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A New Species of *Dendrophryniscus* Jiménez de la Espada 1871 (Amphibia: Anura: Bufonidae) from Mantiqueira Mountain Range, State of Minas Gerais, Brazil

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Abstract: We described a new species of *Dendrophryniscus* from the Mantiqueira Mountains, a mountain range in the Atlantic Forest in the state of Minas Gerais, Brazil. *Dendrophryniscus cuca* sp. nov. is known only from its type locality, the private Protected Area Chapadão da Serra Negra, municipality of Santa Bárbara do Monte Verde. The new species is morphologically distinguished from its congeners mainly by the combination of the following characters: medium size for the genus, longitudinal set of granules posterior to the corner of mouth, fingers neither fringed nor webbed, and presence of nuptial pads in males. Molecular analysis of the 16S mitochondrial DNA indicates a genetic distance range from 2.39% (*D. brevipollicatus*) to 17.92% (*D. carvalhoi*). Individuals were found in bromeliads, and we described a male–male amplexus with the presence of release vibrations, but calling activity was not observed. This is the fourth endemic anuran species of the Serra Negra da Mantiqueira, part of the Mantiqueira mountain range (Serra Negra da Mantiqueira), highlighting the importance of the region for the Neotropical herpetofauna.

Key words: *Dendrophryniscus cuca* sp. nov.; Endemism; Release vibrations; Species delimitation; Taxonomy

The Atlantic Forest (AF) is one of the most diversified ecoregions in the world (Mittermeier et al. 2003), harboring many endemic species, especially in mountainous areas, such as the Mar and Mantiqueira mountain ranges in southeastern Brazil (Cruz and Feio 2007; da Silva et al. 2018; Neves et al. 2018). New species of amphibians have been frequently described in recent years from areas >1000 m above sea level (m a.s.l.) in the AF (Taucce et al. 2018; de Carvalho et al. 2019; Cruz et al. 2019; Nunes et al. 2021). Because of the increase in deforestation in this ecoregion (Mittermeier et al. 2004), most of these endemic and rare species are threatened or, due to the lack of studies, do not have enough data to be assigned a conservation status (Neves et al. 2018), which draws attention to these amphibians.

Among several lineages that are endemic to the AF, *Dendrophryniscus* is a Neotropical monophyletic genus of bufonids (Fouquet et al. 2012). It is a sister taxon of the genus *Amazophrynella* (endemic to Amazonia), which diverged ca. 35 Mya (Fouquet et al. 2012; Moraes et al. 2022). Currently, *Dendrophryniscus* is composed of 16 species, all with reduced body size. Regarding their habitats, they often occur associated with bromeliads, but information about larvae and calls remains scarce (Izecksohn 1993; Cruz et al. 2019). Most species occur in coastal forests and adjacent mountains, such as the Mar mountain range (Cruz and Fusinatto 2008; Recoder et al. 2010; Caramaschi 2012; Cruz et al. 2019). In the Mantiqueira mountain range, *Dendrophryniscus carvalhoi* Izecksohn 1993 is the only species described until now, occurring in the northern portion.

During fieldwork in recent years along an AF area within the Mantiqueira mountain range, state of Minas Gerais, we collected specimens of *Dendrophryniscus*. Herein, we describe them as a new species, which is the second species from Mantiqueira and first in the southern portion of this mountain range. This new species is supported by morphological and molecular data, and we provide information on its natural history.

Materials and Methods

Fieldwork

We observed the first individual of the new species by visual searches on 4 March 2014 at the private protected area Chapadão da Serra Negra (CSN; –21.964305°S, –43.800136°W; datum = WGS84 in all cases; ca. 1110 m a.s.l.), municipality of Santa Bárbara do Monte Verde, state of Minas Gerais, Brazil. In recent years, in the same locality, we observed and captured individuals for the type series. The toads were manually collected, kept within a plastic bag, and euthanized using a liquid solution of 2% lidocaine chlorhydrate. Specimens were fixed in 10% formalin and transferred to permanent storage in 70% ethanol (Heyer et al. 1994). We also collected liver tissue samples before specimen fixation and stored them in 100% ethanol. The samplings were authorized by the System of Authorization and Information in Biodiversity, Ministry of the Environment (nos. 69659 and 45889). Vouchers are housed in the following collections: Coleção de Anfíbios da Universidade Federal de Juiz de Fora (CAUFJF), Juiz de Fora, Minas Gerais, Brazil; Coleção Zoológica da Universidade Federal de Mato Grosso do Sul (ZUFMS-AMP), Campo Grande, Mato Grosso do Sul, Brazil; and Coleção de Anfíbios do

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Morphology

We used the following morphological characters from Cruz and Fusinatto (2008), Recoder et al. (2010), and Cruz et al. (2019) in our analysis: snout–vent length (SVL, in millimeters), granules posterior to the corner of mouth, fringe and web on fingers, nuptial pad in males, third and fourth fingers, body shape, and tubercles in dorsal surface. We followed Heyer et al. (1990) and Kok and Kalamandeen (2008) for morphological terminologies. The sex of an individual was determined based on the absence and presence of nuptial pads in males. Additional specimens of other *Dendrophryniscus* species used to provide morphological comparisons are listed in the Appendix.

Morphometric measurements of the specimens were obtained using a digital caliper to the nearest 0.01 mm and follow Duellman (1970) and Carvalho-e-Silva et al. (2010): SVL; head length (HL), head width (HW), internarial distance (IND), eye–nostril distance (END), eye-to-snout distance (ESD), eye diameter (ED), interorbital distance (IOD), thigh length (THL), tibia length (TL), tarsal length (TAL), foot length (FL), humerus length (HUL), forearm length (FAL), hand length (HAL), width of third finger (F3), width of middle of third finger (F3), length of second finger (F2), and length of fourth finger (F4). We measured F2 and F4 from the insertion of Fingers III and IV in the palm until the tip of each finger, respectively, differently from Carvalho-e-Silva et al. (2010), who apparently used the hand tubercles as landmarks.

Molecular Analysis

We extracted the genomic DNA from muscle of three specimens by using the DNeasy blood and tissue kit (Ludwig Biotechnology). We amplified a section of the mitochondrial 16S ribosomal RNA gene by using the primers 16Sa (5′-CGC CTG TTT ATC AAA AAC AT-3′) and 16Sb (5′-CCG GTC TGA ACT CAG ATC ACG T-3′) according to Palumbi et al. (2002). Polymerase chain reaction (PCR) conditions for amplification consisted of 1× buffer, dNTP at 0.2 mM, each primer at 0.2 μM, $MgCl_2$ at 2 mM, 1 U of Taq polymerase, and 2 μL of template DNA, in a total reaction volume of 25 μL. The PCR cycling program used the following conditions: 94°C for 2 min, followed by 35 cycles of 94°C for 45 s, 50°C for 30 s, and 72°C for 1 min, and concluded with a 5-min extension at 72°C (Costa et al. 2016). The PCR products were purified by ethanol–sodium acetate and sequenced with an ABI 3130 genetic analyzer (Applied Biosystems). We edited the sequences by aligning forward and reverse reads by using Geneious (v.9.0.5, Biomatters, Ltd., San Diego, CA; Kearse et al. 2012).

We aligned the three mitochondrial sequences (605–492 bp; GenBank accessions OM339549–OM339551) with 34 selected sequences from the alignment in Cruz et al. (2019). As with Cruz et al. (2019), the sequences' alignments were computed with MAFFT (v.7; Katoh and Standley 2013) following the fast Fourier transform FFT-NS-i strategy and default parameters. After alignment, we also eliminated regions of missing data and kept only the fragment amplified for all samples with 300 bp (true length from 289 to 296 bp because of indels). We adopted the p-distance and computed intraspecific and interspecific p-distance with corresponding standard errors with 10,000 bootstrap replications and pairwise deletions for gaps by using MEGA11 software (Tamura et al. 2021). Using this same software, a tree was built with the neighbor-joining algorithm, based on the p-distance model and 10,000 replications in the bootstrap test.

Results

Dendrophryniscus ***cuca*** sp. nov.
(Figs. 1–3; Table 1)

Dendrophryniscus sp. Neves et al. (2017).
Dendrophryniscus brevipollicatus da Silva et al. (2018).

Holotype.—CAUFJF 1931, adult male, collected in a private protected area (CSN), municipality of Santa Bárbara do Monte Verde, state of Minas Gerais, Brazil, –21.964305°S, –43.800136°W, ca. 1110 m a.s.l., 18 May 2019, collected by R.C. Nali, R.F. Souza, and L.M.C. Lima.

Paratypes.—A total of 11 paratypes: 5 adult males (CAUFJF 1932, CAUFJF 1937, MNRJ 93961 (ex-CAUFJF 1934), MNRJ 93962 (ex-CAUFJF 1936), and MNRJ 93963 (ex-CAUFJF 1938), 1 adult female (CAUFJF 1933), and 1 juvenile (CAUFJF 1935), collected along with the holotype by the same collectors and date; 2 adult males (ZUFMS-AMP 15668 and ZUFMS-AMP 15670) and 1 juvenile (ZUFMS-AMP 15669) collected in the type locality on 2 November 2021 by D.J. Santana, P.S. Carvalho, and M.M.P. Müller.

Diagnosis.—The new species is diagnosed from other *Dendrophryniscus* spp. by the following combination of characters: (1) medium size for the genus (mean SVL = 19.77 mm in males); (2) longitudinal set of white granules posterior to the corner of mouth; (3) fingers neither fringed nor webbed; (4) moderately developed nuptial pad in males; (5) tip of Finger III poorly expanded laterally, and tip of Finger IV poorly or not expanded laterally; (6) slender body; and (7) rounded granules in dorsal surface.

Description of holotype.—Body small, slender; head triangular, longer than wide. Head length 24.31% of SVL. Snout mucronate in dorsal view and acute in lateral view. Nostrils located laterally, near to the tip of the snout and in the interior of canthus rostralis in lateral view. Morphometric relative lengths were IND < END < ED < ESD < IOD. Canthus rostralis slightly curved and loreal region vertical. Tympanum absent. Tongue lanceolate, narrow and free in the posterior half, with posterior margin rounded. Choanae circular, maxillary and palatine teeth absent. Vocal sac and vocal slits absent. Dorsal surface of body highly granulose, with numerous large irregular warts distributed. Warts more numerous in sacral region and forming lines or clusters of enlarged granules in the dorsolateral region. Ventral and lateral surfaces highly granulose, but with shallow and smaller warts. Forearms and hindlimbs with the same granular patterns of body. Forearms longer and more robust than arms. Hands with four slender fingers, neither fringed and nor webbed, without discs. Fingers I and II rounded, not expanded and Fingers III and IV poorly expanded laterally. Inner finger short and robust, with a distinct moderately and pigmented nuptial pad. Relative lengths of fingers: I < II < IV < III. Subarticular tubercles single and globular; supernumerary tubercles present; outer

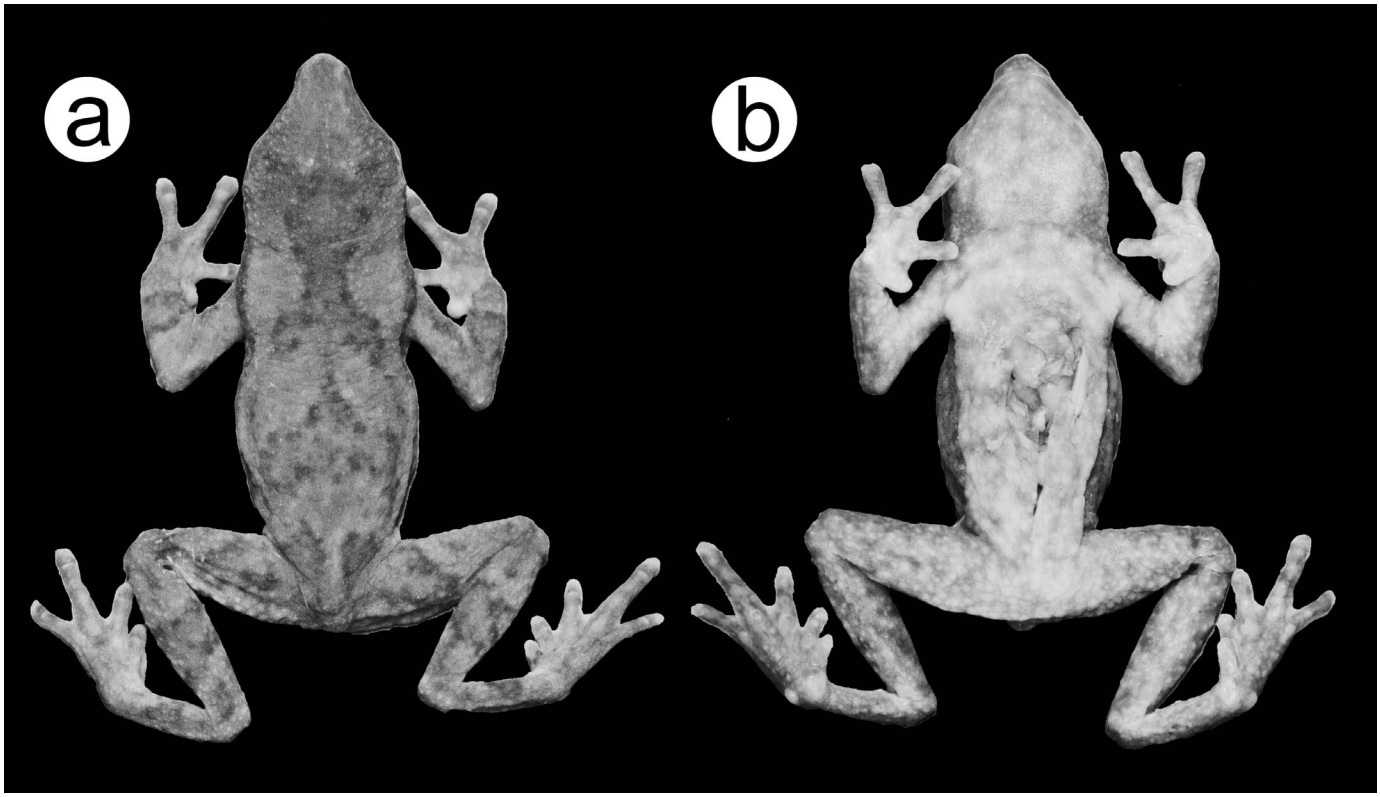

FIG. 1.—Holotype of *Dendrophryniscus cuca* sp. nov. (CAUFJF 1937; snout–vent length = 18.73 mm) in (a) dorsal and (b) ventral views. A color version of this figure is available online.

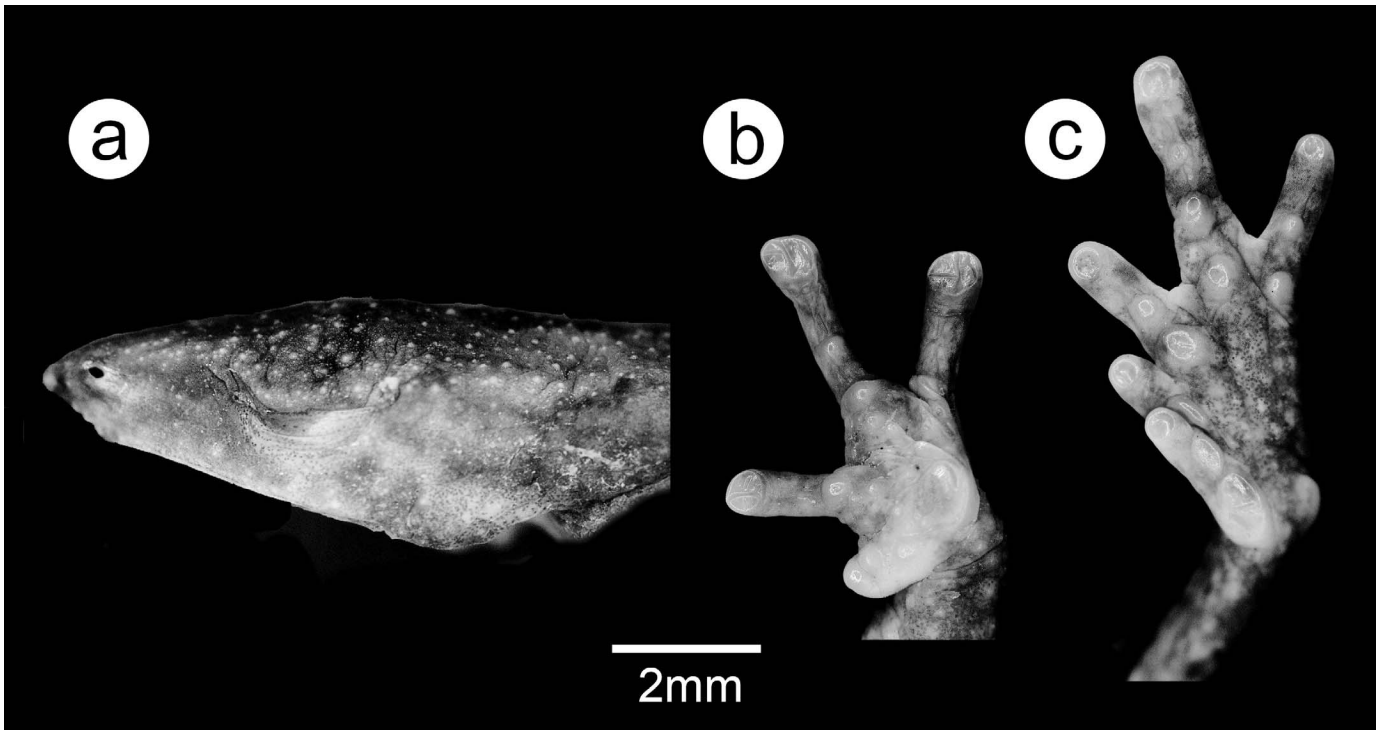

FIG. 2.—Holotype of *Dendrophryniscus cuca* sp. nov. (CAUFJF 1937): (a) lateral view of the head and ventral views of the (b) hand and the (c) foot. A color version of this figure is available online.

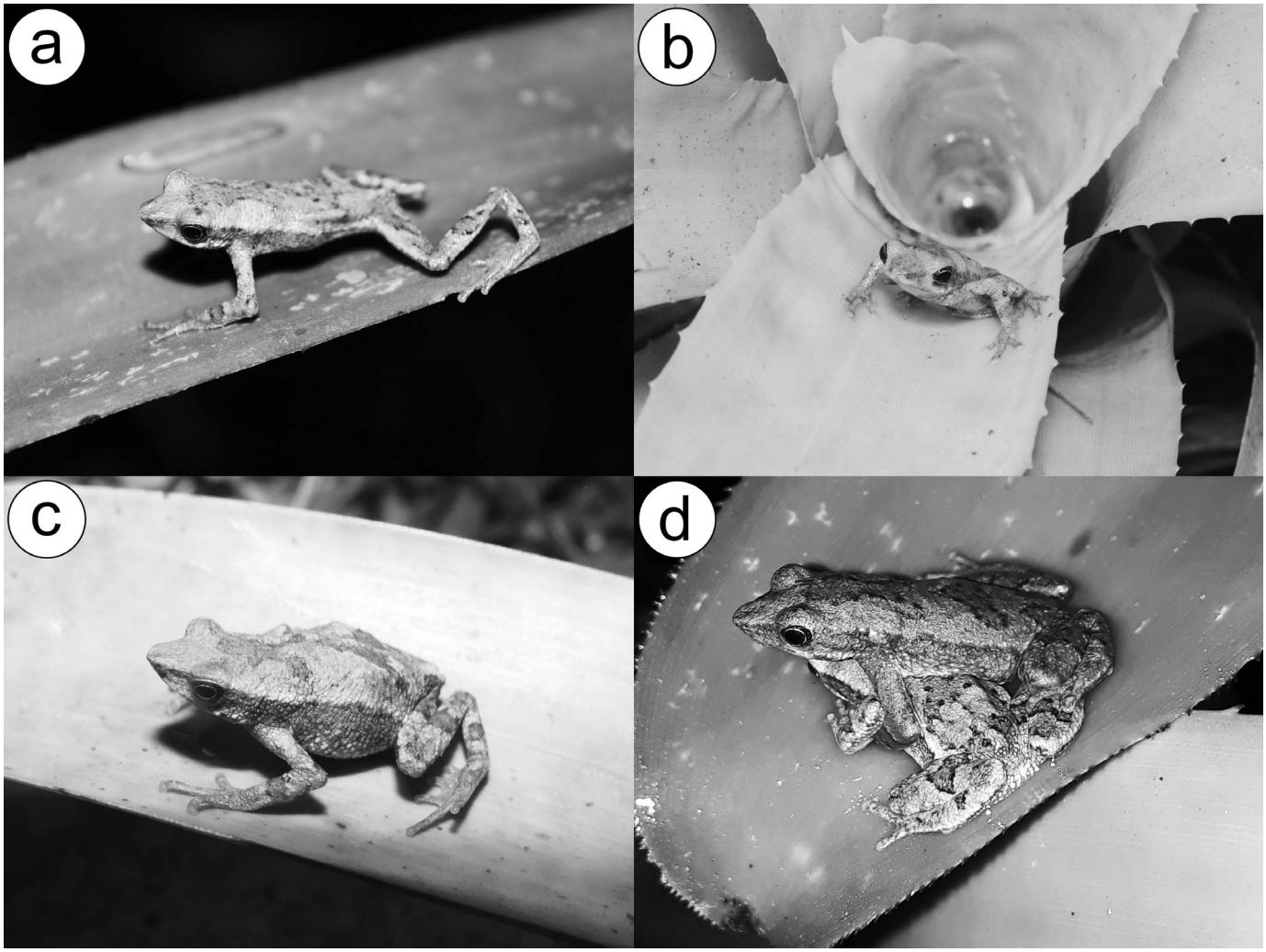

FIG. 3.—*Dendrophryniscus cuca* sp. nov. individuals in life. (a–c) Adults on top of a bromeliad (*Vriesea gradata*) and (d) a male–male amplexus during which the bottom individual displayed release vibrations. A color version of this figure is available online.

metacarpal tubercle globular, large and partially ovoid and inner metacarpal tubercle small and rounded. Hindlimbs slender with thigh and tibia with similar length. Sum of thigh, tibia, tarsal and foot lengths (THL + TL + TAL + FL) ca. 135% of SVL. Foot with slender toes, webbed just at the base, not fringed, distal end rounded, without discs. Relative lengths of toes: I < II < III < V < IV. Subarticular tubercles single, ovoid; supernumerary tubercles present; inner and outer metatarsal tubercle large and elliptical, with the inner metatarsal tubercle a little higher than the outer tubercle.

Comparison with congeneric species.—*Dendrophryniscus cuca* sp. nov. has moderate body size (SVL range = 18.17–21.39 mm) and is larger than *D. imitator* (15.1–17.4 mm) and smaller than *D. oreites* (25 mm), *D. organensis* (23.3–23.8 mm), *D. proboscideus* (39.2–46.4 mm), and *D. skuki* (26.2 mm). The new species can be distinguished from *D. brevipollicatus*, *D. carvalhoi*, *D. haddadi*, *D. imitator*, *D. izecksohni*, *D. jureia*, *D. krausae*, *D. lauroi*, *D. organensis*, *D. proboscideus*, *D. skuki*, and *D. stawiarskyi* by the presence of a longitudinal set of white granules posterior to the corner of mouth (absent in *D. carvalhoi*, *D. haddadi*, *D. organensis*, *D. proboscideus*, *D. skuki*, and *D. stawiarskyi*; an elliptical set in *D. brevipollicatus*, *D. izecksohni*, *D. jureia*, and *D. lauroi*; and just two pronounced granules in *D. imitator*). *Dendrophryniscus cuca* sp. nov. has fingers neither fringed nor webbed, different from *D. brevipollicatus* and *D. davori* (fingers slightly fringed and webbed only at base), *D. izecksohni* and *D. jureia* (fingers fringed and webbed about one third), and *D. krausae* (fingers not fringed, but webbed only at base). The new species can be distinguished from *D. berthalutzae*, *D. carvalhoi*, *D. davori*, *D. izecksohni*, *D. leucomystax*, and *D. stawiarskyi* by the nuptial pad moderately developed in males (absent in *D. berthalutzae* and *D. leucomystax* and very developed in *D. carvalhoi*, *D. davori*, *D. izecksohni*, and *D. stawiarskyi*). *Dendrophryniscus cuca* sp. nov. has the tip of Finger III poorly expanded laterally and Finger IV poorly or not expanded laterally, different from *D. brevipollicatus* and *D. lauroi* (the tip of third and fourth fingers expanded laterally). The new species is distinguished from *D. krausae* and *D. organensis* by its slender body (body robust in *D. organensis* and *D. krausae*). In addition, *D. organensis* has many scattered small conical granular in dorsal surface and the granules of the dorsal surface of *D. cuca* sp. nov. are rounded.

TABLE 1.—Measurements of the holotype and entire type series of *Dendrophryniscus cuca* sp. nov. (average ± SD; range in parentheses). Snout–vent length (SVL), head length (HL), head width (HW), internarial distance (IND), eye–nostril distance (END), eye-to-snout distance (ESD), eye diameter (ED), interorbital distance (IOD), thigh length (THL), tibia length (TL), tarsal length (TAL), foot length (FL), humerus length (HUL), forearm length (FAL), hand length (HAL), width of third finger (F3), width of middle of third finger (M3), length of second finger (F2), and length of fourth finger (F4).

Measurement (mm)	Holotype	Female (n = 1)	Males (n = 7)
SVL	21.35	18.22	19.55 ± 1.2 (18.17–21.39)
HL	5.19	5.16	5.19 ± 0.55 (4.55–6.02)
HW	5.97	5.35	5.61 ± 0.22 (5.33–5.93)
IND	1.85	1.44	1.65 ± 0.17 (1.44–1.98)
END	1.97	1.93	1.91 ± 0.21 (1.58–2.15)
ESD	2.8	2.47	2.63 ± 0.28 (2.34–3.04)
ED	2.23	2.32	2.41 ± 0.16 (2.2–2.65)
IOD	3.05	2.57	2.74 ± 0.2 (2.52–3.14)
THL	8.8	7.62	7.72 ± 0.30 (7.38–8.22)
TL	8.3	7.81	7.81 ± 0.21 (7.45–8.3)
TAL	4.45	4.4	4.27 ± 0.31 (4.01–4.86)
FL	7.37	6.42	6.88 ± 0.5 (6.48–7.72)
HUL	4	4.05	4.05 ± 0.39 (3.73–4.73)
FAL	3.98	4.3	3.99 ± 0.58 (3.34–4.63)
HAL	5.33	4.86	5.05 ± 0.28 (4.73–5.47)
F3	0.88	0.81	0.79 ± 0.1 (0.58–0.88)
M3	0.65	0.62	0.55 ± 0.08 (0.44–0.68)
F2	1.47	1.52	1.51 ± 0.1 (1.39–1.65)
F4	1.96	1.83	1.80 ± 0.15 (1.59–2.08)

Coloration.—In preservative, dorsal pattern of the body and limbs light brown with tiny dark pigmentation with darker blotches, sometimes imperceptible in preserved specimens. Presence of a dark brown band like a bracelet on the forearm and vertical bands on legs. Two dark brown dorsolateral stripes that arise behind the eyes to the insertion of the thighs. Light ventral region with dark blotches. Also, the light texture of the ventral region contains tiny dark pigmentations forming light spots in nonpigmented regions. In life, the brown dorsal and laterodorsal pattern of the body and limbs is more visible and contrasted.

Variation.—Patterns of dark brown blotches in the dorsal region vary across specimens. In most specimens from the type series, the blotches are composed of two or three triangular shapes connected by a band in the vertebral region. The intensity of pigmentation in the ventral region also varies across individuals, with some individuals darker than others. The holotype has the vertical loreal region, but it can also be slightly curved (ZUFMS-AMP 15670). Sexual dimorphism can be observed on nuptial pad in the base of the Finger I in males.

Natural history.—The population was found in two types of cloud forest: dwarf cloud forest and cloud shrubland. Individuals were always observed associated with different species of bromeliads, especially *Vriesea gradata*. The dwarf cloud forest harbors a higher concentration of bromeliads than the cloud shrubland, and individuals of *D. cuca* were found most often in this type of phytogeographic formation (Fig. 4). Juveniles were observed in November, March, and April. Since 2015, we observed the species in multiple occasions while doing fieldwork in the type locality, but calling activity was never heard. On 18 May 2019, we observed many individuals on bromeliad leaves, and at 1950 h (air temperature = 20.1°C), we observed an axillary amplexus between two males at the tip of a bromeliad leaf (Fig. 3d). The

FIG. 4.—Type locality environment of *Dendrophryniscus cuca* sp. nov. in Serra Negra da Mantiqueira. (a) Dwarf cloud forest with species of bromeliads and (b) *Vriesea gradata*, the species of bromeliad most commonly used by the new species. A color version of this figure is available online.

bottom male displayed inaudible release vibrations, also known as warning vibrations (video in FIGSHARE, available at https://dx.doi.org/10.6084/m9.figshare.21357663.v1) at a rate of ca. 20 vibrations/min, as measured from 30 s of the video. We continued to observe the individuals by using red lights. The top male first released the axillary clasp at ca. 2001 h, but continued on top of the bottom male, which remained emitting release vibrations. They ceased all physical contact at ca. 2006 h, when the vibrations also stopped. Only then were the two individuals (CAUFJF 1931 and CAUFJF 1932) collected. We dissected the stomach of CAUFJF 1932 and found items from the taxa Formicidae, Araneae, Coleoptera, and Diptera. Individuals collected on 2 November 2021 were active and exposed on bromeliad leaves during the afternoon, near dusk (ca. 1700 h).

Distribution.—The new species is only known from its type locality. Neves et al. (2017) collected from 13 points along the Serra Negra, including in the CSN and Serra Negra da Mantiqueira State Park (SNMSP). However, the species was found only in the Jardim das *Vrieseas* (type locality) in the private reserve, 0.5 km away from the SNMSP.

Etymology.—The epithet "*cuca*" refers to a mythological entity, belonging to the Brazilian and Iberian folklore, of an old and evil woman who kidnaps disobedient children at night. The choice of the name is related to the promotion and valorization of Brazilian folklore. In Serra Negra da Mantiqueira, there is another endemic species, *Hylodes perere* Silva and Benmaman 2008, that also was named after a mythic creature from Brazilian folklore, and we follow the same line for this new species.

Phylogenetic inferences and mitochondrial DNA divergences.—We recovered *D. cuca* as sister group of *D. brevipollicatus* (Fig. 5). Intraspecific genetic distances ranged from 0% to 2.72% (±0.96%; Table 2). Interspecific distances ranged from 2.39% (±0.77%) between *D. cuca* and *D. brevipollicatus* to 20.15 (±2.36%) between *D. berthalutzae* and *D. carvalhoi* (Table 3; Supplemental Table S1, available online).

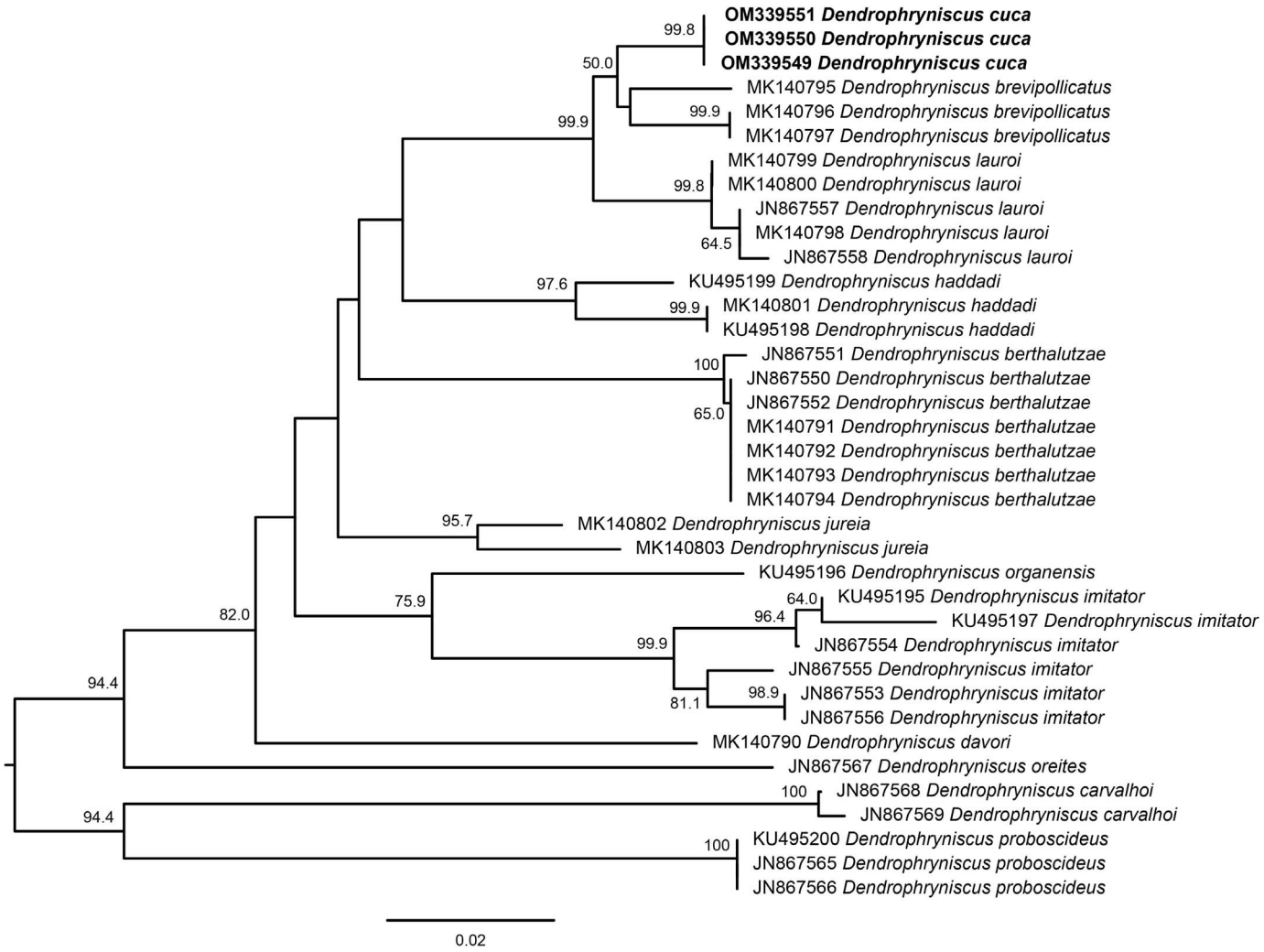

FIG. 5.—Neighbor-joining tree of *Dendrophryniscus* based on 16S RNA ribosomal gene fragment. Bootstrap values (≤50%) are shown on branch nodes. Scale bar corresponds to substitutions per site.

DISCUSSION

Our phylogenetic analysis provided clear support for *D. cuca* as a new species close to *D. brevipollicatus* and *D. lauroi* (Fig. 5). Accordingly, these three species would be sister to almost all species of *Dendrophryniscus* with low support, with the exception of *D. proboscideus* and *D. carvalhoi*, which form an external group to the entire genus. Although it is already evident that this type of analysis is not the most appropriate (see Collins and Cruickshank [2013]), values between 2% and 3% for 16S have been the most accepted. Fouquet et al. (2007) suggested a threshold of 3% for the 16S marker for Neotropical frogs (Vieites et al. 2009), whereas Crawford et al. (2010) working in a more restricted geographical area used a 2% limit for 16S. Although the values presented were near the threshold, morphological and morphometric features also support the status of new species for *D. cuca*. Identification of *Dendrophryniscus* species is difficult due to the reduced body size, few numbers of individuals collected in each locality and/or a long time ago, and scarce larval and acoustic data (Fouquet et al. 2012; Cruz et al. 2019). Therefore, we used different taxonomic tools, applying an integrative approach to support our results, and also revealed important findings about the *Dendrophryniscus* taxonomy and natural history.

TABLE 2.—Intraspecific genetic distances (16S RNA gene fragment) expressed in values of p-distance and corresponding standard errors (SE) of species of *Dendrophryniscus*.

Dendrophryniscus species	p-distance (%)	SE (%)
cuca	0.00	0.00
brevipollicatus	1.60	0.60
lauroi	0.34	0.24
jureia	2.72	0.96
haddadi	1.83	0.64
berthalutzae	0.10	0.10
organensis	n/c[a]	n/c
davori	n/c	n/c
imitator	2.43	0.62
oreites	n/c	n/c
proboscideus	0.00	0.00
carvalhoi	0.34	0.34

[a] n/c = not computed in cases with only one sample for species.

Release vibrations, or warning vibrations, are known in true toads from the family Bufonidae, including the genera *Rhinella* (Vieira et al. 2014; Ávila et al. 2018) and *Anaxyrus* (Schmidt 1990). These vibrations can be characterized as inaudible sounds produced without the participation of vocal

TABLE 3.—Genetic distance (16S RNA gene fragment) between 12 *Dendrophryniscus* species expressed in values of p-distance (below diagonal) and corresponding standard errors (above diagonal).

Dendrophryniscus species	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)	7 (%)	8 (%)	9 (%)	10 (%)	11 (%)	12 (%)
1. *cuca*		0.77	0.98	1.42	1.47	1.64	1.79	1.84	1.79	2.06	2.18	2.26
2. *brevipollicatus*	2.39		0.94	1.46	1.37	1.63	1.71	1.86	1.76	2.04	2.21	2.21
3. *lauroi*	3.00	3.35		1.48	1.46	1.67	1.82	1.90	1.82	2.04	2.20	2.25
4. *jureia*	7.17	8.04	7.78		1.40	1.49	1.62	1.54	1.56	2.03	2.12	2.24
5. *haddadi*	7.42	7.09	7.58	7.42		1.61	1.52	1.69	1.53	2.00	2.23	2.18
6. *berthalutzae*	8.59	9.12	8.87	7.61	9.01		1.86	1.70	1.75	2.21	2.30	2.36
7. *organensis*	10.27	9.95	10.89	9.22	7.90	11.43		1.94	1.51	2.18	2.22	2.31
8. *davori*	10.73	11.68	11.49	7.79	9.61	9.05	12.15		1.75	2.14	2.15	2.16
9. *imitator*	11.47	11.73	12.15	9.56	8.99	11.18	8.34	11.05		1.98	2.17	2.26
10. *oreites*	14.33	14.59	14.61	15.02	14.27	17.18	16.44	15.92	14.50		2.17	2.34
11. *proboscideus*	16.38	17.67	17.27	16.55	18.26	18.90	17.12	16.26	18.09	16.72		2.15
12. *carvalhoi*	17.92	17.73	18.46	18.54	17.41	20.15	19.22	16.09	19.86	19.97	15.87	

cords (Aronson 1944), unlike release calls that are audible sounds also common in Bufonidae, especially *Rhinella* (Toledo et al. 2015; Sugai et al. 2021). The vibrations can be produced by rapid inflation and deflation of lungs, which causes expansion and retraction movements of the body sides (Schmidt 1972, 1990). Release calls and vibrations are advantageous during mating activity, because they prevent amplexus that will not result in egg laying. This is the first time that this type of vibration has been documented on video and described in *Dendrophryniscus*. One study with *D. imitator* (Malagoli et al. 2017) provides insight into the reproduction of species associated with bromeliads, which also seems to be the case of *D. cuca*. Paternal care and male–male competition for females have been found in *D. imitator* (Malagoli et al. 2017), but warning vibrations were not described. Further behavioral studies are sorely needed with *D. cuca* to uncover its full mating system and reproductive mode and confirm possible similarities with *D. imitator*, toward which this species description is the first step. Although *D. cuca* appears to reproduce in bromeliads, similar to *D. imitator*, these species occur in different environments and altitudinal ranges (100–1100 m a.s.l. for *D. imitator* and 1110 for *D. cuca*) and have different geographical distributions (Cruz et al. 2019).

Dendrophryniscus cuca is the second species of the genus described for the Mantiqueira Mountain Range (MMR) and one of the most continental *Dendrophryniscus* species. There are records of *Dendrophryniscus* sp. collected in Itatiaia National Park (meridional portion of MMR) in 1958 by Haroldo Strang (MNRJ 22068–22073), but their precise identification is not possible due to the preservation state of the specimens. *Dendrophryniscus carvalhoi* is restricted to the northern portion of the MMR, in the municipality of Santa Tereza, and Caparaó National Park, state of Espírito Santo, distant ca. 280 km in a straight line from the type locality of *D. cuca* (Izecksohn 1993; Cassimiro and Rodrigues 2018; da Silva et al. 2018). The southern and northern portions of the MMR are divided by a valley with some tributary rivers of the Paraíba do Sul River such as the Muriaé, Pomba, Itabopoana, and Carangola rivers. Species that are phylogenetically close and occupy similar habitats in different mountain ranges suggest biogeographical homologies, i.e., these ranges have already been linked in evolutionary history (Morrone 2001). This pattern is frequently found among anuran species of the Mantiqueira, Mar, and Espinhaço mountain ranges (Cruz and Feio 2007; da Silva et al. 2018; Neves et al. 2018) such as *Physalaemus deimaticus* and *P. erythros* in Espinhaço Mountain Range and *P. rupestris* in the MMR. We suggest that the same pattern might occur with *D. cuca* and *D. carvalhoi* in MMR and *D. brevipollicatus*, *D. imitator*, and *D. lauroi* in the Mar mountain range.

These highlands of the AF in southeastern Brazil harbor high biodiversity and endemism due to the great altitudinal and climatic variation that generated a structural complexity of habitats contributing to speciation (Cruz and Feio 2007). New species of amphibians are described in the MMR each year due to exploration of new areas (Haga et al. 2017; Nunes et al. 2021) or by the use of different taxonomic tools that uncover cryptic species (Taucce et al. 2018; da Silva et al. 2020). Some of these new species include first records for genera in the MMR that were previously thought to occur only in the Mar mountain range, such as *Dendrophryniscus* and *Fritziana* (Peixoto et al. 2016; Folly et al. 2018). This fact reinforces the relationship between these two mountainous ranges (Cruz and Feio 2007; Guedes et al. 2020) and draws attention to more sampling in areas of AF, mainly in MMR. The fieldwork in the Serra Negra da Mantiqueira (one of the MMR highlands), type locality of *D. cuca*, began in mid-2014, where two new species have already been described (*D. cuca* and *Boana cambui*; Pinheiro et al. 2016) and one more is in the description phase. Currently, Serra Negra da Mantiqueira has four endemic species (*Brachycephalus* sp., *Hylodes perere*, *Boana cambui*, and now *D. cuca*), highlighting the importance of this region for Neotropical herpetofauna.

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SUPPLEMENTAL MATERIAL

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APPENDIX

Material Examined in Addition to the New Species

Dendrophryniscus berthalutzae: MNRJ 49537, 74220, 74224, 74225; *Dendrophryniscus brevipollicatus*: MNRJ 60701, 60702, 86388, 86389, 92631–92637; *Dendrophryniscus carvalhoi*: MNRJ 38364, 38365, 40678; *Dendrophryniscus davori*: MNRJ 58300 (Holotype), 58301 (Paratype), 58302 (Paratype); *Dendrophryniscus haddadi*: MNRJ 2430, 92123, 92459, 92460, 92462–92465; *Dendrophryniscus imitator*: MNRJ 22054–22060; *Dendrophryniscus izecksohni*: MNRJ 2416 (Paratype); *Dendrophryniscus jureia*: MNRJ 91510 (Holotype), 40007 (Paratype); *Dendrophryniscus krausae*: MNRJ 50290 (Paratype); *Dendrophryniscus lauroi*: MNRJ 57799, 57800, 71511, 71512, 90575–90587; *Dendrophryniscus leucomystax*: MNRJ 60131 (Paratype); *Dendrophryniscus organensis*: MNRJ 2249, 58291–58293; *Dendrophryniscus stawiarskyi*: MNRJ 2650 (Holotype), 22075.