are widespread and abundant where they occur across South America east of the Andes (Fouquet et al. 2014; Carvalho et al. 2019a; Cassini et al. 2020). Despite their conspicuous vocalizations and high local abundance, the taxonomy of *Adenomera* is still partially understood in certain regions and for certain clades (Fouquet et al. 2014; Carvalho et al. 2020a,b,c, 2021). Most of the recent descriptions of new *Adenomera* species have focused on populations from lowland Amazonian forests (Carvalho et al. 2019b,c; 2020b,c; 2021), whereas their counterparts that occur in open habitats and disturbed areas in other biomes remain understudied to a certain extent.

The genus *Adenomera* comprises 31 species, of which 15 were described or redescribed in the last decade. According to the available phylogeny of *Adenomera* (Fouquet et al. 2014), the species diversity remains underestimated, and at least 15 candidate new species, 6 of which are distributed in the Atlantic Forest, still need a taxonomic evaluation. The recent contributions to the systematics of *Adenomera* highlight the need for increasing sampling efforts in an attempt to understand variation and distribution patterns and provide formal taxonomic descriptions to the many candidate new species that remain unnamed throughout South America (Carvalho et al. 2019a, 2021; Cassini et al. 2020).

Here we focused on populations of the *Adenomera marmorata* clade from the Atlantic Forest of southeastern and southern Brazil, namely *A. ajurauna* (Berneck et al. 2008) and a closely related candidate new species: *Adenomera* sp. S (sensu Fouquet et al. 2014). Our analysis of morphological, acoustic, and DNA sequence data sustains our decision to name and describe populations previously assigned to *Adenomera* sp. S as a new species. We also provide additional data on phenotypic variation and distribution of the closely related *A. ajurauna*.

Materials and Methods

Field Data Collection

We conducted fieldwork in the Brazilian states of São Paulo (SP), Paraná (PR), and Santa Catarina (SC). We collected at two urban and human-altered areas in the surroundings of

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TABLE 1.—Primers used to amplify mitochondrial and nuclear gene fragments from *Adenomera* species.

Primer	Direction	Gene	Sequence	Reference
dgLCO1490	F	COI	GGTCAACAAAATCATAAAGAYATYGG	Meyer (2003)
dgHCO2198	R	COI	TAAACTTCAGGGTGACCAAARAAYCA	Meyer (2003)
Cytb2	F	cytb	AAACTGCAGCCCCTCAGAAATGATATTTGTCCTCA	Kocher et al. (1989)
MVZ15	F	cytb	GAACTAATGGCCCACACWWTACGNAA	Moritz et al. (1992)
CbR2	R	cytb	GTGAAGTTRTCYGGGTCYCC	Fouquet et al. (2012)
16SAR	F	16S	CGCCTGTTTATCAAAAACAT	Palumbi et al. (1991)
16SWilk2	R	16S	GACCTGGATTACTCCGGTCTGA	Wilkinson et al. (1996)
MartFL1	F	RAG1	AGCTGGAGYCARTAYCAYAARATG	Hoegg et al. (2004)
Ad2R	R	RAG1	ATTGGCTCTCCATGTTTCATAG	Fouquet et al. (2012)
POMC 1	F	POMC	GAATGTATYAAAGMMTGCAAGATGGWCCT	Wiens et al. (2005)
POMC 2	F	POMC	TCTGCMGARTCWCCYGTGTTTCC	Wiens et al. (2005)
POMC 3	R	POMC	TAYTGRCCCTTYTTGTGGGCRTT	Wiens et al. (2005)
POMC 4	R	POMC	TGGCATTYTTGAAAAGAGTCAT	Wiens et al. (2005)

the municipality of Iguape, SP: (1) Escola Técnica Estadual Engenheiro Agrônomo Narciso de Medeiros (24.670086°S, 47.549536°W; 4 m above sea level [asl]; in all cases datum = WGS84); and (2) Mirante do Cristo (24.704684°S, 47.547765°W; 69 m asl). We sampled three other localities inside forest habitats: (3) Miracatu, SP (24.255330°S, 47.481677°W; 120 m asl); (4) Guaratuba, PR, at Fazenda Creminácio, on the foothill of Serra do Araraquara (25.956314°S, 48.894109°W; 149 m asl); and (5) Garuva, SC (26.026690°S, 48.867510°W; 59 m asl). Specimens were deposited in the Célio F. B. Haddad (CFBH) collection at Universidade Estadual Paulista, in Rio Claro, São Paulo, Brazil. Other specimens examined in CFBH and other herpetological collections are listed in Appendix I. Institutional abbreviations followed Sabaj (2020).

Taxon Sampling and Molecular Data Acquisition

We compiled a molecular data set of *Adenomera* available in GenBank, aiming to sample species from all the named clades indicated by Fouquet et al. (2014), totaling 21 nominal and 11 unnamed candidate species (including the new species described herein; Supplemental Table S1, available online). We also added three leptodactylid species outside *Adenomera*: *Lithodytes lineatus* (Schneider 1799), the sister group of *Adenomera* (Fouquet et al. 2014), *Hydrolaetare caparu* (Jansen et al. 2007), and *Leptodactylus rhodomystax* (Boulenger 1884). We used a clade formed by the two latter species to root our tree. We selected partial sequences of the mitochondrial genes 16S rRNA (16S), cytochrome c oxidase subunit I (COI), and cytochrome b (CYTB), and the nuclear genes recombination activating gene 1 (RAG1) and pro-opiomelanocortin C (POMC) to build our phylogenetic hypothesis, because they have already been used successfully for *Adenomera* and were available for all species included in the analysis. Gene fragments, primers, and respective sequences are provided in Table 1.

We extracted total DNA from 99.5% ethanol-preserved muscle or liver tissues following Lyra et al. (2017). We conducted PCR amplifications following Cassini et al. (2020) and PCR product cleaning following Lyra et al. (2017). We sent purified PCR products to Macrogen Inc. (Seoul, Republic of Korea), where they conducted sequencing in an ABI 3730XL sequencer. All sequences were deposited in Genbank (Supplemental Table S1).

Alignment, Partition Schemes, and Nucleotide Substitution Model Selection

We performed alignment using MAFFT v7.490 (Katoh and Standley 2013), with the G-INS-i algorithm for the coding gene fragments (COI, CYTB, POMC, and RAG1) and the E-INS-i algorithm for the 16S. We searched for the best partition scheme and best-fitting nuclear models with PartitionFinder v2.1.1 (Lanfear et al. 2017) using the corrected Akaike information criterion (AICc; Hurvich and Tsai 1989) and considering each one of the codon positions as a separate partition for the coding genes and the 16S as a single partition, totaling 13 partitions a priori.

Phylogenetic Analyses, Genetic Distances, and Haplotype Genealogies

We conducted tree searches using both maximum-likelihood (ML) and Bayesian inference (BI) optimality criteria. We computed the ML analysis in RAxML-NG v1.1.0 (Kozlov et al. 2019), starting the search for the most likely tree with 50 random trees and 50 parsimony trees, conducting 1000 nonparametric bootstrap replicates. We computed the BI analysis in MrBayes v3.2.6 (Ronquist et al. 2012) using two independent runs of 2.0×10^7 generations, starting with random trees and four Markov chains (one cold), sampled every 2000 generations. We discarded 25% of generations and trees as burn-in and performed the run with unlinked character state frequencies, substitution rates of the GTR model, gamma shape parameters, and proportion of invariable sites between partitions. We used the standard deviation of split frequencies (<0.01), effective sample size (ESS > 200), and potential scale reduction factor (PSRF, should approach 1.0 as runs converge; Gelman and Rubin 1992) to assess run convergence. We drew phylogenetic trees using FigTree v1.4.4 (available at http://tree.bio.ed.ac.uk/software/figtree/). We computed uncorrected pairwise distances (p distances) for two of the mitochondrial gene fragments, COI and 16S. For this purpose, we used the packages APE v5.6-1 (Paradis and Schliep 2019) and SPIDER v1.5.0 (Brown et al. 2012) of the R v4.1.2 platform (R Core Team 2021).

We reconstructed haplotypes of the nuclear gene fragments using PHASE v2.1.1 (Stephens et al. 2001), performing 10 independent runs of 10,000 iterations, with thinning interval of 100 and burn-in of 10,000. We investigated

TABLE 2.—Best partition scheme of the 12 partitions and corresponding best-fitting nucleotide substitution models. Numbers after gene names stand for codon positions.

Partition	Model
16S	GTR+I+Γ
COI 1	SYM+I+Γ
COI 2	F81+I
COI 3	GTR+Γ
cytb 1	SYM+I+Γ
cytb 2	GTR+I+Γ
cytb 3	GTR+Γ
POMC 1 + RAG1 1	GTR+I+Γ
POMC 2	GTR+Γ
POMC 3	SYM+Γ
RAG1 2	HKY+I
RAG1 3	HKY+Γ

haplotype distribution for the two nuclear gene fragments separately using Haplotype Viewer beta version (Salzburger et al. 2011; available at http://www.cibiv.at/~greg/haplowiewer.shtml) to convert maximum-likelihood trees we generated with RAxML-NG in gene haplotype genealogies. For this analysis, we used only the new species and its sister taxa, *Adenomera ajurauna*.

Morphology and Calls

We analyzed the morphology of 65 specimens of *Adenomera* sp. S (32 males and 33 females) that were genotyped for this study, as well as those linked to call data and, in a few cases, specimens from localities where we are aware that only one *Adenomera* lineage is expected to occur. The morphologically examined specimens of *Adenomera* from the Atlantic Forest are listed in Appendix I; information on analyzed calls of *Adenomera* sp. S and *A. ajurauna* is provided in Appendix II. We measured the morphometric traits using an ocular micrometer (±0.1 mm) fitted to a dissecting microscope, except snout to vent length (SVL), measured using digital calipers (±0.1 mm). The measured traits were: head length (HL), head width (HW), eye diameter (ED), tympanum diameter (TD), eye to nostril distance (EN), internarial distance (IND), hand length (HAL), thigh length (THL), tibia length (TL), and foot length (FL). We followed Watters et al. (2016) for eight measurements (SVL, THL, TL, FL, ED, TD, EN, and IND) and Carvalho et al. (2019b) for three measurements (HAL, HL, and HW). Snout shape was assessed according to Heyer et al. (1990). Character states of toe tips followed Heyer (1973), with definitions modified by Carvalho et al. (2019a). We characterized the internal features of the mouth of a paratype (CFBH 45190) to prevent damaging the holotype of the new species.

We recorded *Adenomera* calls from several localities in the Atlantic Forest of the states of São Paulo and Paraná (183 calls from 8 males of *A. ajurauna*; 782 calls from 21 males of *Adenomera* sp. S). We stored the sound files as monochannel uncompressed wave files at a sampling rate of 44.1 kHz and a sample size of 16 bits. Sound files were deposited in the acoustic repositories of CFBH (CFBH-voc) and AAG-UFU collections. We also analyzed *Adenomera* sound recordings available at these acoustic repositories in addition to those we recorded in the field. See Appendix II for detailed information on localities, repository numbers, and voucher specimens. We analyzed the calls using a customized version (v0.9.6.1) of Soundruler (Gridi-Papp 2007) interfacing with Matlab v6.5.2 scripts (Matlab 2004) through automated procedures for quantification of acoustic traits. We set analytical settings for the automated analysis in Soundruler as follows: window type = Hann, fast Fourier transform (FFT) size = 1024 samples, FFT overlap = 90%; signal detection with smoothing = 500 samples, resolution = 1 sample; signal delineation with smooth factor = 1, smoothing = 13 samples, resolution = 1 sample. We applied a high-pass filter up to 500 Hz to sound files in Soundruler before conducting the acoustic analyses to reduce background noise. We quantified the note repetition rate manually in Audacity v3.0.0 (Audacity Team 2021). Analytical parameters and acoustic definitions followed Carvalho et al. (2019b). We produced sound figures using seewave v2.1.6 (Sueur et al. 2008) and tuneR v1.3.3 (Ligges et al. 2018) in R v4.1.2 (R Core Team 2021). We used the following spectrogram settings: window type = Hann, fast Fourier transform (FFT) size = 256 samples, FFT overlap = 90%; the darkness of frequency components indicates intensity in a relative 30-dB scale (0 dB = maximum amplitude of signal).

Species Concept and Interspecific Comparisons

We used the unified species concept (de Queiroz 2007) following the rationale of species delimitation based on (1) cladogram topology (monophyly) and (2) identification of at least one diagnostic phenotypic character that supports the independence of evolutionary lineages from its sister clade.

RESULTS

Phylogenetic Affinities and Genetic Divergence

We obtained a final aligned data set of 3335 base pairs (bp) among mitochondrial and nuclear gene fragments: 16S (559 bp), COI (657 bp), CYTB (666 bp), POMC (608 bp), and RAG1 (845 bp). The best partition scheme included 12 partitions from the 13 divided a priori, with the first positions of both nuclear gene fragments treated as a single partition. The best partition scheme and corresponding best-fitting nucleotide substitution models used in both BI and ML analyses are presented in Table 2. The standard deviation of split frequencies was 0.005725, all ESS values were above 1000, and PSRF was between 0.999 and 1.003 for all parameters, indicating that the Bayesian analysis converged as expected.

Both BI and ML analyses yielded similar topologies, recovering the *Adenomera marmorata* clade with high support (ML bootstrap = 100%, Posterior Probability, PP = 1.0; Fig. 1). We recovered *A. ajurauna* and *Adenomera* sp. S with high support (ML bootstrap = 74%, PP = 0.98) as reciprocally monophyletic sister taxa, including their sympatric (but not syntopic) occurrence in one of the study areas (Miracatu; Figs. 1 and 2). The uncorrected *p* distances between *A. ajurauna* and *Adenomera* sp. S varied from 3.3 to 5.5% in 16S and 7.5 to 11.5% in COI (Table 3).

Adenomera ajurauna and *Adenomera* sp. S were not completely separated from each other in the two haplotype genealogies, because these two species share haplotypes in the two nuclear DNA genes (Fig. 2). The POMC genealogy contains two conserved haplotypes (putative ancestral

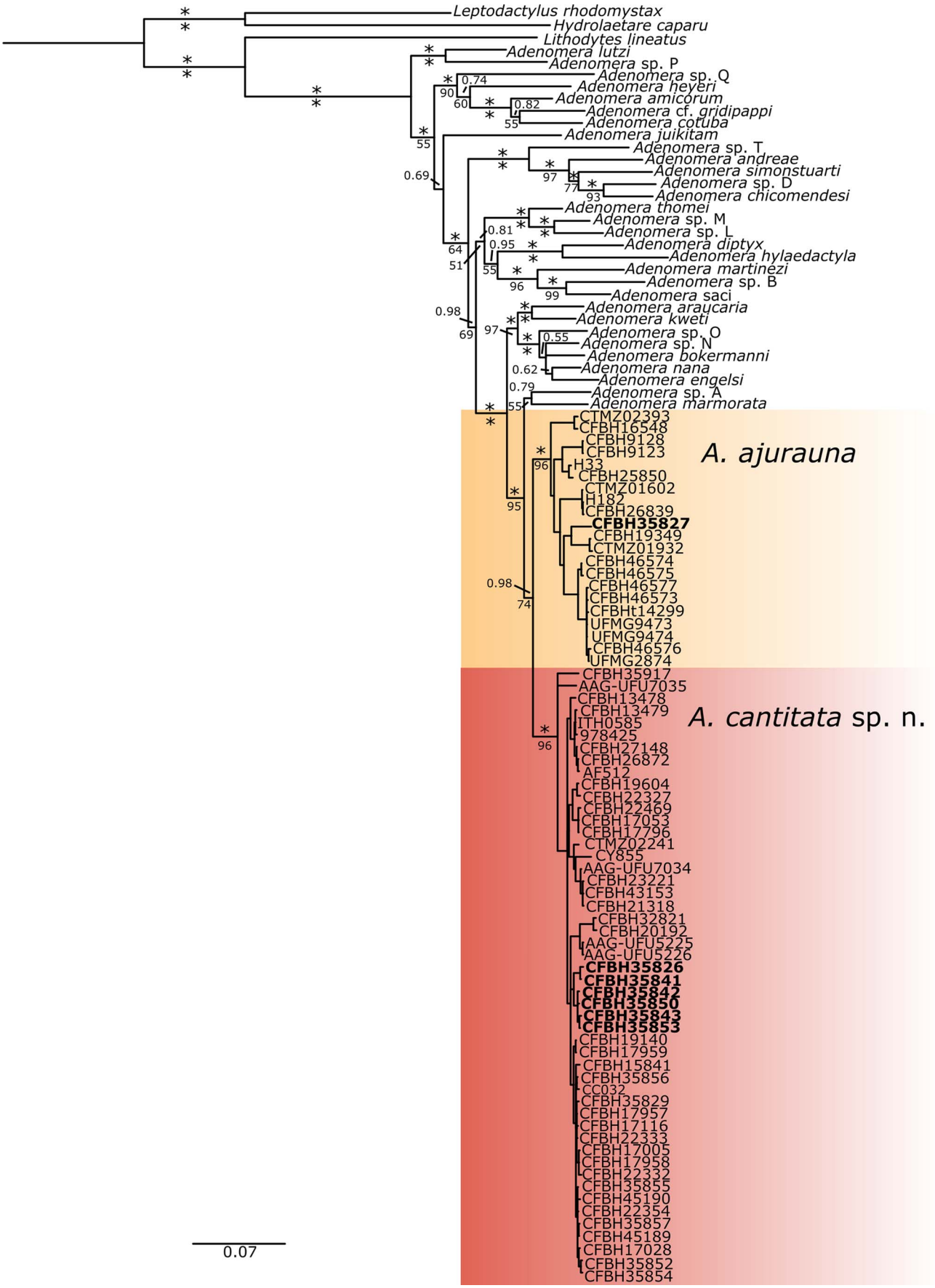

FIG. 1.—Relationships within *Adenomera* based on the 50% majority-rule consensus tree from Bayesian inference of the three mitochondrial (COI,

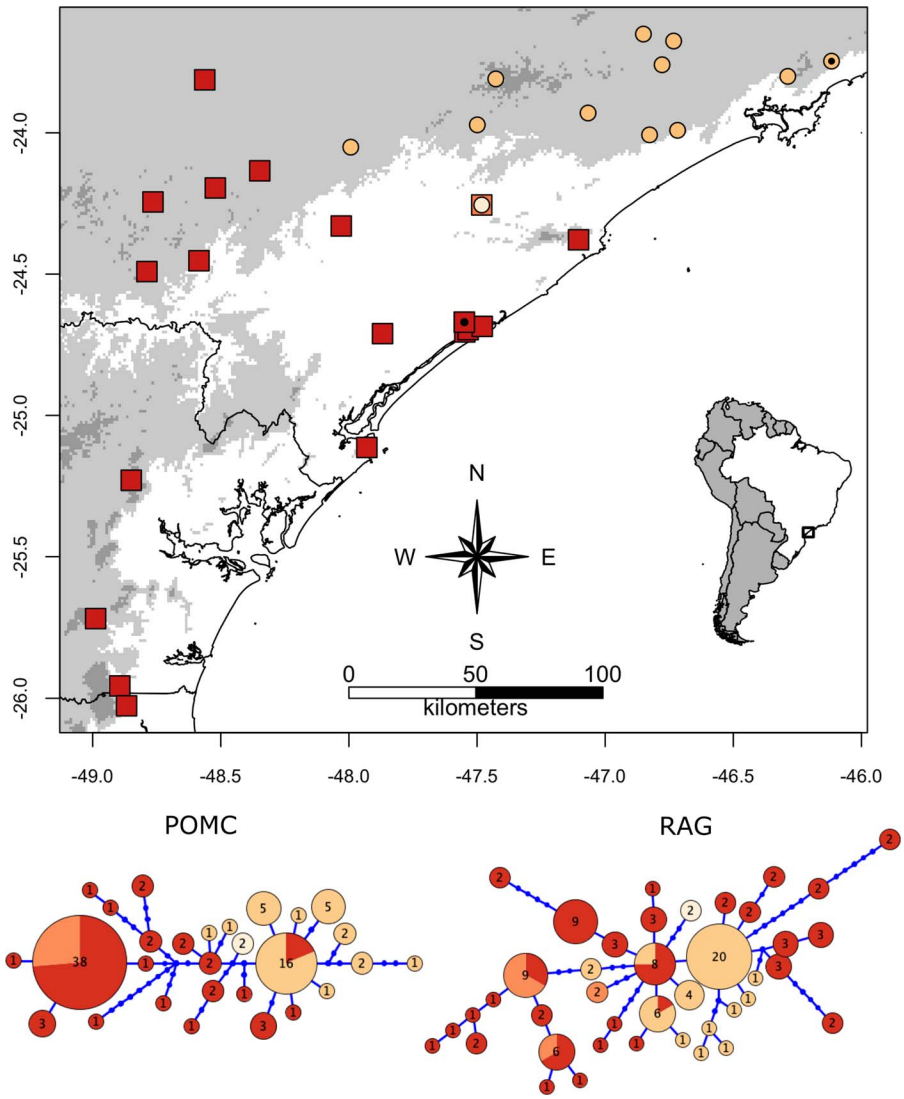

FIG. 2.—Above: Geographic distribution map of *Adenomera ajurauna* and *A. cantitata* sp. nov. in the Atlantic Forest of southeastern and southern Brazil. Red squares represent the occurrence records of *A. cantitata* sp. nov., yellow circles are those of *A. ajurauna*, and type localities are indicated by a black dot. Note the single case of sympatric occurrence between these two species indicated by a circle within a square and lighter colors. Areas above 500- and 1000-m elevation are shaded gray, and black lines correspond to Brazilian state limits. Below: Haplotype genealogies of the two nuclear genes, with the same color scheme of the map. Numbers within the circles represent the number of haplotypes.

alleles), one of these shared by the two species, and the network showed an overall trend towards splitting. In contrast, the admixture is evident in the RAG1 genealogy, with three haplotypes shared by the two species and most of the haplotypes from *Adenomera* sp. S deriving from *A. ajurauna*.

Morphology

The closely related *Adenomera* sp. S and *A. ajurauna* share a small SVL (Table 4) and toe tips expanded into discs (Figs. 3 and 4). *Adenomera ajurauna* generally has a homogeneous solid-brown coloration on the dorsum (Fig. 3D), whereas *Adenomera* sp. S has lighter gray or brown shades (Fig. 3A,B). However, a darker and more solid dorsal coloration can also be observed in specimens of *Adenomera* sp. S (Fig. 3C). Despite being infrequent, a dorsolateral stripe (Fig. 3A) is present in some specimens of *Adenomera* sp. S ($n = 6$ of 42 examined specimens). Such stripe was reported as absent in *A. ajurauna* (Berneck et al. 2008), also rarely observed in specimens of this species ($n = 2$ of 11 examined specimens). The flank of *A. ajurauna* often has darker brown blotches, whereas the flank of *Adenomera* sp. S generally has a marbled pattern

←
CYTB, and 16S) and two nuclear (POMC and RAG1) gene fragments. Numbers above branches are posterior probabilities and numbers below branches are maximum likelihood bootstrap replicates. Bootstrap below 50 and support within species are not shown. Asterisks (*) indicate maximum support. Sympatric individuals of *A. ajurauna* and *A. cantitata* sp. nov. are in bold. The scale bar represents the number of nucleotide substitutions per site.

TABLE 3.—Uncorrected pairwise distances (*p* distances, reported in %) within and among the eight species of the *Adenomera marmorata* clade, based on partial COI (lower diagonal) and partial 16S (upper diagonal) mitochondrial DNA genes. Distances between *A. ajurauna* and *A. cantitata* sp. nov. are highlighted in bold. Data are reported as min–max, when applicable.

	Within species		Among species							
p distance / Species	COI (*n*)	16S (*n*)	1	2	3	4	5	6	7	8
1. *A. ajurauna*	0.0 to 8.0 (19)	0.0 to 3.1 (8)		5.3 to 6.4	9.4 to 10.2	**3.3 to 5.5**	6.3 to 7.7	5.7 to 7.4	3.1 to 7.7	6.1 to 7.2
2. *A. araucaria*	0.0 to 9.8 (9)	— (1)	11.9 to 15.7		7.3	5.9 to 6.9	6.3	4.6	4.8 to 6.5	5.0 to 5.0
3. *A. bokermanni*	6.5 (2)	— (1)	11.1 to 15.6	10.6 to 16.7		9.1 to 10.4	7.1	5.5	7.3 to 9.1	3.6 to 3.6
4. *A. cantitata* sp. nov.	0.0 to 6.5 (39)	0.0 to 2.6 (13)	**7.5 to 11.5**	11.8 to 15.0	9.8 to 14.6		6.1 to 7.1	5.9 to 7.2	3.3 to 6.2	5.8 to 6.1
5. *A. engelsi*	0.5 to 3.6 (4)	— (1)	10.1 to 13.6	11.4 to 15.0	8.7 to 10.0	10.3 to 13.6		5.6	4.4 to 7.3	4.6 to 4.6
6. *A. kweti*	0.0 to 7.3 (8)	— (1)	11.5 to 14.9	9.8 to 12.1	11.1 to 14.4	11.0 to 15.0	10.5 to 12.4		5.0 to 8.0	4.8 to 4.8
7. *A. marmorata*	0.0 to 12.2 (146)	0.0 to 6.7 (39)	7.3 to 14.2	11.5 to 19.1	10.7 to 17.5	7.5 to 14.5	10.9 to 15.4	9.6 to 15.9		4.8 to 7.0
8. *A. nana*	3.6 to 4.6 (3)	— (1)	9.7 to 13.2	10.6 to 15.6	7.9 to 10.7	9.7 to 13.2	6.7 to 8.4	9.6 to 13.0	9.9 to 15.9	

with brown dots and speckles. Both species can also exhibit a homogeneously colored flank. Therefore, both dorsal and lateral color patterns are variable enough to prevent forming a distinguishing pattern (i.e., diagnostic character) between the two species.

Advertisement Call

The calls of *Adenomera* sp. S and *A. ajurauna* consist of single nonpulsed notes with upward frequency modulation and the dominant frequency coinciding with the fundamental harmonic (Fig. 5). The main differences between these species (allopatric populations) are related to two temporal traits: note duration and note repetition rate (Table 5). *Adenomera ajurauna* has a much longer note (151 to 285 ms; Fig. 5A) than that of *Adenomera* sp. S (19 to 89 ms; Fig. 5B). Also, notes of *A. ajurauna* are given at a slower repetition rate (17 to 36 per minute) when compared to those of *Adenomera* sp. S (36 to 213 per minute).

In Miracatu, the only sympatric occurrence known of *Adenomera ajurauna* and *Adenomera* sp. S, both species displayed the shortest values of note duration in comparison with allopatric populations (Table 5). The single male of *A. ajurauna* recorded from Miracatu was found to have a remarkably shorter note duration (82 to 97 ms) in comparison with allopatric populations ($n = 7$ males) of *A. ajurauna* (151 to 285 ms). Likewise, the two males of *Adenomera* sp. S recorded from Miracatu had shorter notes (10 to 29 ms) relative to the allopatric populations ($n = 19$ males) of *Adenomera* sp. S (19 to 89 ms). Therefore, both allopatric and sympatric populations of the two species can be distinguished from each other in note duration (Table 5), and sympatric populations can also differ in dominant frequency: *A. ajurauna* (4285 to 4802 Hz; $n = 1$ male) and *Adenomera* sp. S (5146 to 5405 Hz; $n = 2$ males).

Taxonomic Account

We recognize *Adenomera* populations assigned to *Adenomera* sp. S as an unnamed taxon distributed in southeastern and southern Brazil, based on the combined use of morphology, calls, and DNA sequences. Next, we name and describe these populations as a new species, sister to *A. ajurauna*.

Adenomera ***cantitata*** sp. nov.
Adenomera sp. III: Kwet (2006, 2007)
Adenomera sp. S: Fouquet et al. (2014)

Holotype.—CFBH 35852 (Field PPGT 474), adult male from Escola Técnica Estadual Engenheiro Agrônomo Narciso de Medeiros (24.670086°S, 47.549536°W; 4 m asl), municipality of Iguape, São Paulo State, Brazil, collected on 23 October 2013 by C.S. Cassini, P.P.G. Taucce, and T. Silva-Soares.

Paratypes.—Five adult females (CFBH 17028, 17116, 17959, 22333, and 22354) and two subadults (CFBH 15841 and 19140) from the type locality, collected by Juliana Zina: CFBH 15841 collected on 9 March 2007; CFBH 17028 collected on 15 June 2007; CFBH 17116 collected on 19 August 2007; CFBH 17959 collected on 1 November 2007; CFBH 19140 collected on 21 February 2008; CFBH 22333 collected on 24 October 2008; and CFBH 22354 collected on 10 November 2008. Seven adult males collected within the municipal limits of Iguape, São Paulo State, Brazil:

TABLE 4.—Values for morphometric traits (mm) for the type series of *Adenomera cantitata* sp. nov. and comparative specimens of *A. ajurauna*. Measurements are reported as mean ± SD (range). Abbreviations are defined in the Materials and Methods section. The two subadult paratypes (CFBH 15841 and 19140) were excluded from the morphometric analysis. The measured males of *A. ajurauna* are CFBH 35827 (sympatric occurrence with *A. cantitata* sp. nov.) and CFBH 46575–7 (topotypes). See Appendix I for locality data.

	A. cantitata sp. nov.			*A. ajurauna*
Trait	Holotype (CFBH 35852)	Male paratypes, $n = 7$	Female paratypes, $n = 5$	Males, $n = 4$
SVL	18.8	17.9 ± 1.0 (16.4 to 19.3)	20.1 ± 0.3 (19.7 to 20.4)	18.7 ± 0.9 (17.7 to 19.8)
HL	6.6	6.4 ± 0.4 (5.7 to 6.9)	6.6 ± 0.1 (6.6 to 6.7)	6.1 ± 0.4 (5.7 to 6.7)
HW	6.9	6.8 ± 0.3 (6.5 to 7.3)	7.3 ± 0.2 (7.0 to 7.4)	7.0 ± 0.2 (6.8 to 7.3)
ED	1.5	1.7 ± 0.1 (1.5 to 1.9)	1.8 ± 0.1 (1.7 to 1.9)	1.6 ± 0.2 (1.4 to 1.7)
TD	0.9	0.9 ± 0.1 (0.8 to 0.9)	1.1 ± 0.1 (1.0 to 1.1)	1.0 ± 0.1 (0.9 to 1.0)
EN	1.5	1.4 ± 0.2 (1.2 to 1.6)	1.6 ± 0.1 (1.5 to 1.7)	1.4 ± 0.3 (1.1 to 1.6)
IND	1.7	1.6 ± 0.1 (1.5 to 1.8)	1.8 ± 0.1 (1.7 to 1.9)	1.7 ± 0.1 (1.6 to 1.7)
HAL	4.4	4.1 ± 0.3 (3.7 to 4.5)	4.0 ± 0.2 (3.7 to 4.1)	4.0 ± 0.3 (3.7 to 4.4)
THL	8.4	8.0 ± 0.4 (7.5 to 8.6)	9.2 ± 0.3 (8.9 to 9.5)	7.8 ± 0.4 (7.4 to 8.3)
TL	8.3	8.4 ± 0.3 (8.0 to 9.0)	9.7 ± 0.2 (9.4 to 10.0)	8.2 ± 0.3 (7.8 to 8.5)
FL	9.5	9.2 ± 0.4 (8.6 to 9.9)	9.9 ± 0.3 (9.5 to 10.2)	9.0 ± 0.7 (8.5 to 10.0)

CFBH 35829 and 35854–7 from Mirante do Cristo (24.704684°S, 47.547765°W; 68 m asl), with the same collection date and collectors of the holotype; CFBH 45189, from Restaurante do Engenho (24.696640°S, 47.534713°W; 14 m asl) on 3 December 2011 by C.S. Cassini, C. Canedo, V. Trevine, and L.R. Malagoli; CFBH 45190, from Vila Barra do Icapara (24.685034°S, 47.479579°W; 58 m asl) on 4 December 2011 by C.S. Cassini, C. Canedo, V. Trevine, and L.R. Malagoli.

Additional material.—Specimens collected in southeastern and southern Brazil. Molecular vouchers collected in SP: one male (CFBH 13479) and one female (CFBH 13478) from Guapiara; two females (CFBH 17957–8) from Pariquera-Açu; two males (CFBH 22468–9), two females (CFBH 17053 and 22327), and one juvenile (CFBH 35917) from Cananeia; three males (CFBH 35826, 35850, and 35853) and three females (CFBH 35841–3) from Miracatu; two females (CFBH 26872 and 27148) from Ribeirão Grande. Molecular vouchers collected in PR: one male (CFBH 21318) and one juvenile (CFBH 23221) from Guaratuba, one male (AAG-UFU 7034; call voucher) from São José dos Pinhais, and one female (AAG-UFU 7035) from Campina Grande do Sul. Vouchers of call recordings collected in SP: four males from Apiaí (AAG-UFU 5225–8; the first two specimens are also molecular vouchers). Voucher of call recording collected in PR: one male from Guaratuba (MCP 7689). Voucher of call recording (and also a molecular voucher) collected in SC: one male from Garuva (CFBH 43153).

Diagnosis.—*Adenomera cantitata* is recognized within *Adenomera* by the following combination of phenotypic traits: (1) small size of adult males (SVL = 16.4 to 19.5 mm); (2) antebrachial tubercle absent; (3) toe tips expanded into discs; (4) belly cream-colored in life; (5) robust body shape;

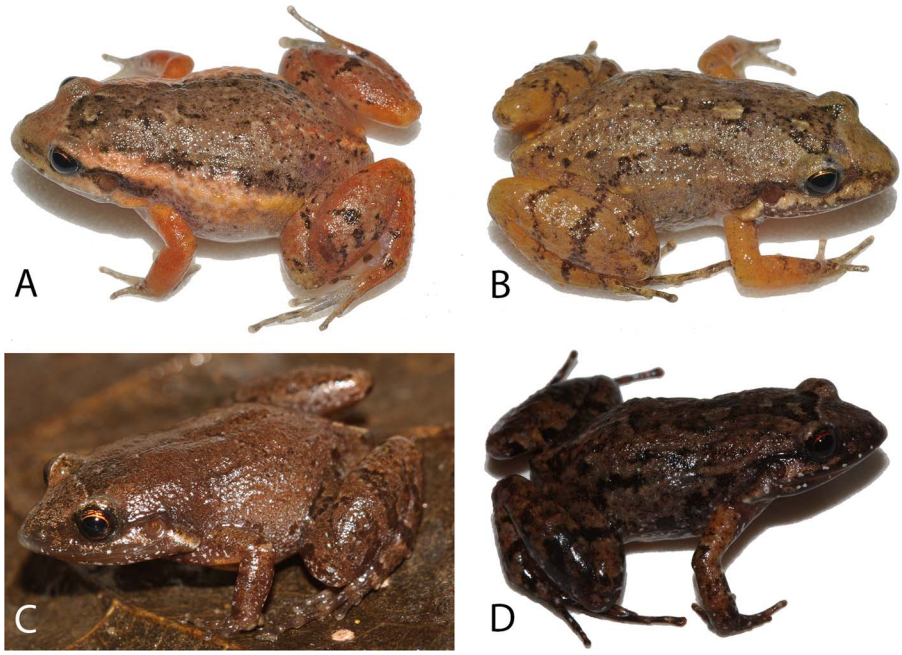

FIG. 3.—Life colors of *Adenomera cantitata* sp. nov. (A to C) and *A. ajurauna* (D). Two chromotypes associated with a pale-colored dorsum: (A) AAG-UFU 5525, dorsolateral stripe present; (B) AAG-UFU 5526, dorsolateral stripe absent. Photographs in A and B by A.A. Giaretta. (C) CFBH 43153 and (D) AAG-UFU 5024, dark-colored dorsum and dorsolateral stripe absent.

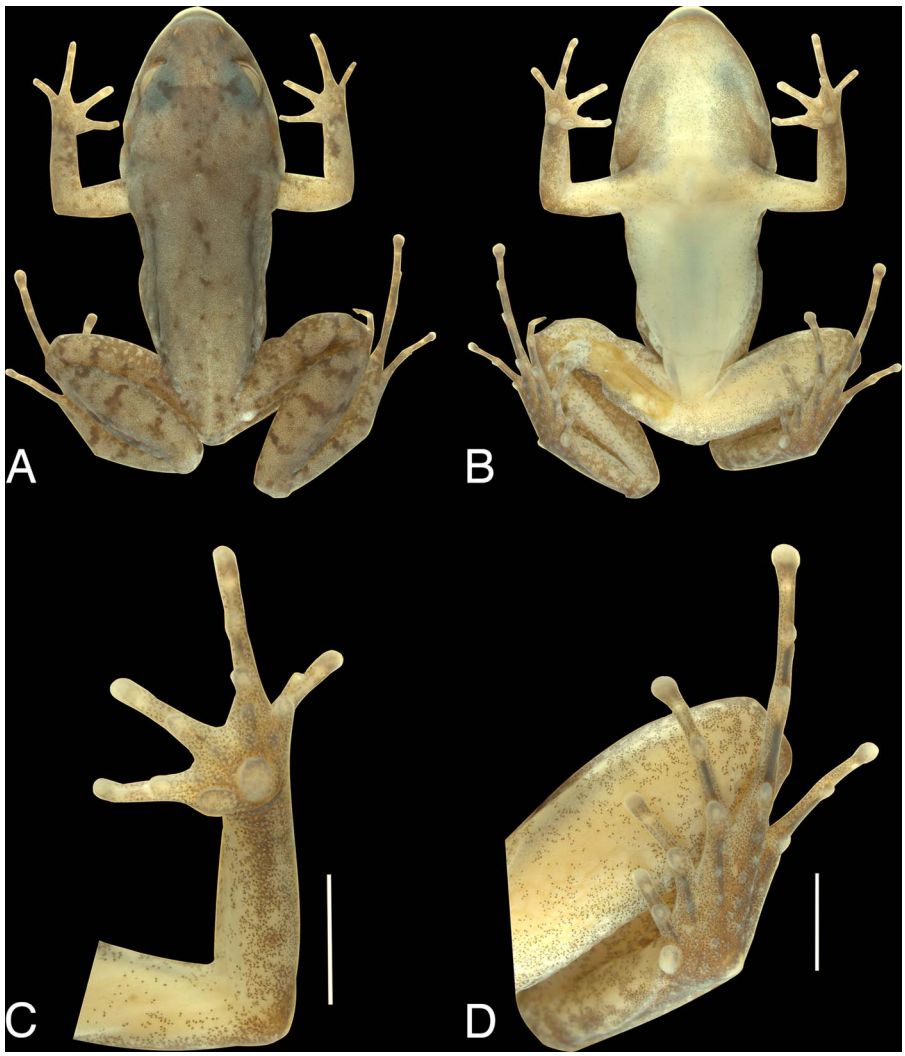

FIG. 4.—(A, B) Dorsal and ventral body, (C) hand, and (D) foot of the preserved holotype of *Adenomera cantitata* sp. nov. (male, CFBH 35852: SVL = 18.8 mm). Scale bars = 2 mm.

(6) vertebral pinstripe absent or indistinct; (7) advertisement call composed of a single nonpulsed note; (8) note dominant frequency (3961 to 5749 Hz) coinciding with the fundamental harmonic; (9) short note duration (10 to 89 ms).

Morphological comparisons with congeners.—*Adenomera cantitata* has small adults for the genus, with SVL varying from 16.4 to 19.3 mm in the 8 specimens composing the species' type series (Table 4) and from 16.9 to 19.5 mm in 14 other sequenced males to the species (listed in the Additional material section). The new species can be distinguished from three of four species of the Amazonian endemic *A. andreae* clade by the smaller size of adult males (SVL = 16.4 to 19.5 mm, $n = 22$), in comparison with those of *A. albarena* (SVL = 21.2 to 23.0 mm, $n = 21$; Martins et al. 2024), *A. chicomendesi* (SVL = 21.3 to 24.0 mm, $n = 13$; Carvalho et al. 2019b), *A. guarayo* (SVL = 19.5 to 22.6 mm, $n = 10$; Carvalho et al. 2020c), and *A. simonstuarti* (SVL = 19.5 to 22.6 mm, $n = 10$; Carvalho et al. 2020a). The new species does not differ consistently from *A. andreae* in any of the analyzed morphological traits, but they are clearly distinguished from each other in acoustic traits (see below).

Adenomera cantitata is distinguished from the two species belonging to the *A. lutzi* clade by the smaller size of adult males (SVL = 16.4 to 19.5 mm, $n = 22$), in comparison with those of *A. glauciae* (SVL = 27.0 to 34.0 mm, $n = 14$; Carvalho et al. 2020b) and *A. lutzi* (SVL = 25.7 to 33.5 mm, $n = 26$; Kok et al. 2007). The new species is also distinguished by lacking an antebrachial tubercle, which is present in both species of the *A. lutzi* clade (Kok et al. 2007; Carvalho et al. 2020b).

Adenomera cantitata is distinguished from 8 of 10 species belonging to the *A. heyeri* clade by lacking an antebrachial tubercle, which is present in *A. amicorum*, *A. aurantiaca*, *A. cotuba*, *A. inopinata*, *A. gridipappi*, *A. kayapo*, *A. phonotriccus*, and *A. tapajonica* (Carvalho et al. 2021). From *A. heyeri* and *A. juikitam*, which also lack the antebrachial tubercle, the new species is distinguished by having toe tips expanded into discs (not expanded or slightly expanded in *A. juikitam*; Carvalho and Giaretta 2013a) and the belly cream-colored in life (yellow-colored in life in *A. heyeri*; Boistel et al. 2006).

Adenomera cantitata is distinguished from all five open-habitat species distributed across the South American Dry Diagonal and the Bolivian Yungas (*A. hylaedactyla* and

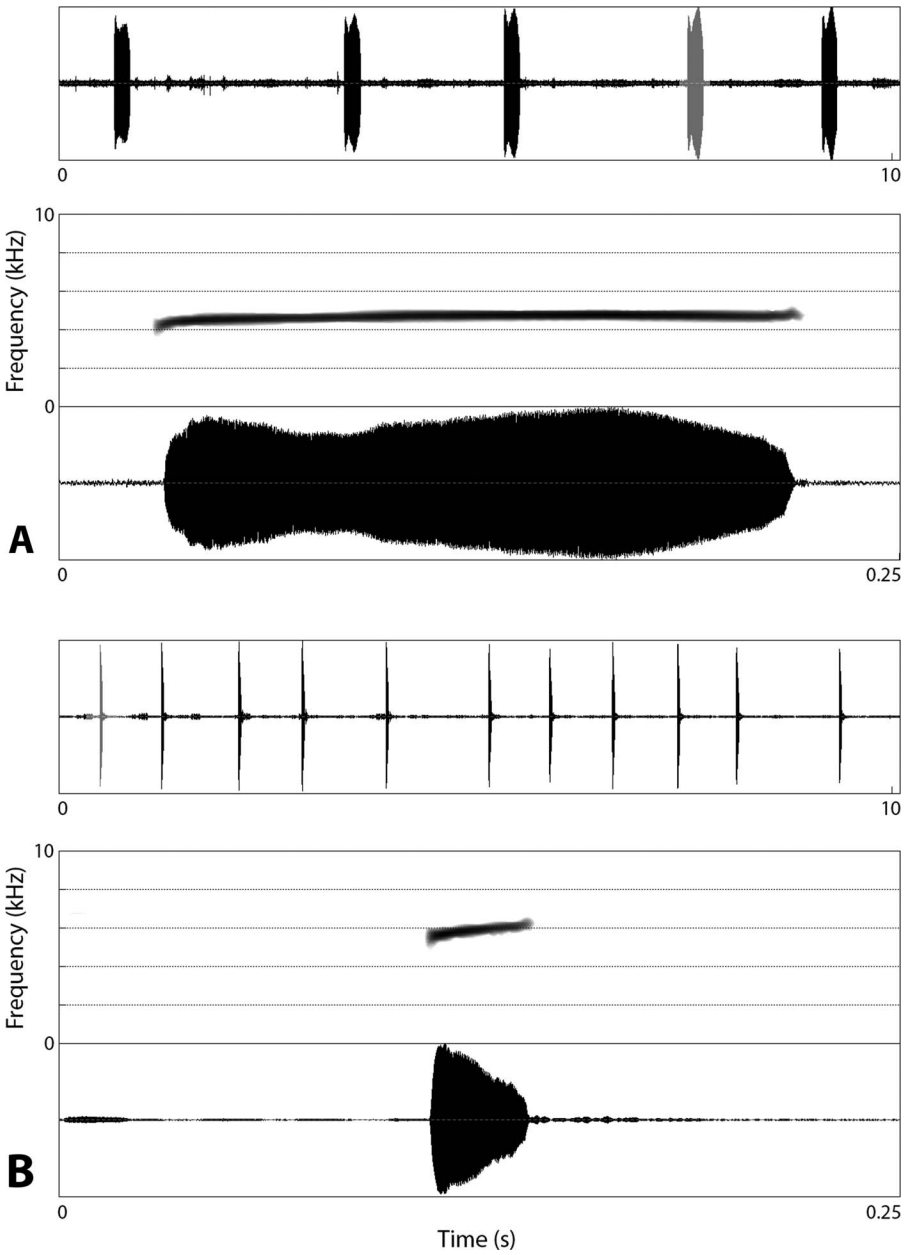

FIG. 5.—Advertisement calls of (A) *Adenomera ajurauna* (voucher AAG-UFU 5024) and (B) *A. cantitata* sp. nov. (voucher CFBH 35852, the holotype). Oscillogram sections (10 s along the x-axis), and spectrogram and oscillogram (250 ms along the x-axis and 10 kHz along the y-axis) detailing one call (highlighted in gray). See Appendix II for locality data.

A. martinezi sister clades) by having toe tips expanded into discs (not expanded in *A. coca*, *A. diptyx*, *A. guarani*, *A. martinezi*, and *A. saci*, and not expanded or slightly expanded in *A. hylaedactyla*; Angulo and Reichle 2008; Carvalho and Giaretta 2013b; Carvalho et al. 2019a; Zaracho et al. 2023). The new species is also distinguished by lacking a conspicuous vertebral pinstripe, which is present in *A. martinezi* and *A. saci* as a complete stripe (Carvalho and Giaretta 2013b), and generally present in *A. diptyx*, *A. guarani*, and *A. hylaedactyla* as an incomplete stripe extending to the posterior half of the body length (Carvalho et al. 2019a; Zaracho et al. 2023). *Adenomera cantitata* has a robust body, as opposed to the slender body of *A. diptyx*, *A. martinezi*, and *A. saci* (Carvalho and Giaretta 2013b; Zaracho et al. 2023).

Adenomera cantitata is distinguished from *A. thomei* (not part of other *Adenomera* clades) by having toe tips expanded into discs (not expanded or slightly expanded in *A. thomei*; Carvalho and Giaretta 2013a). From the other seven species of its own clade (i.e., the Atlantic Forest endemic *A. marmorata* clade), *A. cantitata* is distinguished from *A. bokermanni* (21.2 to 24.3 mm, $n = 18$; specimens listed in Appendix I) and *A. engelsi* (20.9 to 22.7 mm; $n = 12$; Kwet et al. 2009) by having small adult males (SVL = 16.4 to 19.5 mm; $n = 22$). The new species is also distinguished by having toe tips expanded into discs, whereas they are unexpanded or slightly expanded in *A. araucaria*, *A. bokermanni*, *A. engelsi*, and *A. kweti* (Carvalho et al. 2019d). *Adenomera cantitata* (SVL = 16.4 to 19.5 mm; $n = 22$) cannot be consistently distinguished from *A. ajurauna* (SVL = 17.2 to 20.0 mm,

TABLE 5.—Advertisement call data of *Adenomera ajurauna* and *A. cantitata* sp. nov. in the Atlantic Forest of southeastern and southern Brazil, including the single known sympatric occurrence of both species in Miracatu (São Paulo State). See Appendix II for locality data. Values are presented as mean ± SD (range).

Call traits (allopatric populations)	*A. ajurauna*, $n = 7$ males	*A. cantitata* sp. nov., $n = 19$ males
Note duration (ms)	202.9 ± 34.7 (151 to 285), $n = 161$	51.0 ± 19.6 (19 to 89), $n = 679$
Repetition rate (notes per minute)	25.7 ± 6.3 (17 to 36), $n = 7$	80.5 ± 48.3 (36 to 213), $n = 19$
Relative rise time (% note duration)	46.3 ± 16.5 (2 to 82), $n = 161$	19.1 ± 12.0 (3 to 69), $n = 679$
Dominant frequency (,)	4564.4 ± 82.0 (4328 to 4716), $n = 161$	4701.9 ± 479.5 (3961 to 5749), $n = 679$
Frequency modulation (Hz)	458.6 ± 130.4 (215 to 991), $n = 161$	457.8 ± 206.5 (86 to 938), $n = 679$
Call traits (sympatric population)	***A. ajurauna*, $n = 1$ male**	***A. cantitata* sp. nov., $n = 2$ males**
Note duration (ms)	85.9 ± 2.7 (82 to 97), $n = 22$	20.0 ± 7.0 (10 to 29), $n = 103$
Repetition rate (notes per minute)	26.8, $n = 1$	77.1 ± 0.5 (76 to 77), $n = 2$
Relative rise time (% note duration)	35.9 ± 16.8 (13 to 67), $n = 22$	12.5 ± 5.0 (5 to 34), $n = 103$
Dominant frequency (Hz)	4502.4 ± 215.3 (4285 to 4802), $n = 22$	5296.3 ± 83.4 (5146 to 5405), $n = 103$
Frequency modulation (Hz)	665.6 ± 47.4 (603 to 818), $n = 22$	145.8 ± 64.4 (43 to 258), $n = 103$

$n = 7$; Berneck et al. 2008), *A. marmorata* (SVL = 18.0 to 25.6 mm, $n = 26$; specimens listed in Appendix I), and *A. nana* (SVL = 16.3 to 19.4 mm, $n = 12$; Kwet 2007) in adult male size or any of the other analyzed morphological traits, but see the next section for an acoustic diagnosis.

Acoustic comparisons with congeners.—*Adenomera cantitata* is distinguished by a nonpulsed call (Fig. 5B), in comparison with the pulsed calls of all species belonging to the *A. andreae* clade (Carvalho et al. 2020c; Martins et al. 2024), *A. heyeri* clade (Carvalho et al. 2021), *A. hylaedactyla* clade (Carvalho et al. 2019a; Zaracho et al. 2023), in addition to *A. thomei* (Almeida and Angulo 2006). From the two species of the *A. lutzi* clade, *A. cantitata* is distinguished by having a single-note call, in comparison with the multinote call of *A. glauciae* (Carvalho et al. 2020b). The new species is distinguished from *A. lutzi* by a higher dominant frequency (3961 to 5749 Hz), which coincides with the fundamental harmonic, whereas the dominant frequency of *A. lutzi* (3252 to 3682 Hz) coincides with the second harmonic (Carvalho et al. 2020b). From the two species of the *A. martinezi* clade, *A. cantitata* is distinguished by having a nonpulsed call, in comparison with the pulsed call of *A. martinezi* (Carvalho and Giaretta 2013b). The new species is distinguished from *A. saci* by a much higher fundamental harmonic, with the frequency peaking from 3961 to 5749 Hz, whereas the fundamental harmonic of *A. saci* peaks from 1690 to 2250 Hz (Carvalho and Giaretta 2013b).

From the other species of the *Adenomera marmorata* clade, *A. cantitata* is distinguished by a nonpulsed call (pulsed call in *A. araucaria*; Kwet and Angulo 2002) and the dominant frequency coinciding with the fundamental harmonic (coincident with the second harmonic in *A. bokermanni*, *A. engelsi*, *A. kweti*, and *A. nana*; Kwet 2007; Kwet et al. 2009; Carvalho et al. 2019d). The high acoustic variation within *A. marmorata* contains that of the allopatric *A. cantitata* (Cassini et al. 2020, their table 3), but these two species do not share an immediate common ancestor, with *A. cantitata* being the sister taxon to *A. ajurauna* (Fig. 1). This makes *A. cantitata* and *A. marmorata* phenotypically indistinguishable but geographically distinct species in the Atlantic Forest. Regarding the allopatric populations of *A. cantitata* and *A. ajurauna* (Table 5; Fig. 5), they can be distinguished from each other in note duration: *A. cantitata* (19 to 89 ms) and *A. ajurauna* (151 to 285 ms). The only recorded male of *A. ajurauna* in sympatric occurrence with *A. cantitata* overlaps in the value range of note duration when compared to the entire value range of *A. cantitata* (Table 5). However, the value ranges of note duration only between the sympatric populations of *A. cantitata* (10 to 29 ms) and *A. ajurauna* (82 to 97 ms) do not overlap with each other, thus indicating that note duration is a distinguishing feature between the two species in their contact zone in Miracatu (Fig. 6). Sympatric males (but not observed in allopatry; see Table 5) of these two species also differ in the dominant frequency: *A. cantitata* (5146 to 5405 Hz) and *A. ajurauna* (4285 to 4802 Hz).

Description of holotype (Fig. 4).—Snout subovoid in dorsal view, acuminate in lateral view; nostril rounded and nonprotruding, positioned dorsolaterally, closer to the tip of the snout than to the eye; canthus rostralis indistinct; loreal region oblique, straight. Tip of the snout with a horizontal ridge. Tympanic membrane and annulus present; supratympanic fold extending from the posterior corner of the eye, passing over the dorsal edge of the tympanic annulus, to the base of the arm; postcommissural gland elliptical. Dorsum and limbs mostly smooth, with a few warts on the flank and inguinal region. Dorsolateral fold running from the scapular region to the groin. Vocal sac subgular, barely expanded laterally. Ventral surfaces smooth, granular between the underside of the thigh and cloaca. Fringes and ridges on fingers absent. Relative finger lengths IV < I ~ II < III; fingertips not expanded; subarticular tubercles single, nearly rounded; external metacarpal tubercle nearly rounded, covering half of the carpal region; internal metacarpal tubercle elliptical, half the maximum width of the external metacarpal tubercle; few and poorly developed supernumerary tubercles on the palm of the hand. Tubercles on the dorsal surface of hindlimbs absent, present on the posterior surface of the tarsus. Paracloacal gland rounded. Fringing and webbing on toes absent; relative toe lengths I < II < V < III < IV; tips of the toes III and IV fully expanded (character State D), the tip of toes II and V swollen (character State C), tip of Toe I unexpanded (character State B). Tarsal fold extending from the posterior edge of the inner metatarsal tubercle, almost reaching the heel. Subarticular tubercles nearly rounded or elliptical; internal metatarsal tubercle elliptical; external metatarsal tubercle rounded; supernumerary tubercles small and low on the sole of the foot. Morphometric measurements of the holotype are presented in Table 4.

Color of holotype in preservative (Fig. 4).—Snout tip with a faded white coloration (coincident with the fleshy ridge). Upper lip pale speckled. Tympanum light brown,

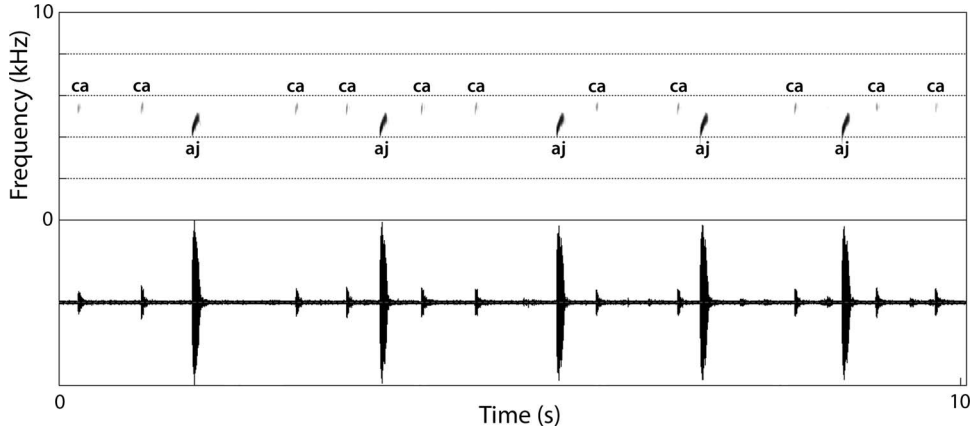

FIG. 6.—A 10-s section containing calls of *Adenomera ajurauna* ("aj") and *A. cantitata* sp. nov. ("ca") from Miracatu (São Paulo State, southeastern Brazil), the single locality of sympatric occurrence record confirmed for these two closely related *Adenomera* species. Calls of *A. ajurauna* (voucher CFBH 35827) were recorded from inside the forest and background calls of *A. cantitata* sp. nov. from the forest border and adjacent open area.

covered with dots off-white and outlined in brown color. Postcommissural gland off-white, covered with dark melanophores. Supratympanic and dorsolateral folds coincident with dark brown stripes. Interorbital blotch as an inverted triangle. Dorsolateral stripe absent. Dorsum and flank grayish brown; dorsum with brown speckles irregularly distributed; flank with small scattered dark brown dots and blotches in contact with the belly. Hindlimbs with transverse dark brown bars. Throat and ventral surface of limbs covered with melanophores, with greater density laterally on the throat. Sparse melanophores on the chest and the belly (laterally). Abdominal region homogeneously cream.

Variation.—The type series is generally homogeneous in morphology and coloration. There are dimorphic differences related to snout shape from above (subovoid in males, rounded in females) and in profile (acuminate in males, rounded in females), which is observed in all *Adenomera* species. Also, females (SVL = 19.7 to 20.4 mm; $n = 5$) are larger than males (SVL = 16.4 to 19.3 mm; $n = 8$). The amount of blotches, dots, and bars forming the dorsal color pattern varies from an almost completely homogeneous coloration to strongly blotched patterns on the background dorsal coloration. Among the specimens with homogeneous coloration, most specimens have a medium or light brown dorsum and only two specimens have a solid-dark brown dorsum (CFBH 26771 and 43153). A dorsolateral stripe is absent in the 15 type specimens, but is present in 6 of 27 examined additional specimens (AAG-UFU 5225, 5227–8, CFBH 17957–8, and 22327). Internal features of the mouth were assessed from the paratype CFBH 45190: vomerine teeth as a straight row posterior and medial to the choana; tongue oval, free at its posterior third length.

Etymology.—The specific epithet *cantitata* is derived from Latin and refers to the feminine nominative case of cantitatus, which means "sing often, repeatedly" in allusion to the vocalization of the species, which can be commonly heard in urban gardens, disturbed areas, and at forest borders in urban and rural areas.

Advertisement call.—The quantitative characterization of the call is derived from 782 calls of 21 males. Sample sizes for each acoustic trait and descriptive statistics, that is, means and standard deviations, are provided in Table 5. The call of *Adenomera cantitata* (Fig. 5B) consists of a single nonpulsed note with a duration varying from 10 to 89 ms, emitted at a relatively fast rate of 36 to 213 per minute. The rise time varies from 3 to 69% of note duration. Notes have an upward frequency modulation throughout their duration, ranging from 43 to 938 Hz. The dominant frequency coincides with the fundamental harmonic, ranging from 3961 to 5749 Hz.

Remarks on *Adenomera* call descriptions from southern Brazil.—Kwet (2006, 2007) was the first to describe the call of *Adenomera cantitata*, referred to at that time as *Adenomera* sp. III from Guaratuba, in the southern limit of the state of Paraná and on the border with the state of Santa Catarina. We obtained DNA sequences from Guaratuba and calls from Garuva, an adjacent area in Santa Catarina (Fig. 2), and found that molecular and acoustic data from this region in southern Brazil match the data analyzed from the remaining populations of *A. cantitata*.

Habitat and distribution.—*Adenomera cantitata* is commonly found in anthropized areas of forest borders and open areas, including urban sites, such as residential areas, public gardens, and sightseeing touristic monuments. Males vocalize on the ground (exposed) or underneath the leaf litter. Calling activity begins in the last hours of the day and goes into the first hours of the night, peaking around the twilight time. *Adenomera cantitata* is endemic to the Atlantic Forest of southern and southeastern Brazil. The species is distributed in two ecoregions: the Serra do Mar coastal forests, which correspond to most of its geographic range, and more inland areas corresponding to the Araucaria moist forests. *Adenomera cantitata* is partially sympatric with other three *Adenomera* species. *Adenomera cantitata* is sympatric with its sister species, *A. ajurauna*, in a single locality in the state of São Paulo (Miracatu; Fig. 2). These two species are ecologically segregated by the use of human-disturbed, open areas (*A. cantitata*) and inside the forest (*A. ajurauna*). In one locality in southern Brazil (Garuva), *A. cantitata* is sympatric with *A. bokermanni*. In this case, *A. bokermanni* was the species using open areas, whereas *A. cantitata* was found a few meters into a small forest stretch. In the same locality, *A. cantitata* and *A. nana* were found calling syntopically inside this fragment.

DISCUSSION

Our phylogenetic analyses recovered *Adenomera ajurauna* and *A. cantitata* as sister species, in agreement with

previous studies (e.g., Fouquet et al. 2014; Carvalho et al. 2019d; Cassini et al. 2020). These species display remarkable divergence in mtDNA and acoustic traits. Furthermore, they are allopatric throughout most of their geographic ranges in the Atlantic Forest. *Adenomera ajurauna* is distributed to the north and *A. cantitata* to the south and were found to be co-distributed in a single locality. In allopatric populations, the note duration is a diagnostic trait, with *A. ajurauna* producing much longer notes (151 to 285 ms) compared to *A. cantitata* (19 to 89 ms). However, when considering the recorded male of *A. ajurauna* sympatric with *A. cantitata*, there is a marginal overlap with the longest durations of analyzed notes of *A. cantitata* (Table 5). Conversely, if we consider only sympatric populations of both species, *A. cantitata* has the shortest note durations for the species (10 to 29 ms), which do not overlap with the shortest notes produced by *A. ajurauna* (82 to 97 ms).

Additionally, the sympatric males of both species differ in the dominant frequency of their calls, which is not observed in allopatric populations. One plausible explanation for the species differentiation observed in the sympatric zone is the reproductive character displacement hypothesis (RCD; Brown and Wilson 1956; Grant 1972), which posits that natural selection can lead to changes in character states in response to the presence of reproductively similar species within the same environment. Alternatively, differences in call frequency could be explained by the size–frequency inverse relationship (Fletcher 2007), which is the case of these species: the only male of *A. ajurauna* collected from Miracatu has a larger SVL (19.8 mm) than all males of *Adenomera* sp. S (17.2 to 18.4 mm; $n = 4$) collected from the same locality.

We observed that allopatric populations and the single known sympatric population of these species use different habitats. *Adenomera ajurauna* is a forest dweller, whereas *A. cantitata* inhabits human-altered, open areas (e.g., urban gardens and plantations). Although the two species do not use the same habitat, we were able to hear both species calling at a short distance from each other in that locality (see Fig. 6). Although some studies support the RCD hypothesis or suggest it as a driver of call variation in frogs (e.g., Gerhardt 1994; Lemmon 2009; Garey et al. 2018), others indicate that environmental factors may play a more prominent role (Mendoza-Henao et al. 2023). It is worth mentioning that populations of *A. marmorata* belonging to the clade J (sensu Cassini et al. 2020) are sympatric with *A. ajurauna* in the state of São Paulo, and this *A. marmorata* clade produces the shortest note durations in the variation reported for the species (Cassini et al. 2020), as opposed to long note duration produced by *A. ajurauna*. This observation might indicate that the RCD hypothesis play a role in acoustic variation between *Adenomera* species with sympatric occurrence.

Regarding our sampling of nuclear DNA genes, the haplotype networks demonstrate a significant level of differentiation, yet *Adenomera ajurauna* and *A. cantitata* share a few alleles in both of them (see Fig. 2). Although accurately determining the precise scenario leading to allele sharing is challenging, we can consider three plausible hypotheses. The first hypothesis suggests ongoing hybridization between the two species, with their contact zone representing a hybrid zone. The intermediate note duration values observed in the sympatric male of *A. ajurauna* (82 to 97 ms) may support this hypothesis (allopatric *A. cantitata* = 19 to 89 ms; allopatric *A. ajurauna* = 151 to 285 ms). However, the substantial divergence in acoustic traits between the two species in sympatry and the absence of allele sharing within the contact zone render this scenario less likely. Furthermore, the few shared alleles appear as more frequent haplotypes and occupy central positions within the networks, suggesting they are the oldest (Posada and Crandall 2001).

This leads us to consider two alternative hypotheses: past admixture and incomplete lineage sorting (ILS). Based solely on our current genetic and phenotypic data, we are unable to differentiate between these two scenarios. In the past-admixture scenario, the two species may have come into contact at some point, leading to the exchange of genetic material, which was subsequently interrupted by geographic isolation. The latter scenario (ILS) occurs when genetic divergence is relatively recent and insufficient time has elapsed for complete lineage sorting. As a result, ancestral polymorphisms or shared ancestral variation can persist (Avise et al. 1983; Maddison 1997).

Adenomera ajurauna and *A. cantitata* inhabit distinct biogeographic regions within the Atlantic Forest, in southeastern and southern Brazil, respectively. These regions are historically divergent in terms of climate and topography and serve as endemism zones for anuran species (Vasconcelos et al. 2014). The current contact zone between these species is geographically linked to the borders of these biogeographic regions, suggesting that both species have been subjected to different environmental conditions over time. It seems unlikely, at least in the recent past, that they were in contact. Moreover, compared to other species within the genus, the speciation event that originated *A. ajurauna* and *A. cantitata* is relatively recent and has probably occurred after some important intraspecific divergence events, such as those within *A. marmorata* (Fouquet et al. 2014). Therefore, we consider the ILS hypothesis as a more plausible explanation for the allele sharing between the two species. Future research focusing on the sympatric area and additional nuclear DNA markers can provide an opportunity to learn more about speciation mechanisms in the Atlantic Forest.

The discovery and formal description of *Adenomera cantitata* bring an interesting look to urban areas, to which an equivocal perspective of biodiversity-poor areas is often attributed (McIntyre et al. 2001; Yamaguchi 2004, 2005). Urban areas are susceptible to constant human activities and represent greatly modified areas, where nonnative species often dominate the landscape. These areas are manipulated to fulfill new purposes, such as activities related to tourism, agriculture, and gardening (McIntyre et al. 2001). These environments are often neglected in biodiversity assessment studies, even though new species have recently been described from urban sites. Some striking examples are those of *Lithobates kauffeldi* (Feinberg et al. 2014), a leopard frog described in the 2010s from New York City (Feinberg et al. 2014), one of the largest urban centers in the world, and *Hylarana urbis* Biju et al. 2014, a golden-backed frog from urban areas in India's Western Ghats (Biju et al. 2014). In the Brazilian Atlantic Forest, an emblematic example is that of *Dendropsophus nekronastes*, only known from two anthropized areas close to Cemitério de Almadina, a graveyard next to Almadina, a small town in the state of Bahia (Dias et al. 2017). Species discoveries also take place in smaller urban areas, such as the newly described *A. cantitata*, distributed in human-altered areas of

small towns in southern and southeastern Brazil that were originally covered by the Atlantic Forest.

In a 13-yr Citizen Science Program survey in Australia, Westgate et al. (2015) found that urban wetland areas have low but stable anuran species richness. The effects of urbanization strongly influence population trajectories, with wetland vegetation structure and canopy cover in the surrounding city landscapes as important elements for the management and rehabilitation of urban gardens and wetlands (Smallbone et al. 2011; Westgate et al. 2015). Future studies on the phenology of natural populations of anurans in urban areas in Brazil can be a good strategy to understand the minimum requirements for their existence and viability, and future conservation planning in these areas. This is especially relevant in Brazil, the world's richest country in biodiversity levels of several taxonomic groups. At the same time, Brazil is facing a rampant loss of natural areas and wildlife, as well as the ecological degradation of small fragments minimally viable for the maintenance of the anuran species occurring in urban and rural areas throughout the country.

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RESUMO: A Mata Atlântica é reconhecida como um *hotspot* de biodiversidade devido à sua alta riqueza de espécies e pelas áreas remanescentes compreenderem menos de 30% de sua vegetação original. Embora muitas espécies de anuros desse bioma estejam ecologicamente restritas a áreas pristinas, há exemplos de novas espécies sendo descobertas em ecossistemas antropizados. O gênero *Adenomera* é um grupo de anuros abundante e amplamente distribuído nos ecossistemas da Mata Atlântica, com a ocorrência de espécies em áreas com diferentes graus de perturbação humana. Neste artigo, nomeamos e descrevemos uma nova espécie de *Adenomera* endêmica da Mata Atlântica tipicamente encontrada em ecossistemas antropizados, como áreas urbanas e rurais. A nova espécie é recuperada dentro do clado de *A. marmorata*, como espécie irmã de *A. ajurauna*. Essas duas espécies têm distribuições alopátricas no sudeste brasileiro, com apenas uma ocorrência simpátrica conhecida. Essas espécies se distinguem por características do canto e pelo uso de diferentes habitats. A espécie de *Adenomera* descrita aqui é mais um caso de uma nova espécie descoberta em áreas urbanas no *hotspot* da Mata Atlântica.

SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at https://doi.org/10.1655/Herpetologica-D-22-00022.S1. See https://github.com/pedrotaucce/new_adenomera_AF for information on scripts, matrices, and software.

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APPENDIX I

Specimens Morphologically Examined (Brazilian Atlantic Forest)

Adenomera ajurauna.—BRAZIL: São Paulo: Bertioga: Parque das Neblinas (topotypes: CFBH 46574–7); Miracatu (CFBH 35827); Piedade (CFBH 25850); Santo André: Paranapiacaba (AAG-UFU 5024; CFBH 46573; MCP 13115); São Paulo: Parque Estadual da Serra do Mar, Núcleo Curucutu (CFBH 9123); Tapiraí (CFBH 16548).

Adenomera araucaria.—BRAZIL: Rio Grande do Sul: Bom Jesus (paratypes: MCP 3345–6); São Francisco de Paula (holotype: MCP 2421; paratypes: MCP 1794, 1849, 3208–9, 3463, 3672, 3676–7; topotypes: CFBH 43927–9, MCP 9896, 11199, 11241, 12055).

Adenomera bokermanni.—BRAZIL: Paraná: Paranaguá (topotypes: LHUFCG 0181–6; UFMG-AMP 10289–91); Santa Catarina: Garuva (CFBH 23216–9, 43152, 43154); Itapoá (MCP 12784; UFMG-AMP 10269–70).

Adenomera engelsi.—BRAZIL: Santa Catarina: Águas Mornas (UFMG-AMP 7106–7); Anitápolis (CFBH 18197, 20266–7); Florianópolis (holotype: MCP 6415; paratypes: MCP 6379, 6439–40, 7704–5, 8255–6, 8266–7); Rancho Queimado (CFBH 13590–1; UFMG-AMP 7001–2, 7017); Santo Amaro da Imperatriz (CFBH 43213–5); São Bonifácio (UFMG-AMP 7065).

Adenomera kweti.—BRAZIL: Santa Catarina: Florianópolis (paratypes: MCP 8285–6, UFMG-AMP 7080, 7111; MCP 1340); Governador Celso Ramos (CFBH 22775); Santo Amaro da Imperatriz (paratype: MCP 8212; CFBH 43213–5); São Bonifácio: Parque Estadual da Serra do Tabuleiro (holotype: CFBH 43184; paratypes: CFBH 43183, 43185–90, UFMG-AMP 7066).

Adenomera marmorata.—BRAZIL: Minas Gerais: Itatiaia (UFMG-AMP 9353–4); Rio de Janeiro: Floresta Nacional da Tijuca (topotypes: CFBH 34401–3, 34406); Maricá (CFBH 34408–9, 34411); São Paulo: Ilhabela (CFBH 15494); Ilha de Alcatrazes (CFBH 17139); Nazaré Paulista (CFBH 36130–2); Santos (CFBH 23923); São Luiz do Paraitinga (CFBH 35995–6, 35998); Ubatuba (CFBH 6396, 32814–5, 36000–2, 36005–6).

Adenomera nana.—BRAZIL: Santa Catarina: Garuva (CFBH 43151); Guaramirim (CFBH 27481); Jaraguá do Sul (MCP 8149–50); Joinville (MCP 8633); Massaranduba (CFBH 43201–3); Porto Belo (CFBH 43156, 43161–3, 43166); São Bento do Sul (MCP 8751–5; UFMG-AMP 10188); São Francisco do Sul (UFMG-AMP 10280).

Adenomera thomei.—BRAZIL: Espírito Santo: Linhares: Povoação (topotypes: AAG-UFU 6185–6; CFBH 5969–70, 18057–60, 18062–4, 18067–9); Minas Gerais: Camanducaia (ZUEC-AMP 11540); Munhoz (ZUEC-AMP 4268); Poços de Caldas (ZUEC-AMP 8376–8); São Paulo: Botucatu (ZUEC-AMP 2503); Campos do Jordão (ZUEC-AMP 980–1); Campinas (ZUEC-AMP 3165); Itatiba (ZUEC-AMP 11400).

Appendix II.—Sound recordings analyzed in this study. Calls were recorded in the Brazilian states of São Paulo (SP), Paraná (PR), and Santa Catarina (SC).

Sound file	Species	Locality	Voucher	Repository	Recordist	Air (°C)
AAG1	*Adenomera ajurauna*	Paranapiacaba, Santo André (SP)		AAG-UFU	A.A. Giaretta	19.5
AAG2	*Adenomera ajurauna*	Paranapiacaba, Santo André (SP)		AAG-UFU	A.A. Giaretta	19.5
AAG3	*Adenomera ajurauna*	Paranapiacaba, Santo André (SP)		AAG-UFU	A.A. Giaretta	19.5
TRC1	*Adenomera ajurauna*	Paranapiacaba, Santo André (SP)	AAG-UFU 5024	AAG-UFU	T.R. Carvalho	
CFBH-voc 8	*Adenomera ajurauna*	Paranapiacaba, Santo André (SP)		CFBH	C.S. Cassini	
CFBH-voc 9	*Adenomera ajurauna*	Parque das Neblinas, Bertioga (SP)		CFBH	C.S. Cassini	
CFBH-voc 10	*Adenomera ajurauna*	Paranapiacaba, Santo André (SP)	MCP 13115	CFBH	P.P.G. Taucce	21.0
CFBH-voc 11	*Adenomera ajurauna*	Miracatu (SP)	CFBH 35827	CFBH	P.P.G. Taucce	
CFBH-voc 12	*Adenomera cantitata*	Iguape (SP)	CFBH 35829	CFBH	C.S. Cassini	
CFBH-voc 13	*Adenomera cantitata*	Iguape (SP)		CFBH	C.S. Cassini	
CFBH-voc 14	*Adenomera cantitata*	Iguape (SP)		CFBH	P.P.G. Taucce	
CFBH-voc 15	*Adenomera cantitata*	Iguape (SP)	CFBH 35852	CFBH	P.P.G. Taucce	
CFBH-voc 16	*Adenomera cantitata*	Miracatu (SP)		CFBH	C.S. Cassini	
CFBH-voc 17	*Adenomera cantitata*	Miracatu (SP)		CFBH	C.S. Cassini	
CFBH35_4	*Adenomera cantitata*	Ribeirão Branco (SP)		CFBH	C.F.B. Haddad	
AAG1	*Adenomera cantitata*	Peruíbe (SP)		AAG-UFU	A.A. Giaretta	28.0
IV6a	*Adenomera cantitata*	Apiaí (SP)		AAG-UFU	I. Vidigal	20.0
LBM5a	*Adenomera cantitata*	Apiaí (SP)		AAG-UFU	L.B. Martins	19.0
AAG3b	*Adenomera cantitata*	Apiaí (SP)		AAG-UFU	A.A. Giaretta	21.0
AAG4a	*Adenomera cantitata*	Apiaí (SP)		AAG-UFU	A.A. Giaretta	21.0
AAG5a	*Adenomera cantitata*	Apiaí (SP)	AAG-UFU 5525	AAG-UFU	A.A. Giaretta	21.0
AAG2b	*Adenomera cantitata*	Apiaí (SP)		AAG-UFU	A.A. Giaretta	22.0
AAG3b	*Adenomera cantitata*	Apiaí (SP)		AAG-UFU	A.A. Giaretta	22.0
AAG4a	*Adenomera cantitata*	Apiaí (SP)		AAG-UFU	A.A. Giaretta	21.0
TRC1	*Adenomera cantitata*	Garuva (SC)	CFBH 43153	AAG-UFU	T.R. Carvalho	21.9
DLB1b	*Adenomera cantitata*	Campina Grande do Sul (PR)		AAG-UFU	D.L. Bang	24.3
DLB1b	*Adenomera cantitata*	Campina Grande do Sul (PR), trilha Pico Paraná		AAG-UFU	D.L. Bang	19.5
DLB2b	*Adenomera cantitata*	Campina Grande do Sul (PR), trilha Pico Paraná		AAG-UFU	D.L. Bang	19.5
DLB1b	*Adenomera cantitata*	PARNA Guaricana, São José dos Pinhais (PR)	AAG-UFU 7034	AAG-UFU	D.L. Bang	22.7