



A new cryptic species of the *Aplastodiscus albosignatus* group (Anura: Hylidae)

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Abstract. The genus *Aplastodiscus* currently includes 15 valid species, and a further five candidate species have already been pointed out in the literature. We here describe one of these candidate species, assigned to the *A. albosignatus* group, from the southeastern slopes of the Espinhaço Range, State of Minas Gerais, Brazil. The new species is cryptic with *A. cavicola*, being morphologically similar in both adult and larval stages. However, the two species are not sister taxa. The advertisement call of the new species is markedly distinct from all other known species of its genus. Examination of topotypic samples of *A. cavicola* corroborates the presence of this species in the Espinhaço Range, but also indicates the need for a revision of the taxonomic status of populations from the state of Rio de Janeiro assigned to this taxon.

Key words. Amphibia, Anura, bioacoustics, conservation status, Neotropics, phylogeny, tadpoles, taxonomy, systematics.

Introduction

The genus *Aplastodiscus* LUTZ, 1950 (Hylinae: Cophomantini), originally monotypic, was described to accommodate *Aplastodiscus perviridis* LUTZ, 1950. Half a century later, GARCIA et al. (2001) transferred *Hyla cochranæ* MERTENS, 1952 to this genus. Subsequently, FAIVOVICH et al. (2005) systematically reviewed the family Hylidae based mostly on DNA sequence data and, on the basis of their results and literature, transferred the species of the former *Hyla albofrenata* and *Hyla albosignata* complexes (CRUZ & PEIXOTO 1985, 1987) to *Aplastodiscus*, recognizing three species groups: *Aplastodiscus albofrenatus*, *A. albosignatus*, and *A. perviridis*.

BERNECK et al. (2016) recovered a molecular phylogeny of *Aplastodiscus* that included all species but *A. musicus* (LUTZ, 1949). Besides the recognized taxa, BERNECK et al. pointed out six new candidate species of this genus, one of which was subsequently described as *A. lutzorum* BERNECK, GIARETTA, BRANDÃO, CRUZ & HADDAD, 2017. Also, they proposed the *A. sibilatus* group in order to accommodate *A. sibilatus* (CRUZ, PIMENTA & SILVANO, 2003) and one

of their candidate species (*Aplastodiscus* sp. 1). BEZERRA et al. (2020) then analysed DNA sequence data of *A. musicus* and recovered it as a member of the *A. albofrenatus* group.

Currently, the genus embraces 15 valid species in four groups (BERNECK et al. 2017): (i) *A. sibilatus* group: *A. sibilatus*; (ii) *A. albofrenatus* group: *A. albofrenatus* (LUTZ, 1924), *A. arildae* (CRUZ & PEIXOTO, 1987), *A. ehrhardti* (MÜLER, 1924), *A. eugenioi* (CARVALHO-E-SILVA & CARVALHO-E-SILVA, 2005), *A. musicus*, and *A. weygoldti* (CRUZ & PEIXOTO, 1987); (iii) *A. perviridis* group: *A. cochranæ*, *A. lutzorum*, and *A. perviridis*; (iv) *A. albosignatus* group: *A. albosignatus* (LUTZ & LUTZ, 1938), *A. cavicola* (CRUZ & PEIXOTO, 1985), *A. flumineus* (CRUZ & PEIXOTO, 1985), *A. ibirapitanga* (CRUZ, PIMENTA & SILVANO, 2003), and *A. leucopygius* (CRUZ & PEIXOTO, 1985).

When examining the material of one of the remaining five candidate species proposed by BERNECK et al. (2016), *Aplastodiscus* sp. 4, we found it to be morphologically cryptic with *A. cavicola*. Although these two were recovered as highly supported members of the *A. albosignatus* group by BERNECK et al. (2016), they were recovered just distantly related to each other. It is important to mention

in this context that BERNECK et al. (l.c.) had not analysed samples from topotypic populations of *A. cavicola* in their analysis.

During field surveys on the eastern slopes of the southern portion of the Espinhaço Range (state of Minas Gerais, southeastern Brazil, the region of provenance of *Aplastodiscus* sp. 4), we had the opportunity collect adult specimens, tadpoles, and to record the advertisement call of this species. The study of this material corroborates BERNECK et al.'s (2016) hypothesis that it is a new species of *Aplastodiscus*, which we describe in the present paper. We also obtained and analysed tissue samples from topotypic *A. cavicola* from Santa Teresa, state of Espírito Santo, southeastern Brazil, and added the resulting DNA sequences to the dataset of BERNECK et al. (2016) in order to compare them with both the sequences of the new species and those of *A. cavicola* previously analysed.

Material and methods

DNA extraction and phylogenetic analysis

For our analysis, we adopted sequences from the dataset of BERNECK et al. (2016), selecting one terminal per species and/or candidate species. For each taxon, we selected the terminals with more gene sequences available. We added to this dataset the sequences of *A. musicus* provided by BEZERRA et al. (2020). Outgroups to *Aplastodiscus* were represented by the same sequences employed by BERNECK et al. (2016; Appendix A). We added to this dataset the sequences from the new species we describe in this work, and from topotypic samples of *A. cavicola* (GenBank accession numbers MT671499–671510 and MT683338–683371; Supplementary document S1).

We amplified and sequenced the same gene fragments as assessed by BERNECK et al. (2016): three fragments of mitochondrial genes (partial sequence of 12S rRNA gene, the intervening tRNA^{VAL}, and partial sequence of the 16S rRNA; a fragment including the downstream region of the rRNA 16S, the intervening tRNA^{LEU}, NADH dehydrogenase subunit 1, and a partial sequence of tRNA^{LEU}; and a fragment of cytochrome oxidase c subunit 1 [COI]); and five fragments of nuclear genes (rhodopsin exon 1, tyrosinase, recombinase-activation 1, tensin 3, and seven in absentia homolog 1) (see Supplementary document S1 for a complete list of employed sequences).

Whole cellular DNA was extracted from ethanol-preserved muscle or liver tissues, following an ammonium acetate precipitation protocol (adapted from MANIATIS et al. 1982, to be employed with microcentrifuge tubes; LYRA et al. 2017). The amplification reactions were carried out in a 22- μ l reaction, using the Ampliqon Taq DNA Polymerase Master Mix (Ampliqon A/S, Odense M, Denmark) with the primers listed in Supplementary document S2.

Polymerase chain reactions (PCRs) were conducted in accordance with the following programs: for mitochondrial genes: 180 s of denaturation at 95°C, followed by 35 cycles of 20 s of denaturation at 95°C plus 20 s of annealing at

50°C plus 80 s of extension at 60°C, followed by 180 s of final extension at 60°C, and stored at 12°C; for nuclear genes: 180 s of denaturation at 95°C, followed by 45 cycles of 20 s of denaturation at 95°C plus 20 s of annealing at 45–56°C plus 60 s of extension at 60–72°C, followed by 180 s of final extension at 60–72°C, and stored at 12°C.

PCR products were then purified using Exo I/SAP (Fermentas) and sent to Macrogen Inc., Seoul, South Korea, for sequencing. All samples were sequenced in both directions. Chromatograms were checked for quality, and contigs were assembled using Sequencher 3.0 software (Gene Codes, Ann Arbor, MI, USA). The sequences of each gene fragment were aligned using MAFFT v.7 (KATO & STANDLEY 2013). We used the E-INS-i strategy for the 12S + tRNA^{VAL} + 16S sequences, and G-INS-i strategy for the other gene fragments. Alignments were then edited with BioEdit software (HALL 1999). Sequence files were merged with SequenceMatrix (VAIDYA et al. 2011).

Phylogenetic analyses were conducted with TNT Willi Hennig Society Edition (GOLOBOFF et al. 2008). Searches were done with New Technology Search at level 15, employing the four strategies available in TNT: Sectorial Search, Parsimony Ratchet, Tree Drift, and Tree Fusing, hitting minimum length 100 times. External gaps were coded as missing data, gaps from the middle of the sequence fragments were treated as a fifth state. Parsimony Jackknife absolute frequencies (FARRIS et al. 1996) were calculated with the parameters of the searches for best score, but requesting the driven search to hit the best length ten times (minimum length is hit with this search strategy) for a total of 1000 replicates. Trees were edited with FigTree (RAMBAUT 2014).

Genetic distances between specimens were estimated for both the final fragment ~590 bp of the 16S (flanked by primers 16Sa-L and 16Sb-H; Supplementary document S2; FOUQUET et al. 2007; VENCES et al. 2005a, b), and a fragment of ~651 bp of the, likewise mitochondrial, protein coding gene COI (flanked by primers AnF1 and AnR1; Supplementary document S2) using MEGA7 (KUMAR et al. 2016). Uncorrected p-distances were calculated by selecting the “pairwise deletion” option of Gaps/Missing Data Treatment.

Adult morphology and species description

Adults were euthanized in 5% lidocaine, fixed in 10% formalin, and preserved in 70% ethanol. Tissue samples from thigh muscles were preserved in pure alcohol for molecular analysis. Voucher specimens employed in this study are housed in the following Brazilian collections: Amphibian Collection of the Centro de Coleções Taxonômicas da Universidade Federal de Minas Gerais, Belo Horizonte, state of Minas Gerais; Amphibian Collection of Museum of Natural Sciences–Pontifícia Universidade Católica de Minas Gerais, Belo Horizonte, state of Minas Gerais; Amphibian Collection of Museu de Zoologia of Universidade de São Paulo, São Paulo, state of São Paulo; Eugenio Izecksohn Amphibian Collection, Universidade Federal do Rio de Janeiro, Rio de Janeiro, state of Rio de Janeiro; Celio F.B.

Haddad Amphibian Collection, Rio Claro, São Paulo, Brazil. Acronyms used follow SABAJ (2019). Additional specimens used for comparisons are listed in the Appendix.

We measured specimens to the nearest 0.1 mm using digital callipers under a ZEISS StEREO Discovery.V8 stereomicroscope. Measurements (in mm) follow DUELLMAN (1970): snout–vent length (SVL), head length (HL), head width (HW), eye diameter (ED), eye–nostril distance (END), internarial distance (IND), eyelid width (EW), interorbital distance (IOD), tympanum diameter (TD), tibia length (TL), tarsal length (TAL), and foot length (FL). We also measured snout length (SL; CEI 1980); thigh length (THL; HEYER et al. 1990); finger IV disc diameter (4FD – originally named finger III diameter, but see “finger numbering” below), and toe IV disc diameter (4TD; NAPOLI & CARAMASCHI 1999). Terminology for external morphology follows DUELLMAN (1970), except for the dorsal outline of the snout, which follows HEYER et al. (1990). Finger numbering follows FABREZI & ALBERCH (1996). Webbing formulae follow SAVAGE & HEYER (1967) as modified by MYERS & DUELLMAN (1982). Sexes were identified by vocalization or the presence of vocal slits in males, and, where possible, the presence of mature oocytes in females.

Larval morphology

Tadpoles were euthanized in 5% lidocaine solution, then preserved in 10% formalin. Some specimens were photographed in life using the apparatus described by ALTIG & McDIARMID (1999) and reared until after metamorphosis. For the external morphological characterization of the tadpoles we analyzed 14 specimens at developmental stages between 30 and 42 of GOSNER (1960). Descriptions of external morphology and proportions are based on four tadpoles at stages between 33 and 35 (lot UFMG-G 2455a). Specimens of other developmental stages (i.e., those not used in the description: lots UFMG-G 2455b–e) were used to assess intraspecific variation. In the description, ratios between measurements are expressed as ranges. Terminology for external morphology follows ALTIG & McDIARMID (1999), except for the lateral line system, which follows LANNOO (1987). Measurements follow ALTIG & McDIARMID (1999) for total length (TL), body length (BL), tail length (TAL), maximum tail height (MTH), internarial distance (IND), interorbital distance (IOD), tail muscle width (TMW), and tail muscle height (TMH); LAVILLA & SCROCCHI (1986) for body width (BW), body width at narial level (BWN), body width at eye level (BWE), body height (BH), eye–snout distance (ESD), eye–nostril distance (END), nostril–snout distance (NSD), eye diameter (ED), narial diameter (ND), snout–spiracle distance (SSD), and oral disc width (ODW; measured across closed oral disc); GROSJEAN (2005) for dorsal fin height (DFH) and ventral fin height (VFH); LINS et al. (2018) for spiracle length (SL), spiracle distal edge height (SDH), dorsal fin insertion angle (DFiA), oral disc position (ODP), and length of anterior gap in the row of marginal papillae of the

oral disc (AGL). Additionally, we measured the vent tube length (distance between the point of anterior insertion of the ventral wall of the tube in the body and its most distal margin, taken in ventral view; VTL). We photographed the preserved tadpoles using an adjustable platform to support the specimens in lateral, dorsal, and ventral views immersed in water (SCHACHT & McBRAYER 2009). Measurements were taken to the nearest 0.1 mm with the aid of ImageJ version 1.50b (SCHNEIDER et al. 2012).

Bioacoustics

Specimens were recorded using a Marantz PMD 660 digital recorder coupled to a Sennheiser K6/ME66 microphone at 44.1 kHz with 16-bit sampling size. The data were analyzed using software Raven Pro 64 v1.5 (Cornell Lab of Ornithology, Bioacoustics Research Program, 2017). Spectrograms and power spectra were produced with Window Type Hann, DFT size of 512 samples; Time Grid with 75% overlap, and hop size of 128 samples. Voucher specimens and recordings are now stored at the Fonoteca Neotropical Jacques Vielliard, Campinas, state of São Paulo, Brazil (FNJV 45025–45032).

For call analysis we adopted the call-centred approach and measured the following parameters sensu KÖHLER et al. (2017): call duration, interval between calls, minimum frequency, maximum frequency, peak frequency (using the Peak Frequency tool of Raven), minimum frequency at 5% of energy (using the Frequency 5% tool of Raven), maximum frequency at 95% of energy (using the Frequency 95% tool of Raven), and Bandwidth 90% (-10 dB; using the Bandwidth 90% tool of Raven).

Nomenclatural acts

The electronic edition of this article conforms to the requirements of the amended International Code of Zoological Nomenclature, and hence the new names contained herein are available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The LSID (Life Science Identifier) for this publication is: urn:lsid:zoobank.org:pub: urn:lsid:zoobank.org:pub:A878B284-5F41-4C04-A1AC-64C293A6DC86. The electronic edition of this work was published in a journal with an ISSN, and has been archived and is available from the following digital repositories: www.salamandra-journal.com, zenodo.org.

Results

Phylogenetic analysis

Our final concatenated dataset produced a matrix of 6631 bp and 49 terminals. Our parsimony analysis recovered 15 equally most parsimonious trees of 9781 steps

(Fig. 1). Conflicts between trees were limited to the clade encompassing terminals of the new species (which we comment on below). Our new samples were recovered as nested within those of *Aplastodiscus* sp. 4 acquired from BERNECK et al. (2016). This clade was recovered with 100% Parsimony Jackknife. Uncorrected p-distances between those samples were 0–0.7% in 16S, and 0–3.8% in COI (Supplementary document S3). This clade was recovered as the sister taxon of *A. ibirapitanga* with 100% Parsimony Jackknife, which is nested within the *A. albosignatus* group (100% Parsimony Jackknife), uncorrected p-distances between the new species and *A. ibirapitanga* varied by 2.6–3% in 16S, and 8.9–10.5% in COI. Uncorrected p-distances between the new species and the other species of the *A. albosignatus* group varied between 4.6–7.4% in 16S and 12.1–15.4% in COI (Supplementary document S3).

Topotypic samples of *Aplastodiscus cavicola* nested with the two samples of this species recovered by BERNECK et al. (2016) (i.e., MNRJ 63689 and FSFL 848) with 99% Parsi-

mony Jackknife (Fig. 1), with MNRJ 63689 being the sister of a clade encompassing FSFL 848 and topotypic samples (100% Parsimony Jackknife). Uncorrected p-distances between those samples were 0–4.9% in 16S, and 0–1.8% in COI (we were unable to acquire COI sequences for MNRJ 63689; Supplementary document S3). This clade was recovered as the sister taxon of *A. leucopygius* with 64% Parsimony Jackknife, which is nested within the *A. albosignatus* group (100% Parsimony Jackknife).

***Aplastodiscus heterophonius* sp. n.**
(Figs 1–5, Tables 1, 2)

ZooBank LSID: urn:lsid:zoobank.org:pub:A878B284-5F41-4C04-A1AC-64C293A6DC86

Remarks: This species has formerly been referred to as *Aplastodiscus* sp. by PIMENTA et al. (2014), *Aplastodiscus* sp. 4 by BERNECK et al. (2016), and *Aplastodiscus* aff. *cavicola* by PENA et al. (2017).

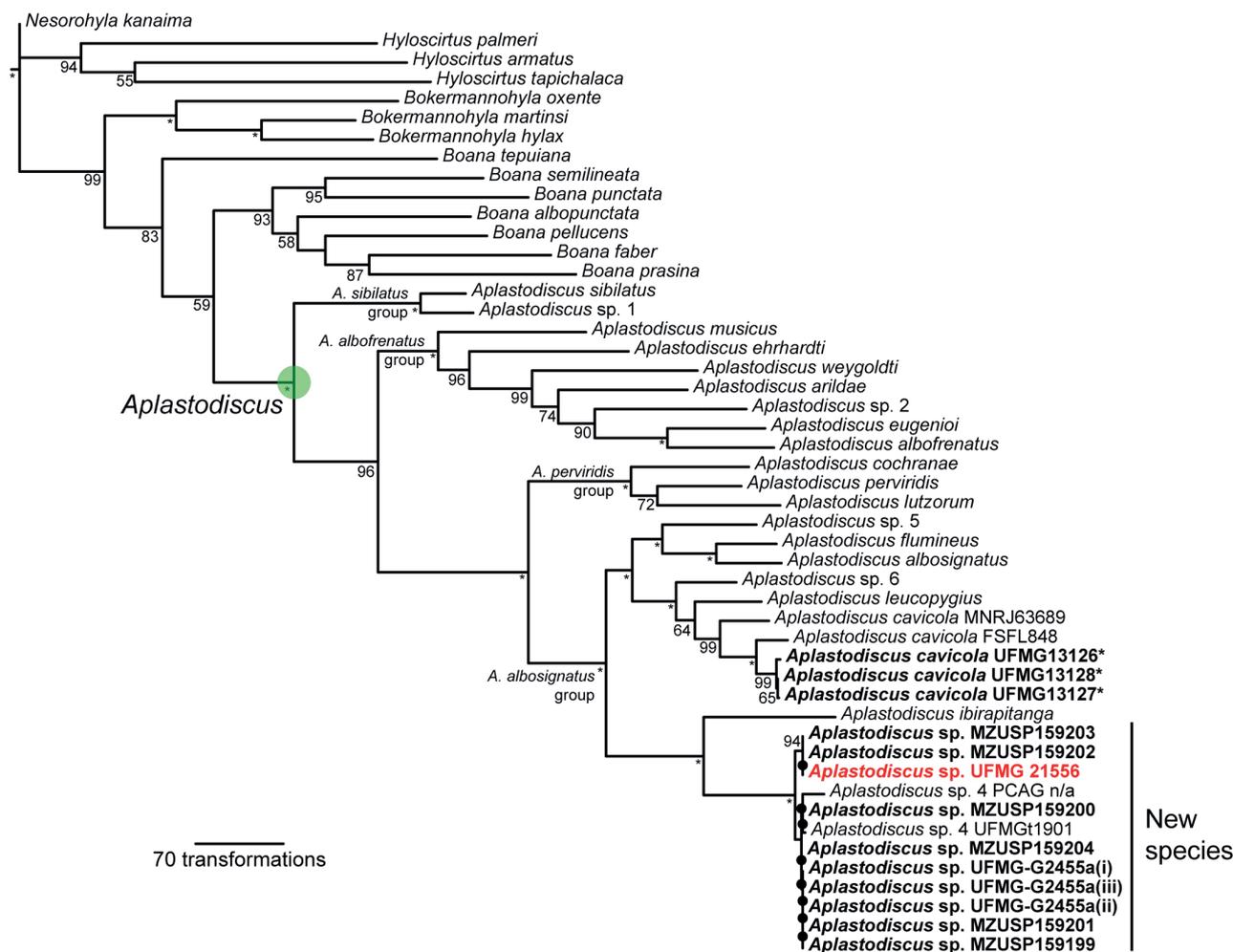


Figure 1. One of the 15 equally most parsimonious trees of 9781 steps recovered with TNT, with gaps treated as a fifth state. Black dots indicate nodes that collapse under strict consensus. Number around nodes are Parsimony Jackknife absolute frequencies; values < 50% are not shown; an * at a node indicates 100% Jackknife. Terminals in bold correspond to new samples employed in this study. Terminal in red mark the holotype of the new species. An * on terminals of *Aplastodiscus cavicola* indicates topotypic specimens.

Holotype: UFMG 21556, an adult male, collected at Fazenda Estiva, Dom Joaquim, Minas Gerais, Brazil (18°53'19.27" S, 43°19'3.39" W; 630 m a.s.l.), 24 January 2019, by F. S. F. LEITE and R. C. L. LIMA.

Paratypes: MZUSP 159203 and 159204, two adult males collected together with the holotype. MZUSP 159199 (froglet), 159200 (juvenile), 159205 (adult male), Serra do Intendente, Santana do Riacho, Minas Gerais, Brazil (19°9'54.99" S, 43°31'43.06" W; 1326 m a.s.l.), 21 January 2019, by F. S. F. LEITE and R. C. L. LIMA. MZUSP 159201, adult male, Morro do Pilar, road from Conceição do Mato Dentro to Morro do Pilar, Minas Gerais, Brazil (19°10'24.92" S, 43°23'57.52" W; 664 m a.s.l.), 22 January 2019, by F. S. F. LEITE and R. C. L. LIMA. MZUSP 159202, adult male, Conceição do Mato Dentro, Minas Gerais, Brazil (19°1'41.12" S, 43°24'9.00" W; 889 m a.s.l.), 23 January 2019, by F. S. F. LEITE and R. C. L. LIMA. UFMG-G 2455, 14 tadpoles sorted in lots a–e, Serra do Intendente, Santana do Riacho, Minas Gerais, Brazil (19°9'54.99" S, 43°31'43.06" W; 1326 m a.s.l.), 21 January 2019, by F. S. F. LEITE and R. C. L. LIMA. UFMG 6668, adult male, collected at Alvorada de Minas, Minas Gerais, Brazil (18°51'46.92" S, 43°24'36.51" W; 668 m a.s.l.), 30 June 2010, by D. P. R. CABRAL and B. C. FIGUEIRO. UFMG 6671, adult male, collected at Dom Joaquim, Minas Gerais, Brazil (18°53'22.73" S, 43°19'1.89" W; 608 m a.s.l.), 29 July 2010, by D. P. R. CABRAL and B. C. FIGUEIRO. UFMG 17524, adult male, Conceição do Mato Dentro, Minas Gerais, Brazil (19°01'54.41" S, 43°22'43.72" W; 713 m a.s.l.), 13 February 2014, by A. L. SILVEIRA and C. R. RIEVERS. UFMG 17525–17526, two adult males, Conceição do Mato Dentro, Minas Gerais, Brazil (19°03'0.40" S, 43°23'57.12" W; 922 m a.s.l.), 13–15 February 2014, by A. L. SILVEIRA and C. R. RIEVERS. UFMG 17527–17528, two adult males, Conceição do Mato Dentro, Minas Gerais, Brazil (19°06'36.68" S, 43°17'53.92" W; 751 m a.s.l.), 20 February 2014, by A. L. SILVEIRA and C. R. RIEVERS. UFMG 17529, subadult, Conceição do Mato Dentro, Minas Gerais, Brazil (18°57'22.87" S, 43°20'52.06" W; 608 m a.s.l.), 10 October 2014, by A. L. SILVEIRA. UFMG 18008, adult male, Santana do Riacho, Minas Gerais, Brazil (19°09'56.17" S, 43°31'44.30" W; 1328 m a.s.l.), 31 October 2015, by P. C. A. GARCIA, I. RODRIGUES, J. V. LACERDA, I. MARQUES, and D. FONSECA. MCNAM 15076, adult male, Morro do Pilar, Minas Gerais, Brazil (19°08'59.21" S, 43°23'23.89" W; 685 m a.s.l.), 19 November 2010, by R. R. CARVALHO JR. MCNAM 17852, adult female, 17859–17860, two adult males, Conceição do Mato Dentro, Minas Gerais, Brazil, between November and December 2012, by D. C. R. COSTA. MCNAM 18236, adult male, Dom Joaquim, Minas Gerais, Brazil (18°53'19.33" S, 43°19'3.57" W; 629 m a.s.l.), 26 June 2013, by R. A. MURTA-FONSECA. MCNAM 18410–18413, four adult females, 18414, adult male, Dom Joaquim, Minas Gerais, Brazil (18°54'16.36" S, 43°20'17.26" W; 643 m a.s.l.), 26 November 2013, by R. A. MURTA-FONSECA and F. QUEIROZ. MCNAM 18415, juvenile, Dom Joaquim, Minas Gerais, Brazil (18°53'21.55" S, 43°19'1.46" W; 631 m a.s.l.), 27 November 2013, by R. A. MURTA-FONSECA and F. QUEIROZ.

MCNAM 18966, adult male, Serro, Minas Gerais, Brazil (18°33'3.33" S, 43°22'38.63" W; 938 m a.s.l.), 28 November 2013, by A. M. LINARES. MCNAM 18987–18988, two adult males, Serro, Minas Gerais, Brazil (18°32'25.53" S, 43°22'46.59" W; 951 m a.s.l.), 1 December 2013, by A. M. LINARES. MCNAM 18990, adult male, Serro, Minas Gerais, Brazil (18°33'21.45" S, 43°24'3.14" W; 757 m a.s.l.), 28 January 2014, by A. M. LINARES.

Etymology: The specific epithet derives from the Greek words ἕτερος (“heteros”), which means different, and φωνή (“phoné”), which means sound (and, also, the voice or cry of animals). The radicals *heter-* and *phon-* are linked through the connecting vowel *-o-*, followed by the Greek suffix *-ικός*, latinized as *-icus*, to form an adjective. The resulting specific epithet, *heterophonicus*, means “the one with a different voice”, in allusion to the very distinct call of the new species of *Aplastodiscus* described here.

Diagnosis: A green treefrog species of the genus *Aplastodiscus* characterized by adults with (1) small size (SVL 26.9–33.6 mm in males; 28.3–33.6 mm in females); (2) a well-developed supraclacal crest (character “present, restricted to the supraclacal region” of BERNECK et al. 2016); (3) presence of webbing between fingers and toes; (4) presence of lateral fimbriae on forearms and shanks; (5) absence of nuptial pad in males; (6) in life, iris red to orange with a dark grey to silvery ring around the pupil; (7) small fringe of guanophores on lower lip margin (same as “white submandibular dermal fold” of BERNECK et al. 2016; BEZERRA et al. 2020); (8) no sexual dimorphism in SVL. Advertisement call (9) composed of scratchy notes partially pulsed to pulsatile; (10) presence of ascending frequency modulation; (11) call duration 167–280 ms; (12) peak frequency between 661.3 and 947.5 Hz. (13) Tadpoles with LTRF 2(2)/4(1); (14) oral disc with single row of marginal papillae; (15) with an anterior gap; (16) absence of posterior gap; (17) presence of few submarginal papillae laterally in the oral disc; (18) absence of small flaps with labial teeth laterally in the oral disc; (19) absence of paired light spots in the posterior portion of the body; (20) presence of dark longitudinal stripes in the tail, one between dorsal and ventral myotomes of muscle, restricted to the proximal quarter of tail length, and an interrupted narrow brown line on the dorsal margin of the muscle; (21) tail homogeneously marbled along its length; (22) presence of translucent fins.

Comparisons with other species of *Aplastodiscus*:

Adults: *Aplastodiscus heterophonicus* can be promptly distinguished from the species of the *Aplastodiscus sibilatus* group by the presence of a supraclacal crest and absence of a subclacal dermal fold (absence of supraclacal crest, and presence of subclacal dermal fold in *A. sibilatus*; CRUZ et al. 2003; BERNECK et al. 2016). From the species of the *A. perviridis* group, *A. heterophonicus* can be distinguished by the presence of a supraclacal crest (absent in *A. perviridis* group), presence of webbing between fingers and toes (absent); presence of lateral fimbriae on forearms

and shanks (absent); iris red to orange with a dark grey to silvery ring around the pupil in life (iris with distinct upper and lower colorations; GARCIA et al. 2001; BERNECK et al. 2017). The presence of a small fringe of guanophores on the lower lip of *A. heterophonicus* differentiates it from all species of the *A. abofrenatus* and *A. perviridis* groups but *A. musicus* (fringe of guanophores absent on the lower lip in the *A. abofrenatus* and *A. perviridis* groups, present in *A. musicus*; BERNECK et al. 2016, BEZERRA et al. 2020). The absence of nuptial pad differentiates the new species from *A. musicus* (present; LUTZ 1949). The presence of a supraclacal crest also promptly distinguishes the new species from *A. ehrhardti*, *A. arildae*, and *A. weygoldti* (cloacal ornamentation in those species is limited to iridophores in the outer cloacal epithelium; BERNECK et al. 2016); and from *A. flumineus*, *A. albosignatus*, and *Aplastodiscus* sp. 5 (cloacal ornamentation elliptical, around the cloacal region in those species; BERNECK et al. 2016). The absence of sexual dimorphism in SVL differentiates *A. heterophonicus* from *A. eugenioi* and *A. ibirapitanga* (females larger than males; CRUZ et al. 2003; CARVALHO-E-SILVA & CARVALHO-E-SILVA 2005). The smaller SVL in males (26.9–33.6 mm) separates the new species from *A. abofrenatus*, *A. ibirapitanga*, and *A. leucopygius* (SVL 37–44.2 mm in those species combined; CRUZ et al. 2003; HEYER et al. 1990). The female SVL of the new species (28.3–33.6 mm) is smaller than in *A. eugenioi* (36–39 mm; CARVALHO-E-SILVA & CARVALHO-E-SILVA 2005). Although the range of SVL of males of *A. heterophonicus* and *A. cavicola* overlaps, that of the new species (26.9–33.6 mm) tends to separate it from *A. cavicola* (32.3–41.7 in specimens measured by us, see Appendix, and 33.2–37.3 mm according to CRUZ & PEIXOTO 1985). According to BERNECK et al. (2016), no diagnostic phenotypic character is known for *Aplastodiscus* sp. 6.

Tadpoles: Among the 15 currently recognized species of *Aplastodiscus*, the tadpoles of five remain undescribed (i.e., *A. ehrhardti*, *A. flumineus*, *A. lutzorum*, *A. musicus*, and *A. weygoldti*). The tadpole of *A. heterophonicus* can be promptly distinguished from those of *A. sibilatus* by the number of anterior (i.e., 2) and posterior (i.e., 4) tooth rows, by presenting a single row of marginal papillae, an anterior gap on marginal papillae, and few submarginal papillae laterally in the oral disc (4 anterior and 5–7 posterior tooth rows, bi- and triserial rows of marginal papillae without an anterior gap, and high density of submarginal papillae in the lateral portions of oral disc in *A. sibilatus*; MERCÊS & JUNCÁ 2010). From species of the *A. abofrenatus* group, *A. heterophonicus* tadpoles differ from those of *A. abofrenatus* by the number of tooth rows and absence of small flaps with labial teeth laterally in the oral disc (3 to 4 anterior and 5 to 7 posterior tooth rows and flaps with labial teeth in *A. abofrenatus*; PEIXOTO & CRUZ 1983). *Aplastodiscus heterophonicus* tadpoles differ from those of *A. arildae* by the tail colour pattern, which is homogeneously marbled with small spots in *A. heterophonicus* and exhibits large transversal dark spots that will be concentrated mainly in the posterior third of the tail in *A. arildae* (LEITE et al. 2007). From *A. eugenioi*, the tadpoles of *A. heterophonicus*

differ by the absence of paired light spots in the posterior portion of the body and by the dark longitudinal stripe between the dorsal and ventral myotomes of the tail muscle, which is restricted to the proximal quarter of the tail (presence of light spots on the body and a brown longitudinal stripe reaching to the end of the tail in *A. eugenioi*; CARVALHO-E-SILVA & CARVALHO-E-SILVA 2005). In the *A. perviridis* group, the tadpoles of *A. heterophonicus* differ from those of *A. cochranae* by the presence of an interrupted narrow brown line along the dorsal margin of the tail musculature (absence of a brown line on the dorsal margin of the tail in *A. cochranae*; GARCIA et al. 2001). *Aplastodiscus heterophonicus* tadpoles differ from those of *A. perviridis* by their homogeneous tail colour pattern and translucent fins (tail gradually darkening along its length, and outer margins of fins reddish in *A. perviridis*; CARAMASCHI et al. 1980). Regarding the *A. albosignatus* group, the tadpoles of *A. heterophonicus* differ from those of *A. albosignatus* and *A. ibirapitanga* by the presence of few submarginal papillae laterally in the oral disc (submarginal papillae absent in these species; PEIXOTO & CRUZ 1983, MERCÊS & JUNCÁ 2010). Tadpoles of *A. heterophonicus* are very similar to those of *A. leucopygius* and *A. cavicola*. They differ from those of *A. leucopygius* by the absence of reddish coloration in the fins (fins with reddish tones in *A. leucopygius*; GOMES & PEIXOTO 2002). From *A. cavicola*, *A. heterophonicus* may differ by the absence of a narrow posterior gap in the row of marginal papillae (narrow posterior gap in *A. cavicola*; PEZZUTI et al. 2010; but see Discussion below).

Advertisement Call: The only species with no advertisement call description is *Aplastodiscus flumineus*. The call of *A. heterophonicus* can be promptly distinguished from the calls of all its congeners by being composed of a scratchy note partially pulsed or pulsatile (whistling tonal notes in the other species; BOKERMANN 1967, HEYER et al. 1990, HADDAD & SAWAYA 2000, GARCIA et al. 2001, HARTMANN et al. 2004, ABRUNHOSA et al. 2005, CONTE et al. 2005, HADDAD et al. 2005, CARVALHO JR. et al. 2006, ORRICO et al. 2006, ZINA & HADDAD 2006, MIRANDA et al. 2016, BERNECK et al. 2017, BEZERRA et al. 2020). The ascending frequency modulation differentiates the call of *A. heterophonicus* from the calls of *A. albosignatus*, *A. arildae*, *A. ehrhardti*, *A. eugenioi*, *A. ibirapitanga*, *A. musicus*, *A. sibilatus*, and *A. weygoldti* (calls without frequency modulation in these species; HADDAD & SAWAYA 2000, HARTMAN et al. 2005, ABRUNHOSA et al. 2005, CONTE et al. 2005, CARVALHO JR. et al. 2006, ORRICO et al. 2006, ZINA & HADDAD 2006, MIRANDA et al. 2016, BEZERRA et al. 2020), and from *A. abofrenatus* (descending frequency modulation; HEYER et al. 1990). The call of the new species has a longer duration (167–280 ms) than the calls of *A. abofrenatus*, *A. arildae*, *A. ehrhardti*, *A. musicus*, *A. leucopygius*, *A. sibilatus*, and *A. weygoldti* (advertisement calls 8.1–140 ms combined; BOKERMANN 1967, HEYER et al. 1990, HADDAD & SAWAYA 2000, ABRUNHOSA et al. 2005, CONTE et al. 2005, CARVALHO JR. et al. 2006, ORRICO et al. 2006, ZINA & HADDAD 2006, BEZERRA et al. 2020), and a shorter duration than the advertisement call of *A. cavicola*

(360–580 ms; ABRUNHOSA et al. 2005). Its peak frequency, normally within the fundamental between 661.3–947.5 Hz, differentiates the call of *A. heterophonicus* from those of species of the *A. albofrenatus*, *A. perviridis*, and *A. sibilatus* groups, which are at higher frequencies between 1700 and 3100 Hz (values combined; BOKERMANN 1967, HEYER et al. 1990, HADDAD & SAWAYA 2000, GARCIA et al. 2001 ABRUNHOSA et al. 2005, CONTE et al. 2005, HADDAD et al. 2005, HARTMAN et al. 2005, CARVALHO JR. et al. 2006, ORRICO et al. 2006, ZINA & HADDAD 2006, MIRANDA et al. 2016, BERNECK et al. 2017, BEZERRA et al. 2020).

Holotype description (Figs 2–3): Adult male, small, SVL 28.8; body slender; head wider than body, nearly as wide

as long (HW/HL = 1.03); snout rounded in dorsal view (SL/HL = 0.45; SL/HW = 0.44), with a tiny anterior projection, round in profile; END almost the same length as ED (END/ED = 1.02); canthus rostralis slightly curved in dorsal view and rounded in cross-section; loreal region slightly concave; lips thin, not flared; internarial region barely depressed; nostrils barely protuberant, dorsolaterally directed; interorbital area flat, larger than ED (IOD/ED = 1.12), about a third of HW (IOD/HW = 0.31). Eyes large and protuberant (ED/HL = 0.29; ED/HW = 0.28); palpebral membrane translucent, without reticulations. Supratympanic fold weakly developed, covering the upper portion of tympanic ring, extending to arm insertion. Tympanum moderately large (TD/ED = 0.64; TD/HL = 0.18),

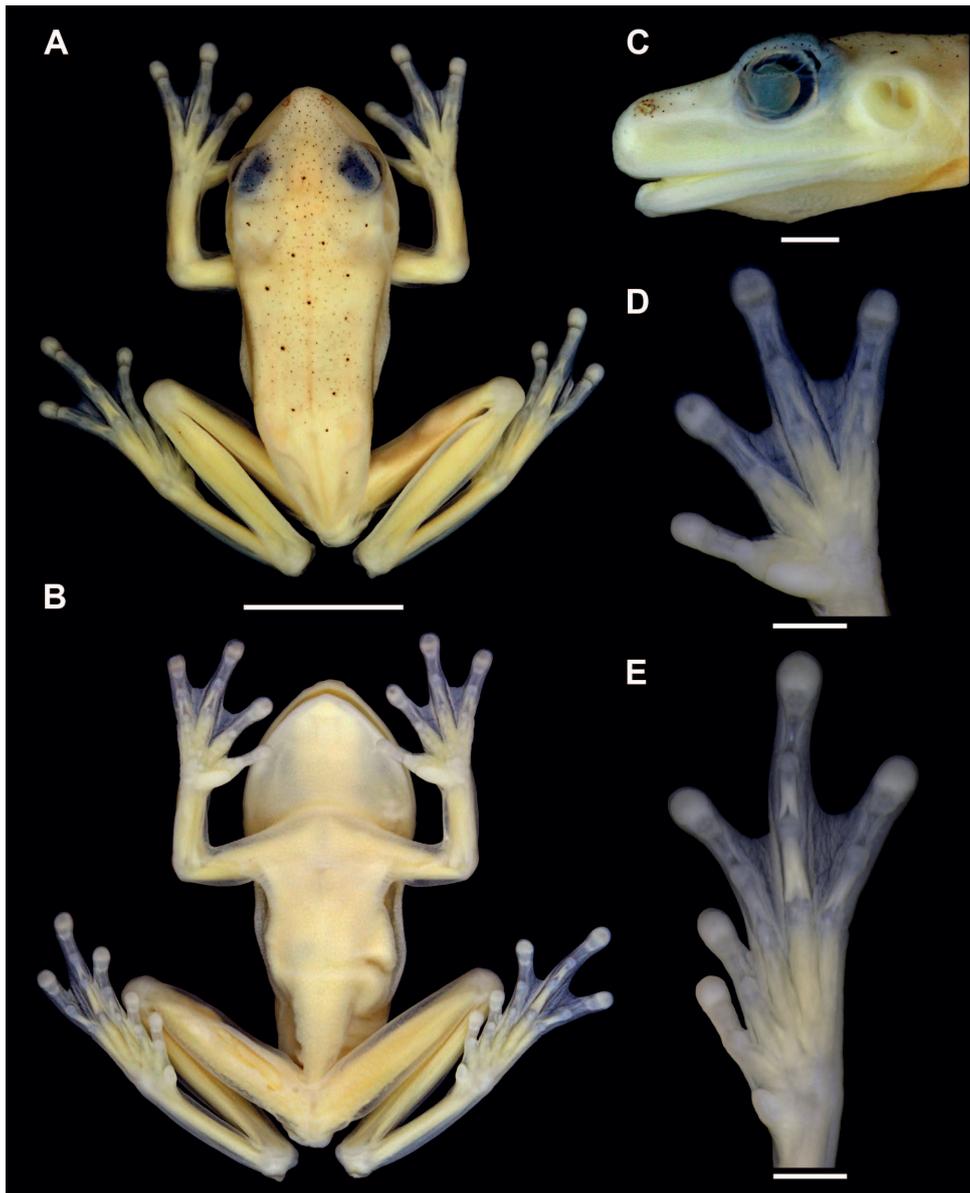


Figure 2. Holotype of *Aplastodiscus heterophonicus* sp. n. (UFMG 21556): A) dorsal and B) ventral views; C) lateral profile of the head; D) palmar surface of left hand; E) plantar surface of tarsal region of left foot. Scale bars of A and B = 10 mm; C–E = 2 mm.

distinct, directed dorsolaterally, separated from eye by a distance of about 0.60 TD.

Arm slender, not hypertrophied, lacking an axillary membrane; a homogeneous fringe extends from the proximal limit of the hand to the elbow. Fingers long, bearing round discs; disc on finger II smaller than all others; disc diameter on finger IV narrower than tympanum ($TD/4FD = 1.46$); relative finger lengths $II < III < V < IV$; webbing formula $II-III 1^{1/2}-2^+ IV 2-2V$; presence of lateral fringes on fingers; subarticular tubercles distinct, nonbifid, and rounded in ventral view, flattened in profile; supernumerary tubercles present; inner metacarpal tubercle flat and elongated; outer metacarpal tubercle bifid, flat, and large. Nuptial pad absent.

Hind limbs long and slender ($THL/SVL = 0.53$; $TL/SVL 0.52$); tarsal fold absent, a homogeneous tarsal fringe extends from Toe V to the lateral margin of the ankle, continuous with the calcar; calcars small, developed as a fringe across the ankle, with a tiny projection laterally. Toes long, bearing round discs of equal diameters, almost equal to the diameters of those on the fingers ($4TD/4FD = 0.96$); relative toe lengths $I < II < III = V < IV$; webbing formula $I 2-2^+ II 1^+-2^{1/2} III 1^{1/2}-2^{1/2} IV 2^{1/2}-1 V$; lateral fringes on toes present. Subarticular tubercles round in ventral view, flattened in profile; supernumerary tubercles present; outer metatarsal tubercle absent; inner metatarsal tubercle distinct, flat, and elliptical in ventral view.

Skin smooth except in the gular, pectoral, and abdominal areas and on the ventral faces of the thighs, where it is granular. Pectoral fold absent. Small fringe of guanophores on lower lip margin. Cloacal opening directed posteroventrally at upper level of thighs; cloacal sheath absent; a large white, supraclacal dermal fold, limited to the supraclacal region, extends posteriorly, covering the cloacal opening in dorsal view; subclacal dermal fold absent; cloacal granules present, heterogeneous, and scattered below cloacal opening.

Tongue slightly cordiform, barely free behind; denticerous processes of vomers prominent, in two separated, curved series converging medially, bearing six teeth each. Choanae large, almost rounded, spaced 2.5 mm from each other. Vocal slits moderately long, extending from about the distal third of tongue length, almost reaching the angle of jaws. Vocal sac poorly developed, single, median, and subgular. Mental gland, if present, not discernible under stereomicroscope.

Measurements of holotype (mm): SVL 28.8, HL 10.7, HW 11.0, ED 3.1, END 3.1, IND 2.2, EW 2.2, IOD 3.4, TD 2.0, TL 15.1, TAL 8.3, FL 13.7, SL 4.9, THL 15.3, 4FD 1.4, 4TD 1.3.

Coloration in life (Fig. 3): Body overall green. Dorsum green, with sparse, yet homogeneously distributed spots of melanophores, and small whitish flecks. Thighs and shanks a little darker than body. Articulations of limbs and limbs insertions bluish green, probably due to accumulations of biliverdin, as is the darker coloration of thighs and shanks. Flanks, belly, and upper eyelids yellowish, probably due to the contrast with the white peritoneum under the skin.

Hidden surfaces of thighs yellowish green. Fringes on forearms, lower lip, and shanks, calcars, supraclacal crest, and clacal tubercles whitish. Iris orange, almost red, with a black peripheral ring. A ring with an irregular margin, formed by dark grey and silvery pigments, surrounds the pupil.

Coloration in preservative (Fig. 2): The green, bluish green and yellow colours due to biliverdin completely vanished in alcohol and the specimen became overall cream. White flecks disappeared completely as well. Spots of melanophores were maintained, and tiny melanophores are still visible, sparsely scattered over the dorsal side of the body. The white colour of the fringes on the forearms, lower lip, shanks, calcars, supraclacal crest, and clacal tubercles almost completely vanished. The tympanum became translucent, revealing the pars externa plectri. The iris lost its orange colour, becoming silvery externally, and black around the pupil.

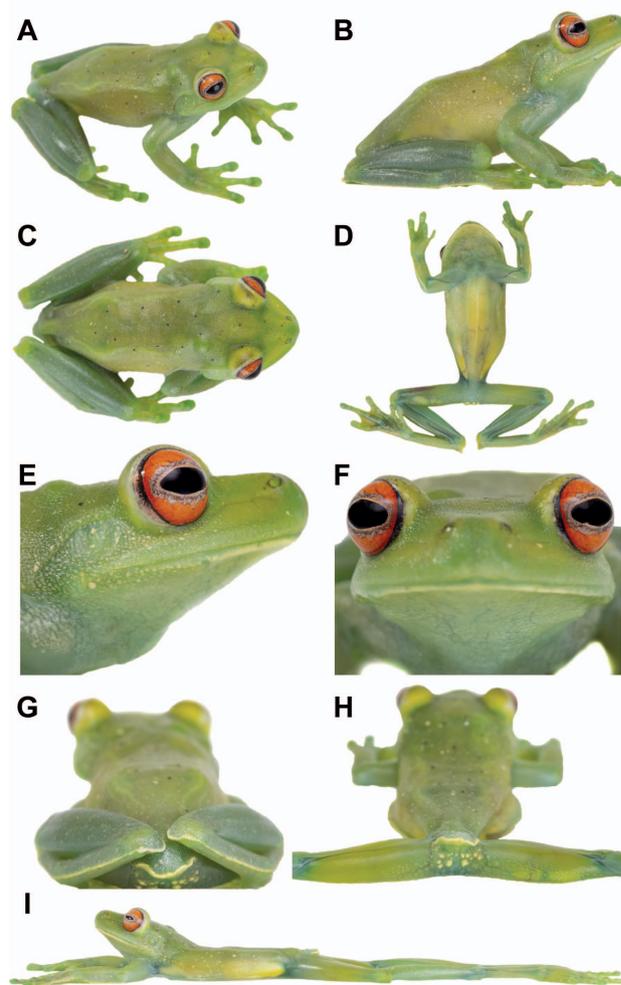


Figure 3. Coloration in life of the holotype of *Aplastodiscus heterophonius* sp. n. (UFMG 21556): A) dorsolateral, B) lateral, C) dorsal, and D) ventral views of body; E) lateral and F) frontal views of head; G) posterior view of body; H) detail of cloacal region; I) flank. Images not to scale. Photo credits: H. Thomassen.

Table 1. Selected morphometric data of the type series of *Aplastodiscus heterophonius* sp. n. Values (in mm) are given as ranges (mean \pm SD). See text for explanation of measurement abbreviations.

Measurements	Body ratio	
	Males (n = 23)	Females (n = 5)
SVL	27.1–33.6 (30.5 \pm 1.7)	28.3–33.6 (30.9 \pm 2.2)
HL	10.4–12.6 (11.3 \pm 0.5)	10.3–12.1 (11.3 \pm 0.7)
HW	9.5–11.8 (10.8 \pm 0.6)	9.9–12.0 (10.9 \pm 0.7)
ED	2.7–3.5 (3.2 \pm 0.2)	3.4–3.6 (3.4 \pm 0.1)
END	2.9–3.7 (3.3 \pm 0.2)	2.9–3.3 (3.1 \pm 0.2)
IND	2.0–2.5 (2.2 \pm 0.2)	1.9–2.3 (2.1 \pm 0.2)
EW	1.9–2.6 (2.2 \pm 0.2)	2.3–2.8 (2.6 \pm 0.2)
IOD	2.9–3.6 (3.4 \pm 0.2)	3.4–3.8 (3.5 \pm 0.2)
TD	1.8–2.7 (2.2 \pm 0.2)	2.0–2.7 (2.5 \pm 0.3)
TL	14.3–17.4 (15.5 \pm 0.7)	14.1–16.1 (15.4 \pm 0.9)
TAL	7.8–9.9 (8.7 \pm 0.5)	7.5–8.9 (8.5 \pm 0.6)
FL	12.1–15.1 (13.6 \pm 0.7)	11.4–13.8 (13.0 \pm 1.0)
SL	4.6–5.6 (5.2 \pm 0.3)	4.6–5.4 (4.9 \pm 0.3)
THL	13.2–16.8 (15.4 \pm 0.8)	14.1–17.0 (15.6 \pm 1.1)
4FD	1.3–1.7 (1.5 \pm 0.1)	1.3–1.8 (1.6 \pm 0.2)
4TD	1.1–1.7 (1.4 \pm 0.1)	1.2–1.7 (1.6 \pm 0.2)

Variation: Morphometric variations are presented in Table 1. Hand webbing formula varies as II–III ($1-1^{1/2}$)–($2-2^{1/2}$) IV ($2-2^+$)–(1^+-2) V. Foot webbing formula varies as I (1^+-2)–($2-3^-$) II ($1-1^{1/2}$)–(2^+-3^-) III ($1-1^{1/2}$)–($2-3^-$) IV ($2-3^-$)–($1-1^{1/2}$) V. Some specimens have a stouter body, with the head as wide as body at the level of the arms (UFMG 6668, 17524, 17526, 17527, MCNAM 17852, 18236, 18410, 18412, 18413, 18987). The snout might be truncated in dorsal view (UFMG 17526, 17529) and without the anterior projection (UFMG 6668, 17524–17529, 18008, MZUSP 159204, MCNAM 15076, 17852, 17859, 17860, 18236, 18410, 18411, 18413, 18966, 18987, 18990). MZUSP 159205 has a barely discernible anterior projection. In profile, the snout is truncated in UFMG 17529 and MCNAM 17852. The internarial region is flat in UFMG 6668, 6671, 17526, 17527, 17529, 18008, MZUSP 159201, MCNAM 15706, 17859, 18236, 18410, 18412–18414, 18966, 18987, 18988, and 18990. Forearm fringes are poorly developed in UFMG 17525, 17528, and MCNAM 17852. Supernumerary tubercles on the hands are barely discernible in MZUSP 159203, MCNAM 17852, 17859, and 18988. The inner metacarpal tubercle is barely discernible in MCNAM 17852. The outer metacarpal tubercle is barely discernible in MCNAM 17852 and 18411.

The tarsal fold is short but present in MZUSP 159205. Tarsal fringes are poorly developed in UFMG 17525 and MCNAM 17852. The calcar varies in having a more distinctly developed tip laterally (UFMG 17524, MZUSP 159203, MCNAM 17860, 18410) or being poorly developed (UFMG 17525, MZUSP 159201, 159202, MCNAM 17852, 18966, 18988). Supernumerary tubercles on feet are evident in UFMG 6671, 17524, 17525, 17529, MZUSP 159202, MCNAM 17860, 18236, 18966, and 18987. Cloacal granules are barely evident in UFMG 17525, MZUSP 159201–159205, MCNAM 18410, 18413, 18987, 18988, and 18990.

Dentigerous processes of vomers vary in being arranged either in a straight transversal line (MZUSP 159203), straight and converging anteriorly (UFMG 17524, 17528, MZUSP 159204, MCNAM 18236, 18411), or arched and transversal (MZUSP 159205 and MCNAM 18966). Numbers of teeth vary between 3 and 9 (6.1 ± 1.6 ; $n = 29$) in the right process; and between 3 and 10 (6.5 ± 1.5 ; $n = 29$) in the left one.

White flecks remained evident after preservation in UFMG 18008, MCNAM 15076, 17852, 17859, 18236, and 18410–18414. Similarly, the white coloration of the lower lip, crests, and fringes remained evident in UFMG 17526–17528, MCNAM 15076, 17852, 17859, 17860, 18236, 18410–18414, and 18987 (in this last specimen, the white coloration is evident only on the cloacal crest). The iris might be completely black in preserved specimens (UFMG 6671), entirely silvery (UFMG 17526, 17528, 18008, MCNAM 17859, 18236, 18410, 18412, 18413), or silvery to copper distally, with a gradient to dark from the periphery inwards to the pupil (MCNAM 15076, 17852, 17860, 18411, 18414, 18966, 18987, 18988, 18990). The mentioned variation in coloration probably is due to different conditions of preservation. The skin on the tympanum is not completely translucent in MCNAM 18236. Small white blotches varying in number and position throughout the body are present in UFMG 17527–17529, MCNAM 15076, 17852, 18236, 18410–18414, and 18987.

Larval morphology: (Fig. 4, Table 2) Body depressed (BH/BW 0.75–0.85; Figs 4A and B); its length 0.27–0.30 total length; elliptical in dorsal view; in lateral view, the ventral contour is slightly convex from the peribranchial region to the final portion of abdomen. Snout rounded in lateral and dorsal views (BWN/BWE 0.71–0.75; Figs 4D and E). Nostrils positioned dorsally (IND/BWN 0.60–0.64), large

(ND/BL 0.04–0.05), elliptical, directed dorsolaterally, with a large triangular fleshy projection on the medial margin that gives each nostril a reniform outline (Fig. 4E); located approximately halfway between eyes and tip of snout (NSD/ESD 0.50–0.54). Eyes positioned dorsally (IOD/BWE 0.65–0.67), medium-sized (ED/BWE 0.20–0.23), directed dorsolaterally. Spiracle sinistral, lateral, visible in dorsal and ventral views (SDH/BH 0.54–0.62), medium-sized (SL/BL 0.11–0.15), directed posterodorsally; its inner wall free from the body wall and slightly longer than its external wall; opening located between the middle and posterior thirds of the body (SSD/BL 0.62–0.68; Fig. 4D). Intestinal tube circularly coiled; its switchback point located at the centre of abdominal region. Vent tube long (VTL/BL 0.16–0.24), with dextral opening; its mid-ventral portion fused to the ventral fin (Fig. 4C). Tail low (MTH/TAL 0.20–0.26), but higher than body (MTH/BH 1.12–1.16); tail musculature robust (TMH/BH 0.59–0.74; TMW/BW 0.50–0.56), reaching the tip of the pointed tail. Dorsal and ventral fins low (DFH/TAL 0.06–0.08; VFH/TAL 0.04–0.06),

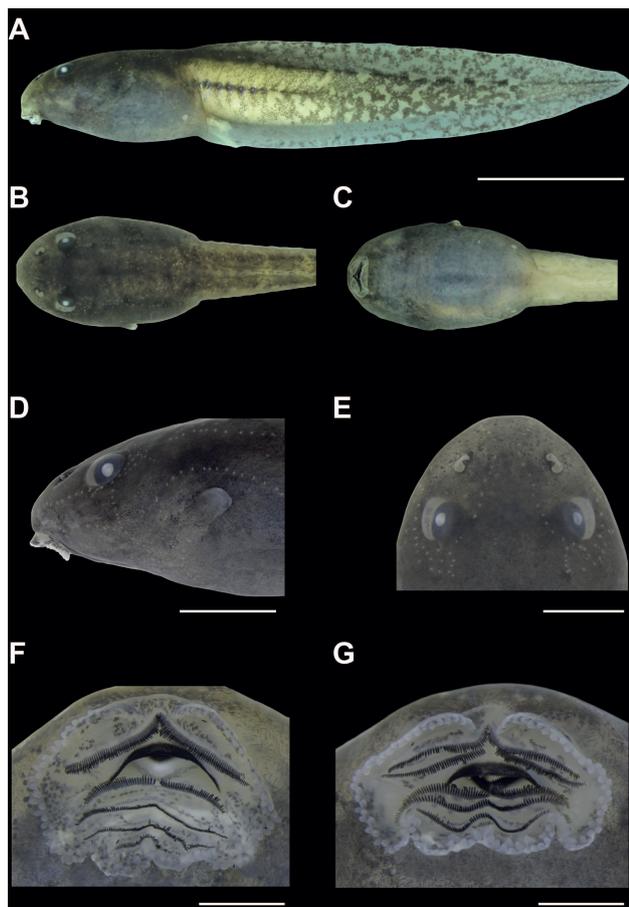


Figure 4. Tadpole of *Aplastodiscus heterophonicus* sp. n. at Gosner stage 34: A) lateral, B) dorsal, and C) ventral views; details of snout in lateral (D) and dorsal (E) views (lot UFMG-G 2455a). Oral disc completely opened at stage 34 (F) (lot UFMG-G 2455a), and partially opened at stage 33 (G) (lot UFMG-G 2455b). Scale bars A–C = 10 mm; D–E = 2 mm; F–G = 1 mm.

Table 2. Morphometric data (in mm) of tadpoles of *Aplastodiscus heterophonicus* sp. n. in two groups of stages of GOSNER (1960). The ranges (in mm) are followed by mean and standard deviation; see text for abbreviations.

Characters	Stages 30–32 (n = 4)	Stages 33–35 (n = 4)
BH	5.6–6.4 (6.1±0.4)	6.4–7.0 (6.7±0.3)
BL	11.2–12.9 (12.3±0.7)	13.1–13.8 (13.4±0.3)
BW	6.6–7.9 (7.3±0.6)	8.5–10.3 (9.2±0.6)
BWE	5.7–6.5 (6.2±0.4)	7.4–8.9 (7.9±0.6)
BWN	4.3–4.7 (4.6±0.2)	5.5–6.7 (6.0±0.5)
DFH	2.1–2.4 (2.2±0.1)	2.2–2.7 (2.5±0.2)
DFiA	0.4°–7.2° (2.5°±3.0)	0.1°–15.3° (6.5°±6.6°)
ED	1.3–1.5 (1.4±0.1)	1.3–1.6 (1.5±0.1)
END	1.8–2.1 (1.9±0.1)	1.9–2.1 (2.0±0.1)
ESD	3.8–3.9 (3.9±0.0)	3.9–4.3 (4.1±0.2)
IND	2.8–3.0 (2.9±0.1)	3.0–3.1 (3.1±0.1)
IOD	4.1–4.6 (4.3±0.2)	4.3–4.7 (4.5±0.2)
MTH	6.6–7.4 (6.9±0.4)	7.3–8.2 (7.7±0.4)
ND	0.6–0.7 (0.7±0.1)	0.6–0.7 (0.6±0.0)
NSD	1.9–2.1 (2.1±0.1)	2.0–2.3 (2.1±0.1)
ODP	21.7°–25.5° (24.2°±1.7)	22.0°–30.0° (28.2°±4.1)
ODW	2.5–2.8 (2.7±0.1)	2.6–3.3 (3.0±0.3)
SDH	2.9–4.1 (3.6±0.5)	3.5–4.3 (3.9±0.3)
SL	1.4–1.6 (1.5±0.1)	1.5–1.9 (1.6±0.2)
SSD	7.0–8.5 (7.9±0.6)	8.5–9.1 (8.8±0.2)
TAL	27.6–31.7 (29.3±1.8)	31.1–36.5 (32.8±2.5)
TL	39.4–44.2 (41.5±2.1)	44.4–50.2 (46.2±2.7)
TMH	3.7–4.1 (3.9±0.2)	4.1–4.8 (4.3±0.3)
TMW	3.7–4.2 (4.0±0.2)	4.0–4.8 (4.4±0.3)
VFH	1.5–1.9 (1.6±0.2)	1.6–2.0 (1.8±0.2)
VTL	1.6–1.9 (1.8±0.1)	2.1–3.3 (3.0±0.3)

their free margins slightly convex; dorsal fin emerging in posterior third of the body at a low angle (DFiA 1–15°); its maximum height in the posterior third of the tail; point of emergence of ventral fin concealed by vent tube.

Oral disc (Figs 4F and G) positioned ventrally (ODP 22.4–30.1°), medium-sized (ODW/BW 0.32–0.42), with three posterior emarginations (one medial and two lateral); 109–125 conical marginal papillae organized in one row, with their bases being offset lateroposteriorly in the oral disc; with a narrow anterior gap (AGL/ODW 0.08–0.15); two to ten submarginal papillae located in each lateral portion of the oral disc. Labial tooth row formula 2(2)/4(1); gaps in A-2 and P-1 about 6 and 2% of the ODW, respectively; A-1 and A-2 of the same length; P-2 slightly longer than P-1 and P-3; P-4 frequently fragmented and smaller than the other rows. Jaw-sheaths narrow, darkly pigmented and with finely serrated margins (50 to 62 conical serrations in the upper jaw-sheath); upper jaw-sheath arc-shaped, lower jaw-sheath V-shaped. Measurements for developmental stages 34–36, and 37–39 are shown in Table 2.

Lateral line system visible, with 10 pairs of neuromast lines. Most lines possess whitish rounded stitches. The su-

praorbital and infraorbital lines extend anteriorly from behind the eyes to the anterior region of the oral disc, and medially and laterally to the nares, respectively. Posterior supraorbital and posterior infraorbital lines are irregularly arranged behind the eyes. Angular lines extend obliquely from below eyes to the ventral contour of body and are distinct in the mid-ventral region of the body. Anterior oral lines extend from the lateral region of oral disc and approach the angular lines ventrally. Longitudinal oral line located between the anterior oral line and angular line. Dorsal and middle lines extend from the mid-dorsal body region to the tail; the first, medially located ones converge anterior to the body–tail junction and continue posteriorly along the dorsal fin to halfway down the tail; middle lines located dorsolaterally reach the body–tail junction where they join the middle caudal series. Along the proximal third of tail, this middle series turns towards the dorsal fin of the tail, reaching the dorsal fin base, and turns inconspicuous in the distal third of the tail. Ventral body lines extend anterodorsally from near the vent tube to the region above the spiracle. Following a small gap without neuromasts, they continue posteroventrally from below the base of the spiracle to the mid-abdominal region.

Coloration: In life, body with a brownish background marbled with dark brown, black, and golden dots (Fig. 5A). Some specimens with regularly scattered black spots all over the body. Spiracle lightly pigmented, mainly in its distal portion. Belly region translucent whitish, facilitating a partial view of the intestinal tube. Iris black with irregularly scattered golden dots and a golden or coppery ring around the pupil. Tail muscle cream with melanophores forming irregular and angular dark brown spots; a continuous dark longitudinal stripe from end of body, between the dorsal and ventral myotomes of the tail, reaches the proximal quarter of the tail; dorsal margin of the caudal musculature with a broken narrow brown line; fins translucent with scattered darker blotches mainly on the dorsal fin. The coloration in preservative is similar to the coloration in life, with the golden tones faded. A pair of whitish rounded spots (KOLENC et al. 2008, QUINZIO & GOLDBERG 2019) becomes evident anterolaterally to the base of the vent tube.

Variation in larval morphology: Four specimens at stages between 31 and 33 (UFMG-G 2455b) with LTRF 2(2)/4, and one at stage 28 with 2(2)/4(1,4) (UFMG 2455b). P4 is frag-

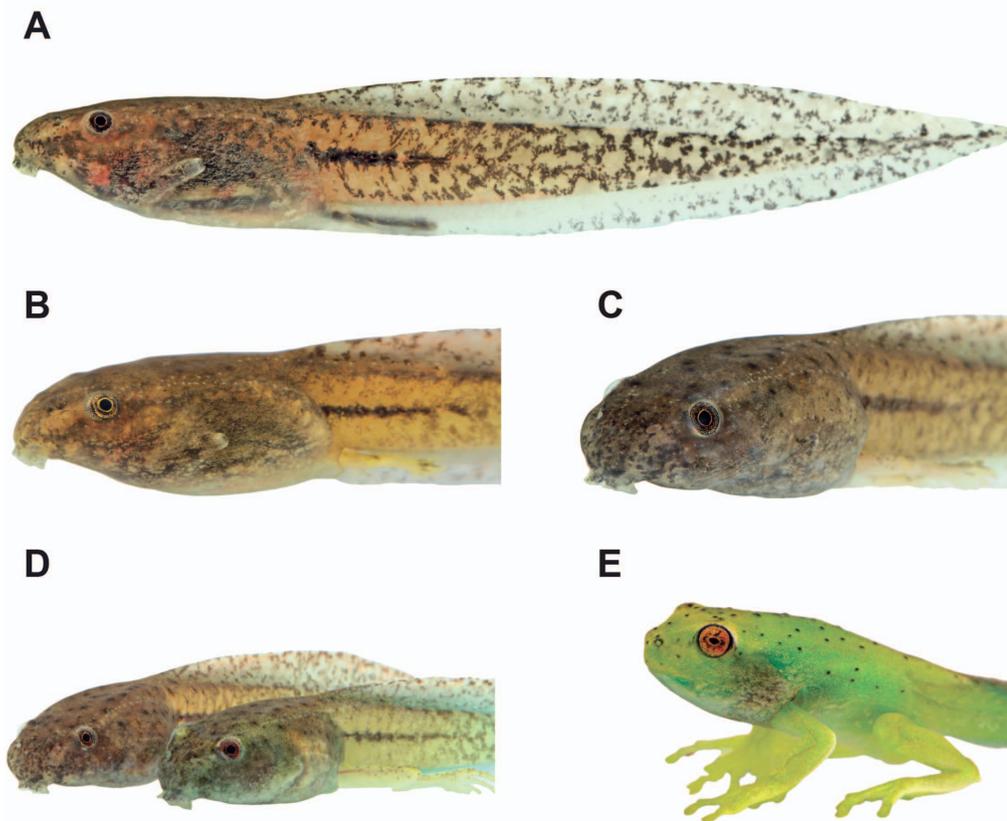


Figure 5. Tadpoles of *Aplastodiscus heterophonicus* sp. n. photographed in life: A) lateral view of a specimen at Gosner stage 33 (lot UFMG-G 2455a); B) detail of the body of a specimen at stage 38 (lot UFMG-G 2455b); C) detail of the body of a specimen at stage 40 with a coppery coloured iris (lot UFMG-G 2455b); D) specimens side by side at stages 40 and 41 (lot UFMG-G 2455b). Note that the one on the first plane (stage 41) has a more greenish background colour on its body and tail, and the eyes are reddish; E) a froglet with a greenish coloration, black dots distributed along the dorsum, and an orange iris (lot UFMG-G 2455c). Images not to scale.

mented in seven specimens at various developmental stages (UFMG-G 2455a–c), and in one at stage 29, it is formed by two barely visible flaps of teeth.

At stage 40, the eyes have a coppery ring around the pupil (Fig. 5C). From stage 41 onwards, the body and tail background colorations take on a more greenish hue, with the regularly scattered black spots becoming more evident all over the body; the tail and limbs become yellowish, and the iris becomes red (Fig. 5D). Dorsal coloration turns bright green in metamorphs, with black dots evenly distributed on the dorsum, iris orange, and yellowish limbs (Fig. 5E).

Remark: Tadpoles of *A. arildae* have a reddish iris from Gosner stage 26 onwards (T. L. PEZZUTI pers. obs.), while in *A. heterophonicus* the iris will turn reddish only much later, at stage 41.

Advertisement call: The advertisement call of *Aplastodiscus heterophonicus* is composed of a single scratchy harmonic note, which can be emitted repeatedly (Fig. 6). The call rate (calls per minute) varies from 6.6–67.2 (36.7 ± 22.8 ; $n = 8$). Each call duration is 167–280 (214.7 ± 42.5 ; $n = 85$) ms, with intervals between two consecutive calls of 0.51–15.9 (3.04 ± 3.41 ; $n = 77$) s. The first part of a call varies from being pulsed, with 17–39 (27.4 ± 7.6 ; $n = 30$) pulses, to pulsatile ($n = 55$), and accounts for 0.55–0.82 (0.7 ± 0.1 ; $n = 85$) % of the call duration. The final part of the call is unpulsed. The frequencies are arranged in an ascending modulation and several harmonic bands, which can vary considerably in number depending on the quality of the recording. Across the 85 calls analyzed, the minimum frequency of the call is at 414.7 Hz, and the maximum at 15306.4 Hz. However, such high maximum values might be a product of the quality of the recording, and/or the distance from the calling male, and are not commonly detected. Usually, the peak frequency lies within the fundamental band at 681.1 ($n = 20$), 947.5 ($n = 18$), 750 ($n = 15$), or 661.3 ($n = 12$) Hz. However, the dominant frequency of some recorded calls was

found to lie in higher bands, with peak frequencies at 2153.3 ($n = 7$), 1378.1 ($n = 6$), 2067.2 ($n = 3$), 1550.4 ($n = 2$), or at 1464.3 ($n = 2$) Hz. The minimum frequency at 5% energy is between 562.5–1894.9 (878.5 ± 410.7 ; $n = 85$) Hz, the maximum frequency at 95% energy between 861.3–2325.6 (1247.8 ± 448.8 ; $n = 85$) Hz, and 90% bandwidth is between 258.5–516.8 (369.3 ± 65.8 ; $n = 85$) Hz.

Geographic distribution and natural history: *Aplastodiscus heterophonicus* occurs on the eastern slopes of the southern portion of the Espinhaço Range, in the municipalities of Conceição do Mato Dentro, Dom Joaquim, Alvorada de Minas, and Morro do Pilar, state of Minas Gerais, Brazil, at altitudes from 608–1326 m a.s.l. (Fig. 7). Aside from the type series records, the new species has been recorded (but not collected) at seven additional localities: Conceição do Mato Dentro, near Road MG-010 (less than 4 km straight line from the road), between Conceição do Mato Dentro and São Sebastião do Bom Sucesso ($18^{\circ}57'20.19''$ S, $43^{\circ}23'48''$ W; $18^{\circ}57'14''$ S, $43^{\circ}25'6''$ W; $18^{\circ}59'21''$ S, $43^{\circ}24'33''$ W); Conceição do Mato Dentro, near the junction of Road MG-010 and Estrada Real ($19^{\circ}3'48''$ S, $43^{\circ}24'22''$ W); and at Morro do Pilar, near Estrada Real between Morro do Pilar and Conceição do Mato Dentro ($19^{\circ}8'43''$ S, $43^{\circ}24'55''$ W; $19^{\circ}9'17''$ S, $43^{\circ}23'5''$ W; $19^{\circ}9'23''$ S, $43^{\circ}22'51''$ W).

Aplastodiscus heterophonicus is a forest dweller occurring inside or on the fringes of semi-deciduous forest fragments. It does not occur in the endangered campo rupestre *sensu stricto* (rupestrian grasslands, as defined by SILVEIRA et al. 2016), but its highest record (1326 m a.s.l., on the Serra do Intendente) is embedded in the campo rupestre *sensu lato* ecosystem (see SILVEIRA et al. 2016) with its transitional vegetation such as gallery forests and relict hilltop forests where the new species was found.

Vocal activity usually is concentrated in the rainy season (November–February), but males were also found calling on one occasion in July (dry season). Males called at night, mainly from inside subterranean nests. They can be also

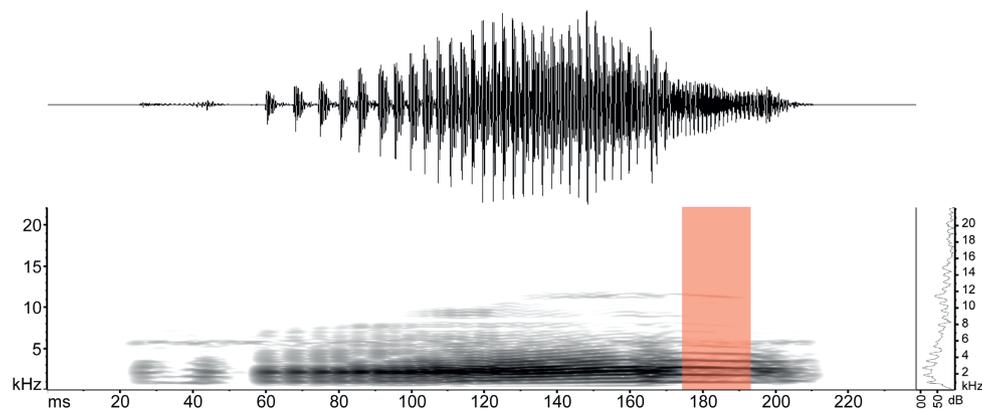


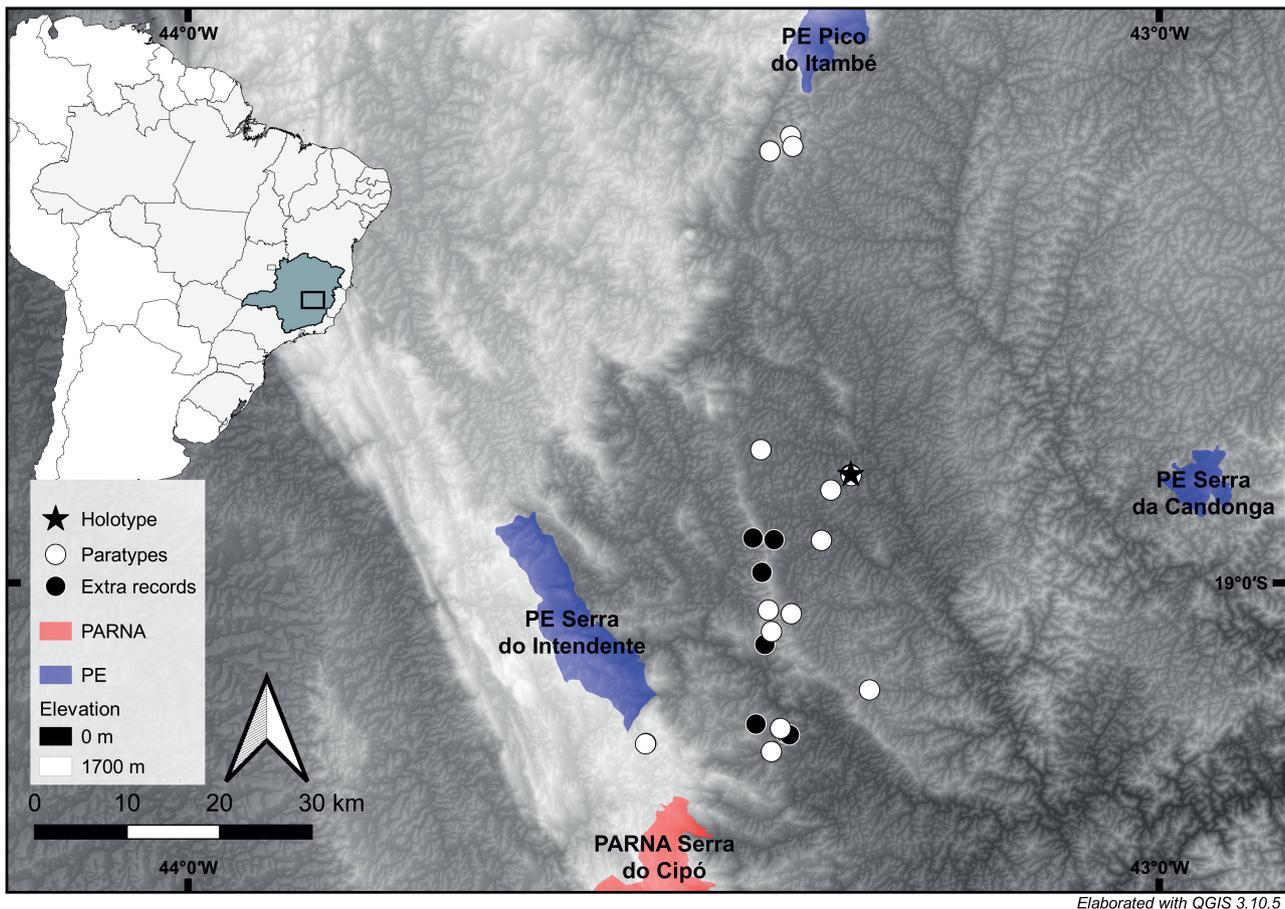
Figure 6. Waveform (top), spectrogram (bottom left), and power spectrum (bottom right; referring to the area highlighted in red) of the advertisement call of *Aplastodiscus heterophonicus* sp. n. holotype (UFMG 21556; recording FNJV 45029), recorded at Dom Joaquim, state of Minas Gerais, on 24 January 2019. For illustration purpose only, spectrogram was generated using the software Raven Pro v1.5 with Window type Blackman, DFT size 512 samples; Time Grid with 94.9% overlap and hop size of 26 samples; Brightness and Contrast adjusted to 66 and 60%, respectively.

found calling on the ground, and rarely perched on vegetation near the forest floor. Nests are excavated in muddy pits and partly covered with dead leaves and debris. They are found in swallow swamps associated with small permanent or temporary forest streams, or in the emerging aquatic vegetation that grows on the margins of artificial ponds formed by dammed-up streams. One nest with unpigmented eggs containing embryos at early stages of development was found in January 2018. This same nest was flooded in the following days, rendering it unsuitable for monitoring these tadpoles further.

Tadpoles were found on the bottom of shallow (less than 20 cm) swamps (lentic environment) or in slow-flowing, small, swampy streams surrounded by semi-deciduous forests. They are benthonic and show greater activity at night, which is when they can be found foraging on the muddy sediment. When disturbed, they will promptly dash away with fast short movements and hide under deposited leaf litter or roots. In January 2018, tadpoles could be found at several developmental stages (including early and metamorphic stages).

Our observations suggest the species reproduces according to the reproductive mode 5 of HADDAD & PRADO (2005), as follows: eggs and early larval stages in constructed subterranean nests; subsequent to flooding, exotrophic tadpoles in ponds or streams.

Conservation status: The Espinhaço Range is the most extensive and continuous Brazilian mountain range. It is characterized by high endemic anuran species richness (TAUCCE et al. 2020) with 41 endemic species, including *Aplastodiscus heterophonicus*. Some of these endemic species are restricted to single or few localities (e.g., LEITE et al. 2011, 2012; BARATA et al. 2013, ARAUJO-VIEIRA et al. 2015, LEAL et al. 2020). This seems to be the case with the new species as well, whose extent of occurrence (measured by a minimum convex polygon) covers only 954.49 km². The geographic coordinates registered for *A. heterophonicus* are proximal to the Parque Nacional Serra do Cipó (Serra do Cipó National Park), Parque Estadual Serra do Intendente (State Park Serra do Intendente), and Parque Estadual Pico do Itambé (State Park Pico do Itambé), three strictly pro-



Elaborated with QGIS 3.10.5

Figure 7. Map of the currently known distribution of *Aplastodiscus heterophonicus* sp. n. Shown on the top left is a part of South America with Brazil highlighted in light grey, and the state of Minas Gerais in dark grey; the black square corresponds to the main map. The black star (holotype) and white dots (paratypes) refer to type material localities (for coordinates see the list of type material); the black dots refer to additional records (for coordinates see Geographic distribution and natural history). Colour-shaded areas point out strictly protected reserves: red (PARNA), Parque Nacional (National Park); blue (PE), Parque Estadual (State Park).

tected nature reserves (Fig. 7). Although these have already been sampled intensively for their herpetofauna (ETEROVICK & SAZIMA 2004, F.S.F. LEITE unpubl. data), and with the Serra do Cipó in fact being one of the best sampled regions in the Espinhaço Range (LEITE et al. 2008), *A. heterophonicus* has apparently never been found within these protected areas. However, the new species occurs in a 6.07-km² private reserve called Fazenda Estiva, where the holotype and some of the paratypes were collected.

The Atlantic Forest is one of the most threatened tropical rainforests in the world (MITTERMEIER et al. 2011). Estimates for the remaining vegetation cover in this biome are a mere 28% at present (REZENDE et al. 2018). The eastern slopes of the southern Espinhaço Range have been, historically and currently, affected by many anthropogenic threats, such as extensive agropastoral use, logging, open-cast mining, and uncontrolled urbanization. The habitat loss driven by these activities is probably the greatest threat factor faced by *A. heterophonicus* populations.

Discussion

We have here described *Aplastodiscus heterophonicus* sp. n., a member of the *A. albosignatus* group, as previously suggested (as “*Aplastodiscus* sp. 4”; BERNECK et al. 2016). The new taxon increases the number of formally recognized species of this group to six. It is morphologically cryptic with *A. cavicola*, although it is phylogenetically closer to *A. ibirapitanga*.

While *A. heterophonicus* can be easily differentiated from its sister taxon, *A. ibirapitanga*, through characters of both adults and larvae, the same does not hold true for *A. cavicola*. There is only a tendency of *A. heterophonicus* adults in being smaller than those of *A. cavicola*, but their SVLs might overlap. The only trait found in the tadpoles that differentiate these two species is the narrow posterior gap in the row of marginal papillae on the oral disc of *A. cavicola* (tadpoles from Congonhas, Minas Gerais; PEZZUTI et al. 2010). However, MERCÊS & JUNCÁ (2010) do not mention such trait when they described the tadpoles of *A. cavicola* from Igrapiúna, Bahia. As it is a very inconspicuous and small gap, we cannot discard the possibility that this character was not noted by these authors. Alternatively, the difference between the tadpoles from Congonhas and Igrapiúna could be due to interpopulation variation.

The advertisement call of *A. heterophonicus*, however, is very distinct from all other species of its genus, including the morphologically similar *A. cavicola*. Within *Aplastodiscus*, *A. cochranæ* and *A. perviridis* are morphologically distinct species, with very similar vocalizations (GARCIA et al. 2001). These somehow contrary conditions within the genus reveal an interesting scenario worth to be investigated further in an ecological-evolutionary context.

Our results also point to the possible existence of another case of morphologically cryptic species that might currently be consolidated under the name *Aplastodiscus cavicola* (CRUZ & PEIXOTO, 1985). We sequenced topotypic

samples of *A. cavicola* from Santa Teresa, state of Espírito Santo, which allowed us to corroborate the assignment of populations from the Quadrilátero Ferrífero region, at the southernmost portion of the Espinhaço Range, state of Minas Gerais to this taxon. However, the genetic distances found might indicate that the populations presently named *A. cavicola* from Duas Barras, state of Rio de Janeiro (MNRJ 63689), could in fact represent a distinct taxon (4.7–5.1% in 16S, compared with topotypic samples of *A. cavicola*; 4.9% compared with the sample from Espinhaço Range; Supplementary document S3), considering average genetic distances between anuran species (e.g., FOUQUET et al. 2007, VENCES et al. 2005a, b, LYRA et al. 2017). Resolving this taxonomic puzzle will require further sampling across populations from the state of Rio de Janeiro.

The observation of *A. heterophonicus* males calling from inside burrows raises a discussion of the evolution of this behaviour in the genus *Aplastodiscus*. The denomination *Aplastodiscus cavicola* was selected by CRUZ & PEIXOTO (1985, as *Hyla cavicola*) due to males of this species being usually found calling hidden from inside burrows. We are not aware of other species of *Aplastodiscus* sharing this behavioural character, at least facultatively. Our phylogenetic hypothesis suggests that it evolved independently twice in this genus (in *A. cavicola* and in *A. heterophonicus*), or alternatively it could have evolved at a deeper node, at least in the *A. albosignatus* group. Field studies of other species of *Aplastodiscus* are crucial to understanding the evolution of this behaviour in this genus. While searching for *A. cavicola*, B. v. M. BERNECK (pers. obs.) was under the impression that males calling from inside burrows could be heard across greater distances. However, this subjective perception remains to be tested.

Comparing our phylogenetic results with those of BERNECK et al. (2016) and BEZERRA et al. (2020), the few topological differences are limited to nodes with lower support, such as the relationships of *Aplastodiscus arildae* and *A. weygoldti* within the *A. albofrenatus* group, or interspecific relationships in the *A. perviridis* group. Probably, they are due to minor differences in the sampling efforts between these studies and ours. Apart from *A. cavicola* and *A. heterophonicus*, we used only one terminal per species for all other *Aplastodiscus* species/candidate species.

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Appendix

Other specimens studied

Aplastodiscus cavicola: Brazil: Espírito Santo: Cariacica: CFBH 22496; Santa Teresa: UFMG 13126–13129, EI 7354–7351; Vargem Alta: CFBH 25543. Minas Gerais: Itabira: MCNAM 16887, 17425, 17426.

Supplementary data

The following data are available online:

Supplementary document S1. GenBank accession codes for gene sequences and respective samples employed in our study.

Supplementary document S2. List of primers employed in our study and respective references.

Supplementary document S3. Table of uncorrected p-distances among *Aplastodiscus* samples employed on our study for the mitochondrial ribosomal gene 16S and cytochrome oxidase c subunit I.