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Unveiling cryptic diversity in the genus *Arcovomer* (Anura: Microhylidae): description of two new species from the Brazilian Atlantic Forest

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Abstract. The Brazilian Atlantic Forest is one of the world's most threatened biodiversity hotspots, harbouring high levels of species richness and endemism, including more than 700 amphibian species. Although considerable progress has been made in describing this diversity, new species continue to be formally recognized each year, and many lineages remain poorly studied. Here, we use an integrative approach combining molecular, morphometric, and bioacoustic data to reassess species diversity within the frog genus *Arcovomer* (Anura, Microhylidae), long regarded as monotypic. Our results reveal the presence of three deeply divergent lineages within the genus *Arcovomer*. One corresponds to *A. passarellii*, the type species from the state of Rio de Janeiro, while the other two represent distinct lineages from the states of Espírito Santo and São Paulo, respectively. Phylogenetic analyses based on mitochondrial and nuclear markers recovered *Arcovomer* as a strongly supported clade, and morphometric and acoustic evidence combined with molecular data support the distinctiveness of these lineages, leading to the description of two new species. These species are geographically structured across distinct ecoregions of the Brazilian Atlantic Forest, highlighting the conservation relevance of recognizing independently evolving, range-restricted taxa. By revealing previously overlooked diversity within this genus, this study highlights the importance of integrative taxonomy, long-term specimen curation, and sound archives for documenting and conserving biodiversity within one of the world's most threatened biomes.

Key words. Amphibia, genetic divergence, bioacoustics, endemism, integrative taxonomy, biological collections.

Introduction

The Atlantic Forest is globally recognized as a biodiversity hotspot, characterized by high species richness and endemism, and harbours nearly 8% of all tetrapod diversity worldwide (Myers et al. 2000, Figueiredo et al. 2021). Amphibians are represented by more than 700 species distributed across forest remnants and environmental gradients (Figueiredo et al. 2021). However, this ecosystem faces several threats, including deforestation and habitat fragmentation, which have caused population declines, increasing extinction risk for amphibian populations (Lima et al. 2020, Anunciação et al. 2023). Despite these conservation threats, the Atlantic Forest remains a critical region for biodiversity research, with nearly 10% of its amphibian's species described within the last 14 years (Figueiredo et al. 2021, Frost 2026), underscoring the importance of continued exploration and conservation actions.

The family Microhylidae Günther, 1858 (1843) is the second most diverse amphibian family (Frost 2026), with 769 valid species arranged in 12 subfamilies. Commonly known as narrow-mouthed frogs, members of this family inhabit a wide range of environments and biomes around the world (Frost 2026). The evolutionary history and taxonomy of narrow-mouthed frogs have been progressively unravelled through molecular studies. Most research has focused on higher-level phylogenetic relationships (Frost et al. 2006, Pyron & Wiens 2011, de Sá et al. 2012, Peloso et al. 2016, Streicher et al. 2020), diversification patterns (Roelants et al. 2007, Kurabayashi et al. 2011), genus-

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level taxonomic revisions (Peloso et al. 2014, de Sá et al. 2019, Novaes-e-Fagundes et al. 2022), and assessments of species diversity (Peloso et al. 2014, Fouquet et al. 2021).

The New World subfamily Gastrophryninae Fitzinger, 1843 (sensu Peloso et al. 2016), comprises 80 species distributed across 11 genera (Frost 2026). Four of these genera are monotypic. Three of them are restricted to the Atlantic Forest biome: *Arcovomer* Carvalho, 1954, *Dasypops* Miranda-Ribeiro, 1924, and *Myersiella* Carvalho, 1954 (Frost 2026). *Dermatonotus* Méhely, 1904 is also currently considered monotypic, but has a broader geographic distribution and includes two major phylogeographic lineages (Oliveira et al. 2018). The genus *Arcovomer* and its type species *Arcovomer passarellii* Carvalho, 1954 were described by Antenor Leitão de Carvalho in his comprehensive review of American microhylids (Carvalho 1954). The description was based on a male specimen collected by Antonio Passarelli in November 1944 in the municipality of Duque de Caxias, Rio de Janeiro, Brazil, which was initially examined for external morphology and coloration and subsequently cleared and stained. Carvalho (1954) emphasized the arched shape of the vomer as a distinctive diagnostic character of the genus.

Carvalho (1954) associated the newly described genus with the Amazonian narrow-mouthed frogs of the genus *Hamptophryne*. This hypothesis has been repeatedly recovered by subsequent molecular phylogenetic studies employing both multilocus Sanger datasets and phylogenomic approaches, which placed *Arcovomer passarellii* as the sister lineage to *Hamptophryne* (de Sá et al. 2012, Tu et al. 2018, and Hime et al. 2021). However, Peloso et al. (2016), using an anchored hybrid enrichment phylogenomic framework, placed *Arcovomer* as sister to a clade comprising *Dermatonotus*, *Elachistocleis*, *Gastrophryne*, and *Hypopachus*, suggesting that the phylogenetic placement of *Arcovomer passarellii* within the subfamily Gastrophryninae remains uncertain.

Currently, *A. passarellii* is known from isolated populations in the coastal Atlantic Forest of the Brazilian states of Espírito Santo (ES), Rio de Janeiro (RJ), and São Paulo (SP) (Frost 2026). de Sá et al. (2012), based on phylogenetic inferences of one mitochondrial and three nuclear genes, suggested that species diversity within *Arcovomer* may be underestimated, proposing that populations from Espírito Santo and São Paulo could represent undescribed species. Jennings et al. (2016) complemented this previously reported pattern by quantifying the genetic divergence of mitochondrial COI sequences between populations from Rio de Janeiro and Espírito Santo, further supporting the recognition of the Espírito Santo lineage as a distinct species. These molecular findings are in line with the earlier hypothesis of Pombal Jr & Bastos (1992), who proposed that the populations from Espírito Santo probably represent a distinct, 'smaller' adult body size species than the one from RJ.

The advertisement call of *A. passarellii* was first described by Nelson (1973), based on recordings from a population in Itaguaí, RJ, obtained at the locality then known as Horto Florestal de Santa Cruz, currently the Floresta Nacional (FLONA) Mário Xavier, municipality of Seropédica. These recordings included data on call duration, dominant frequency, and harmonic structure. Nelson (1973) also discussed the phylogenetic and ecological significance of these calls within Microhylidae. Later, Giaretta & Martins (2009) described the advertisement call of a population from Ubatuba, SP, and provided additional notes on the species' behaviour and natural history. They found that the calls were slightly different from previous publications, but that this could be due individual variation or measurement errors.

The genus *Arcovomer*, so far considered monotypic, seems to harbour more than one species. The aim of this study was to identify and formally describe two new cryptic species, one from ES and another from SP, through an integrative framework combining acoustic, morphometric, and molecular evidence.

Materials and methods

Specimens

We examined adult specimens of *Arcovomer* deposited in the following Brazilian herpetological collections: Coleção Célio Fernando Baptista Haddad, Departamento de Biodiversidade, Instituto de Biociências, Unesp, Rio Claro (CFBH); Departamento de Zoologia, Unesp, São José do Rio Preto (DZSJRP); Museu de Diversidade Biológica (MDBio), Instituto de Biologia (IB), Unicamp (ZUEC-AMP); and Museu Nacional, Universidade Federal do Rio de Janeiro, Rio de Janeiro (MNRJ). The identification of examined material is provided in the results section.

Since the type series of *A. passarellii* consists solely of the holotype MNRJ 1012 (originally MN 1012), a male cleared and stained as described by Carvalho (1954), we compared the new species with topotypic specimens of *A. passarellii* and information available in the literature. The analysed material of *A. passarellii* included individuals from Duque de Caxias, RJ (MNRJ 34590, 43399, 80702) and from the FLONA Mário Xavier, municipality of Seropédica, RJ (ZUEC-AMP 3000, 5206, 21804).

We also examined records (voucher specimens and call recordings) of all congeneric material deposited in the main collections housing *Arcovomer* spp. (Table S1) to assess species distributions (Fig. 1). Populations for which we could not access molecular, morphological, or acoustic data were tentatively assigned to one of the three species only based on proximity. Populations were classified as 'stable' when recent records were available, or as 'missing' when no individuals had been recorded since 1990, following the criteria of Toledo et al. (2023).

Molecular data, genetic diversities and phylogenetic analyses

We gathered ethanol-preserved tissues of *Arcovomer* from herpetological collections for genetic analysis. Genomic

DNA was extracted using standard salt precipitation (Maniatis et al. 1982), adapted for microcentrifuge tubes as described in Lyra et al. (2017). We amplified and sequenced fragments of two mitochondrial genes [the mitochondrially encoded 16S RNA (16S) and mitochondrially encoded cytochrome c oxidase I (COI)], and three nuclear genes [Brain-derived Neurotrophic Factor (BDNF), Cellular Myelocytomatosis Oncogene – Exon 2 (CMYC), and Seven in Absentia homolog 1 (SIAH)]. The amplification reactions protocols follow Peloso et al. (2014) and Lyra et al. (2017) and primers used are given in Supplementary Table S2. The resulting amplified fragments were Sanger sequenced in both directions by Macrogen Inc. of Seoul, South, Korea. Chromatographs were checked manually, assembled, and quality trimmed using Geneious Prime® 2026.0.2 (www.geneious.com). The protein coding gene fragments were translated into amino acids and no stop codons were observed. The newly generated sequences were submitted to GenBank, and the accession numbers of all sequences included in this dataset are listed in Supplementary Table S3.

For the phylogenetic analysis, we added the newly generated sequences to a matrix containing sequences of *Arcovomer passarellii* available in GenBank and representative sequences of all genera of the subfamily Gastrophryninae, following de Sá et al. (2012), Peloso et al. (2016), and Hime et al. (2020). One sample of *Otophryne robusta* (Microhylidae, Otophryninae) was included as an outgroup.

We performed multiple sequence alignments for each gene fragment using MAFFT 7 (Katoh & Standley 2013) plugin in Geneious Prime® 2026.0.2 (www.geneious.com), under the strategies Q-INS-i for the 16S gene fragment and G-INS-i for the other loci. The alignments were visually checked for possibly misaligned regions, concatenated using Concatenator (Vences et al. 2021), and the final matrix consisted of 51 samples and was 3640 bp long. Then, we conducted a maximum likelihood analysis in RAxML 8.2.10 (Stamatakis 2014) using the CIPRES Science Gateway online server (Miller et al. 2010). The analysis was done considering one predefined partition for each gene and, when appropriate, by codon position, employing the GTR+ G model, and with 100 independent searches for the best tree and 500 non-parametric bootstrap replicates. The resulting tree was visualized and edited in Figtree1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/).

We estimated the uncorrected pairwise distances (p-distance) among the samples of *Arcovomer* in Mega X (Ku-

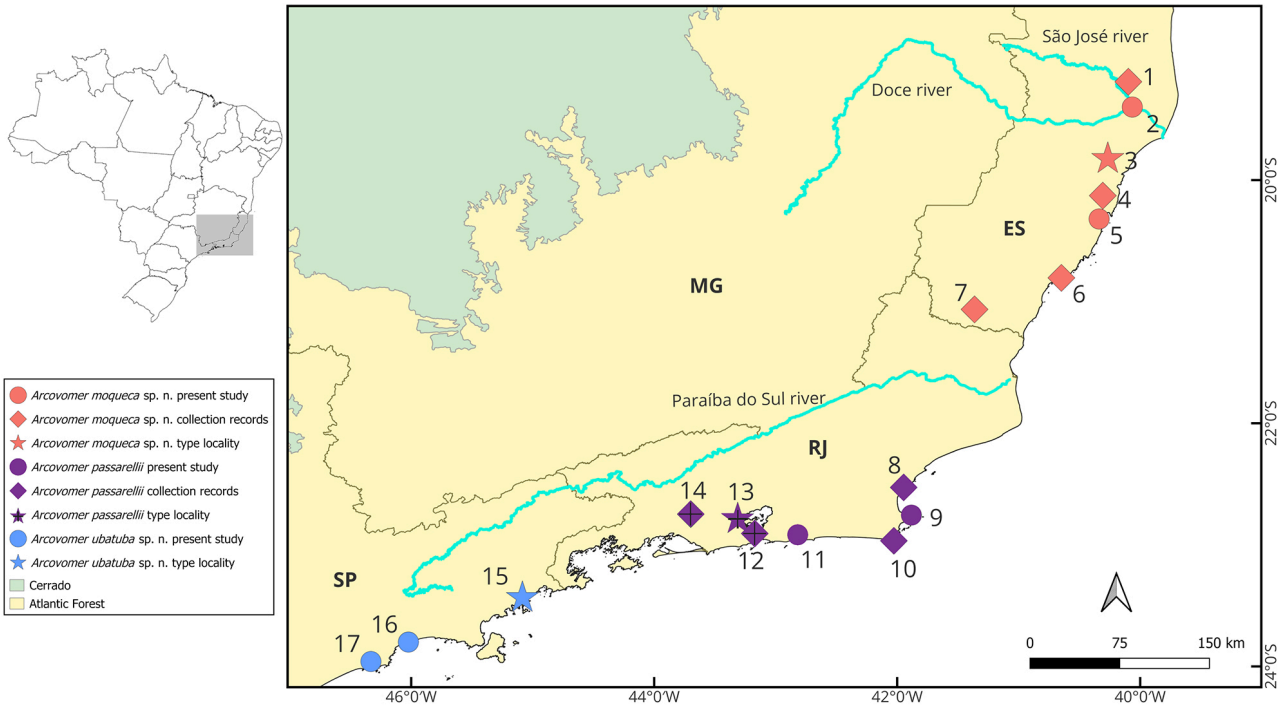

Figure 1. Geographic distribution of the three *Arcovomer* species: *A. passarellii* (purple symbols), *A. moqueca* sp. n. (red symbols), and *A. ubatuba* sp. n. (blue symbols). Type localities of each species are indicated by stars. Municipalities: (1) Sooretama, (2) Linhares, (3) Aracruz, (4) Serra, (5) Vitória, (6) Anchieta, (7) Mimoso do Sul, state of Espírito Santo (ES); (8) Rio das Ostras, (9) Armação dos Búzios, (10) Arraial do Cabo, (11) Maricá, (12) Rio de Janeiro, (13) Duque de Caxias, (14) Seropédica, state of Rio de Janeiro (RJ); (15) Ubatuba, (16) Bertioga, (17) Santos, state of São Paulo (SP). Symbols containing a black cross (+) indicate localities with no records of specimens since 1990; populations from these localities were treated as missing following the criteria of Toledo et al. (2023). Circles and stars indicate localities for which we directly examined specimens and/or associated data, including morphological, molecular, and/or acoustic information. Diamonds indicate records from major Brazilian collections surveyed for which species identifications are considered tentative, inferred solely from geographic proximity to the known distributions of the three species.

mar et al. 2018, Stecher et al. 2020), for the COI fragment and the 16S fragment flanked by primers 16Sar-L and 16Sbr-H (Palumbi et al. 1991), considering pairwise deletion. We also performed a species delimitation analysis for both markers using the 'assemble species by automatic partitioning' (ASAP) method (Puillandre et al. 2021), as an exploratory tool and additional line of evidence to generate preliminary species hypotheses from the sequence data. The analysis was done using the software ASAPy from iTaxoTools 0.1 (Vences et al. 2021).

For the nuclear genes we also constructed haplotype networks to check for congruent differentiation with mitochondrial lineages. For that we used the software Hapsolutely from iTaxoTools 0.1 (Vences et al. 2021) to separate the sequences in haplotypes using the Phase algorithm (Stephens et al. 2001; 0.90 probability threshold) and constructed a haplotype network using TCS method (Templeton et al. 1992).

Morphology and morphometrics

We measured 33 adult males and 12 adult females from the state of Espírito Santo (municipalities of Aracruz and Linhares), eight adult males and two adult females from the state of São Paulo (municipality of Ubatuba), and five adult males and two adult females of *A. passarellii* topotypic material from Duque de Caxias, Rio de Janeiro. Raw morphometric data are in Supplementary Table S4. All 17 morphometric variables (in mm) were taken from adults using the software ZenPro (Zeiss 2024) and include: snout–vent length (SVL, straight line from tip of snout to posterior margin of vent), head length (HL, from angle of jaws to tip of snout), head width (HW, at widest point across jaws), internarial distance (InD, shortest distance between medial margins of nostrils), interorbital distance (IoD, shortest distance between medial margins of orbits), eye diameter (ED, greatest horizontal distance between anterior and posterior margins of eye), eye–nostril distance (END, from anterior margin of eye to posterior margin of nostril), eye–snout distance (ESD, from anterior margin of eye to tip of snout), nostril diameter (ND, greatest horizontal distance between anterior and posterior inner margins of nostril), nostril–snout distance (NSD, from anterior margin of nostril to tip of snout), arm length (AL, from axilla to elbow), forearm length (FaL, from elbow to proximal margin of medial metacarpal tubercle), hand length (HaL, from proximal margin of medial metacarpal tubercle to distal margin of Finger IV), thigh length (ThL, from vent aperture to knee), tibia length (TL, from knee to calcar), tarsus length (TAL, from calcar to proximal margin of medial metatarsal tubercle), and foot length (FtL, from proximal margin of medial metatarsal tubercle to distal margin of adhesive disc of Toe IV).

We numbered adult fingers from II to V following Fabrezi & Alberch (1996), as the first digit was evolutionarily lost in Anura. The sex of specimens was determined by the presence of vocal slits and vocal sacs in males, and by the visualization of unfertilized eggs through the abdominal skin or via lateral dissection in females. Descriptions of coloration pattern were based on field notes and photographs taken in life provided by Célio F. B. Haddad, João L. Gasparini, Edelcio Muscat, and Ariovaldo A. Giaretta.

Bioacoustics

We obtained advertisement calls from the Fonoteca Neotropical Jacques Vielliard (FNJV – MDBio, Unicamp) and from the Arquivo Sonoro da Coleção de Anuros da Universidade Federal de Uberlândia (UFU). These calls were recorded using different recorders and microphones, and other details of each recording (e.g., time, temperature, equipment) are provided in Supplementary Table S5. Recordings made with analog devices were digitized in WAV format using Sound Forge 8.0 at a sampling rate of 96 kHz and 24-bit resolution. Prior to analysis, calls were normalized (DC offset removed, vertically centred at 0.0, and amplitude adjusted to a maximum of -1.0 dB) and processed for noise reduction (12 dB, sensitivity 6.0, no frequency smoothing) using Audacity 2.1.0 (Audacity Team 2015). Treated files were saved as uncompressed 24-bit WAV files. In total, we analysed calls of 10 males: two of *Arcovomer passarellii* from FLONA Mário Xavier, Seropédica, RJ, located about 40 km west of its type locality; two from Aracruz, ES; and six from Ubatuba, SP.

We analysed advertisement calls using Raven Pro 1.5 (64-bit version; Bioacoustics Research Program 2014) with the following settings: Hann window, window size = 256 samples, 3 dB filter bandwidth = 248 Hz, brightness = 50%, contrast = 50%, overlap = 85% (locked), DFT size = 1024 samples (locked), Hop Size = 38, and spectral resolution = 43.1 Hz. Dominant frequency peaks were obtained with the "Peak Frequency" function. Minimum and maximum frequencies were defined as the frequencies containing 5% and 95% of the call energy, extracted with the "Frequency 5%" and "Frequency 95%" functions, respectively. Frequency modulation was assessed using the "1st Quartile Frequency" and "3rd Quartile Frequency" functions, which divide the spectrum into two intervals containing 25 and 75% of the call energy, respectively (Charif et al. 2010). In addition, we used the "Max Time" function to evaluate amplitude modulation. Call figures were produced in R 4.5.1 (64-bit; R Core Team 2025) using the Seewave package v. 1.6 (Sueur et al. 2008) with the following parameters: Hanning window, 90% overlap, and 256-point FFT.

Temporal traits were measured from oscillograms (call duration, inter-call interval, and calls per minute), whereas spectral traits were measured from spectrograms (dominant frequency, minimum and maximum dominant freq. reached, 1st and 3rd quartile frequency, and peak of second harmonic frequency). Terminology for call descriptions follows Köhler et al. (2017). For each male, we calculated mean values of acoustic traits, which were then used to compute overall means and standard deviations. Reported ranges represent the minimum and maximum values

across all samples. We also quantified among-male variation using the coefficient of variation (CV = (SD / mean) × 100). Following Gerhardt (1991), we considered CVs < 11% as indicative of static properties and CVs > 15% as indicative of dynamic properties.

Statistical analysis

We investigated morphometric and acoustic differentiation among species using the 'randomForest' (RF) function (randomForest package v. 4.7–1.2; Liaw & Wiener 2002), which generates multiple classification trees (e.g., 1000) based on bootstrap samples. At each split, the best predictors are selected from a random subset of variables, and final classifications are determined by majority voting (see Liaw & Wiener 2002 for details). Importantly, morphometric and acoustic analyses were conducted without any a priori grouping based on molecular data. RF proximity scores were subsequently used to visualize patterns of similarity among individuals through multidimensional scaling (MDS), implemented with the 'proximityPlot' function from the 'rfPermute' package v. 2.5.5 (Archer 2025). All analyses were performed in R.

For the morphometric multivariate analysis and univariate statistical tests comparing the new species with *Arcovomer passarellii*, we included all variables measured. Data wrangling and reshaping were performed using the dplyr and tidyr packages (Wickham et al. 2023). Pairwise permutation tests for differences among species were conducted using the wilcox_test function from the coin package (Resampling Statistics model; Hothorn et al. 2008). Heatmaps of p-values were visualized using ggplot2 (Wickham 2016), with significant comparisons highlighted in bold. Significance was set at $P \leq 0.05$.

Acoustic multivariate analyses incorporated: call duration, inter-call interval, calls per minute, peak dominant frequency, minimum and maximum dominant frequency, 1st Quartile Frequency, and 3rd Quartile Frequency. Due to the limited number of recordings for the ES lineage of *A. passarellii*, no statistical comparisons were performed.

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:FB550CCA-23B3-48C9-899D-0BB47E262230. 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: salamandra-journal.com, zenodo.org.

Results

Phylogenetic inference and genetic diversity

The maximum likelihood analysis recovered *Arcovomer* as a well-supported monophyletic group composed of three distinct and highly supported clades (Fig. 2). The first clade comprises specimens from Rio de Janeiro (RJ) and is sister to the clade that includes specimens from São Paulo (SP). The third encompasses specimens from Espírito Santo (ES) and is sister to RJ+SP. Furthermore, the analyses placed *Arcovomer* as the sister lineage to *Hamptophryne*.

The three lineages of *Arcovomer* correspond to geographically structured populations, each exhibiting substantial genetic divergence. For the COI fragment, mean uncorrected p-distances were 9.8% between RJ and SP, 13.2% between RJ and ES, and 14.3% between SP and ES. For the 16S fragment, mean uncorrected p-distances were 6.2% between RJ and SP, 5.8% between RJ and ES, and 7.5% between SP and ES. The best scores from ASAP analyses also resulted in three species partition sets for *Arcovomer* for COI and 16S datasets. The analyses recovered almost all species of Gastrophryninae included as different sets, except two species of *Elachistocleis* and four species of *Chiasmocleis*. These two genera are quite diverse and the inclusion of only a few representatives may have biased the analyses for those groups.

For the nuclear loci, we found no sharing haplotypes between SP and ES, supporting the absence of gene flow between these lineages (Fig. S1). There is no nuclear sequence for RJ, preventing us to directly test haplotype sharing for this lineage.

Phenotypic comparisons

The RF multivariate analysis of morphometric data assigned all males of *Arcovomer* sp. from ES to the same group, with a classification error of 0% (Table 1). In contrast, the SP lineage and *A. passarellii* showed classification errors of 25% and 80%, respectively (Table 1). Multidimensional scaling (MDS) further demonstrated complete separation between *Arcovomer* lineages from ES and SP, whereas *A. passarellii* overlapped with both clusters (Fig. 3A). To further explore these results, we plotted boxplots of key traits based on the pairwise non-parametric tests (SVL, AL, and FtL), which revealed consistently smaller values in *Arcovomer* individuals from ES compared to the other two lineages (Fig. 3B). Pairwise non-parametric tests (Fig. S2) expanded this pattern: of the 17 morphometric traits, ES and SP differed in 11 variables, ES and *A. passarellii* in 5 (TL, SVL, IoD, FaL, and AL), while the SP lineage and *A. passarellii* differed only in foot length (FtL). These results indicate that the ES lineage is the most morphometrically distinct, whereas the SP lineage and *A. passarellii* are more similar to each other (Fig. S2). Detailed morphometric data for the three species are in Table 2.

In the acoustic dataset, RF classified all males of *Arcovomer* from SP in a single group (classification error = 0%;

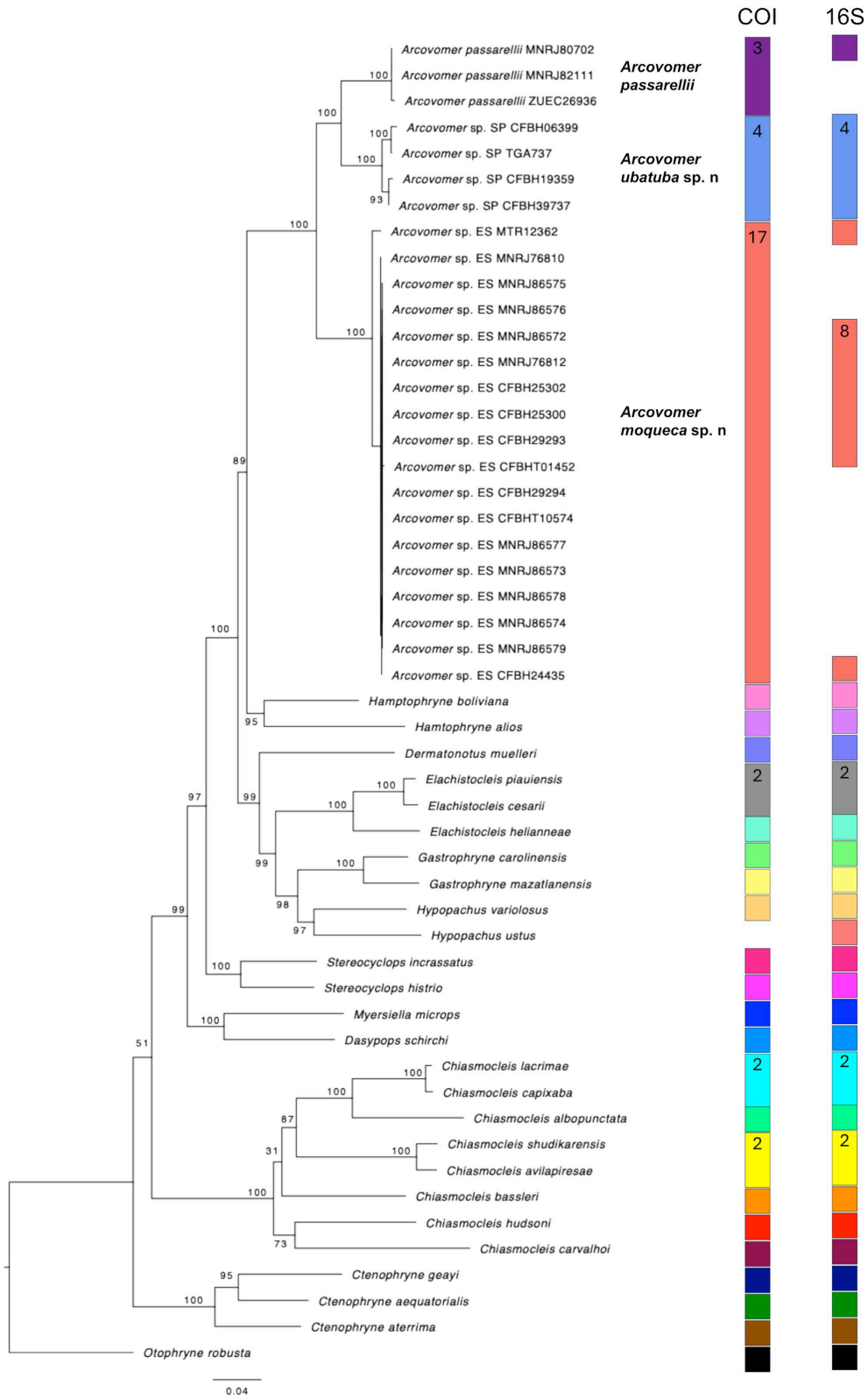

Figure 2. Maximum likelihood tree recovered for the phylogenetic relationships of *Arcovomer* based on the 16S RNA (16S), cytochrome c oxidase I (COI), Brain-derived Neurotrophic Factor (BDNF), Cellular Myelocytomatosis Oncogene – Exon 2 (CMYC), and Seven in Absentia homolog 1 (SIAH). Numbers near the nodes are bootstrap values. Results of the species delimitation analysis using the 'assemble species by automatic partitioning' (ASAP) method are shown to the right of the phylogeny, for both COI and 16S.

Table 1. Confusion matrix for the three known cryptic species of *Arcovomer* based on morphometric and acoustic (values in bold) datasets by means of a Random Forests model. Settings: number of tree permutations = 10000 (both datasets); number of variables tried at each split = 4.0 | 2.0; error rates = 13.0 % | 40.0 %.

	A. moqueca sp. n. Espírito Santo	*A. ubatuba* sp. n. São Paulo	*A. passarellii* Rio de Janeiro	classification error
A. moqueca sp. n.	33 | **0**	0 | **1**	0 | **1**	0% | **100%**
A. ubatuba sp. n.	1 | **0**	6 | **6**	1 | **0**	25% | **0%**
A. passarellii	2 | **1**	2 | **1**	1 | **0**	80% | **100%**

Table 1), whereas males from ES and *A. passarellii* showed substantial overlap in acoustic traits, resulting in higher misclassification rates. MDS analysis of the acoustic dataset revealed complete separation only for the cluster of *Arcovomer* from SP (Fig. S3). Calls per minute and inter-call interval were identified as the main contributors to variation based on both variable importance metrics (Fig. S3). These results suggest that acoustic differentiation is evident only for the SP species, which exhibits longer inter-call intervals and a lower call rate, while the ES lineages and *A. passarellii* are acoustically similar. See Table 3 for more detailed information with the specific values for each acoustic trait.

Taxonomy

Although the morphometric and acoustic evidence does not provide diagnostic traits without overlap, the combined results consistently reveal patterns of differentiation among the studied lineages. Morphometric and phylogenetic analyses indicated that the ES lineage is the most distinct, differing significantly from both the SP lineage and *A. passarellii* in the majority of measured traits. In contrast, acoustic analyses highlighted the SP lineage as the most divergent, especially regarding call rate and inter-call interval. Taken together with the phylogenetic topology recovered and high genetic distances, these findings indicate that the

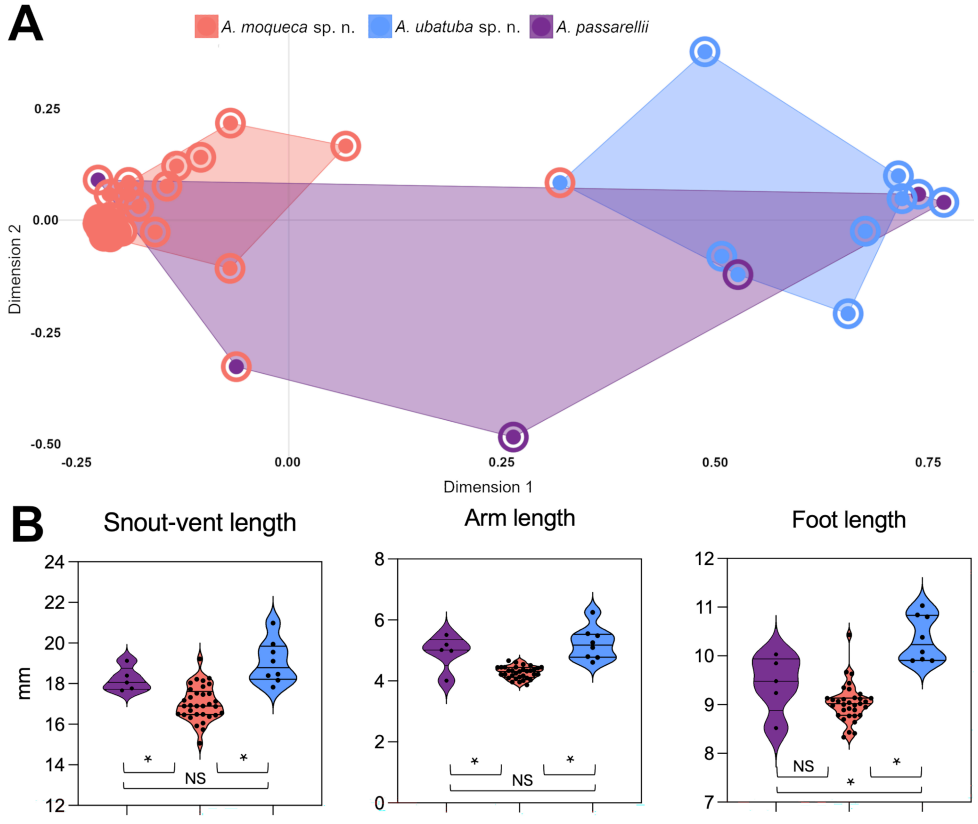

Figure 3. (A) Multidimensional scaling (MDS) plot based on morphometric data of *Arcovomer*, showing complete separation between *A. moqueca* sp. n. from ES and *A. ubatuba* sp. n. from SP, with *A. passarellii* overlapping both clusters. Each dot represents an adult male. Circles around the dots indicate the classification assigned by the Random Forest analysis. (B) Boxplots of the three most important variables (snout–vent length, arm length, and foot length), illustrating consistently smaller values in *A. moqueca* sp. n.

Table 2. Morphometric comparison of the type series of the two new cryptic species with specimens of *Arcovomer passarellii*. Values are given as mean ± standard deviation (minimum–maximum).

	A. moqueca sp. n.		*A. ubatuba* sp. n.		*Arcovomer passarellii*	
Traits	33 males	12 females	8 males	2 females	5 males	2 females
SVL	17.0±0.9 (15.1–19.2)	19.2±0.9 (17.9–20.7)	19.0±1.1 (17.8–21.0)	22.9±0.2 (22.8–23.0)	18.2±0.6 (17.7–19.1)	21.4±1.9 (20.1–22.8)
HL	5.8±0.3 (5.1–6.4)	6.0±0.7 (5.3–7.8)	6.0±0.5 (5.3–6.7)	6.6±0.5 (6.2–7.0)	5.5±0.6 (4.7–6.2)	5.3±1.0 (4.6–6.0)
HW	6.3±0.4 (5.2–7.5)	6.5±0.6 (5.8–7.0)	6.8±0.5 (6.1–7.4)	7.4±0.5 (7.1–7.8)	6.2±0.5 (5.5–6.7)	6.3±0.4 (6.0–6.6)
InD	1.6±0.1 (1.4–1.9)	1.7±0.2 (1.4–2.1)	1.6±0.2 (1.4–1.8)	1.8±0.0 (1.8–1.8)	1.6±0.1 (1.6–1.7)	1.5±0.2 (1.4–1.6)
IoD	2.3±0.2 (2.1–2.7)	2.6±0.3 (2.2–3.1)	2.6±0.3 (2.3–3.1)	3.0±0.1 (3.0–3.1)	2.6±0.2 (2.3–2.9)	3.1±0.4 (2.8–3.4)
END	1.2±0.1 (1.0–1.4)	1.3±0.1 (1.1–1.5)	1.3±0.1 (1.0–1.5)	1.6±0.0 (1.6–1.6)	1.3±0.1 (1.2–1.3)	1.5±0.2 (1.4–1.7)
ESD	1.7±0.3 (1.1–2.1)	2.0±0.2 (1.6–2.2)	1.9±0.3 (1.3–2.3)	2.4±0.1 (2.4–2.5)	1.8±0.2 (1.5–2.0)	2.2±0.4 (1.9–2.5)
NSD	0.3±0.1 (0.2–0.6)	0.4±0.1 (0.3–0.6)	0.4±0.1 (0.2–0.5)	0.6±0.1 (0.5–0.6)	0.4±0.1 (0.2–0.4)	0.5±0.1 (0.4–0.6)
ED	1.5±0.2 (1.2–1.9)	1.7±0.1 (1.4–1.9)	1.6±0.1 (1.4–1.8)	1.8±0.0 (1.8–1.9)	1.7±0.2 (1.3–1.9)	1.6±0.3 (1.5–1.8)
ND	0.2±0.0 (0.1–0.2)	0.2±0.0 (0.2–0.2)	0.2±0.0 (0.1–0.2)	0.2±0.0 (0.2–0.3)	0.2±0.0 (0.1–0.2)	0.2±0.0 (0.2–0.2)
AL	4.3±0.2 (3.9–4.7)	4.5±0.5 (3.6–5.3)	5.2±0.5 (4.6–6.3)	5.4±0.6 (5.0–5.8)	4.9±0.6 (4.0–5.5)	5.5±0.4 (5.3–5.8)
FaL	2.4±0.2 (2.0–2.7)	2.8±0.2 (2.5–3.2)	3.1±0.4 (2.6–3.8)	3.2±0.5 (2.9–3.6)	2.9±0.3 (2.5–3.2)	3.3±0.3 (3.1–3.5)
HaL	4.2±0.2 (4.0–4.5)	4.4±0.2 (4.0–4.8)	5.0±0.2 (4.6–5.3)	5.8±0.1 (5.7–5.8)	4.6±0.4 (4.0–5.2)	5.6±0.2 (5.5–5.8)
ThL	7.2±0.6 (6.0–8.3)	8.3±0.3 (7.8–8.7)	8.6±0.5 (7.9–9.4)	9.8±0.0 (9.8–9.8)	8.2±1.1 (7.2–9.8)	9.0±0.4 (8.7–9.3)
TL	7.4±0.5 (6.2–8.2)	8.5±0.5 (7.6–9.4)	8.5±0.7 (7.6–9.6)	9.1±1.5 (8.0–10.1)	8.1±0.6 (7.6–9.2)	9.5±0.1 (9.4–9.5)
TAL	4.0±0.3 (2.5–4.4)	4.3±0.5 (3.2–4.9)	4.2±0.6 (3.2–4.6)	4.4±0.9 (3.8–5.0)	3.6±0.6 (2.8–4.3)	5.0±0.2 (4.9–5.1)
FtL	9.0±0.4 (8.3–10.4)	10.2±1.1 (9.2–12.9)	10.4±0.5 (9.9–11.0)	12.2±0.6 (11.8–12.6)	9.4±0.6 (8.5–10.0)	12.6±3.3 (10.3–14.9)

A. passarellii complex contains more than one species. Here we describe the lineages from Espírito Santo and São Paulo as two new species. Prior to these descriptions, based on external morphology and coloration, we provide a phenotypic variation in preservative of topotypic material of *A. passarellii*, as well as a redescription of its advertisement call.

Arcovomer passarellii

Variation in preservative: The topotypic material (MNRJ 34590, 43399, 80702) and specimens from FLONA Mário Xavier, Seropédica, RJ (ZUEC-AMP 3000, 5206, 21804) examined herein fully agree with the original description of external morphology and coloration, as well as with Plate 1 (Page 21, Fig. 2 there) in Carvalho (1954). In preservative, all individuals retain a light brown dorsum, with a characteristic dark brown longitudinal blotch extending from the tip of the snout across the top of the head and body to the cloacal region. Darker brown transverse blotches are present on the forearm, thigh, shank, and tarsus. Two dark brown blotches occur on each side of the cloaca. The venter is light brown, bearing numerous rounded white spots on the chest, belly, and fore- and hind limbs. Dermal spines on the tarsus, foot, and toes are present and are more developed in males. Specimens MNRJ 43399, 80702, and ZUEC-AMP 3000 and 5206 exhibit some whitish blotches on the dorsum. ZUEC-AMP 3000 shows a large whitish blotch on the lateral surface of the body, covering nearly the entire area between the axilla and groin.

Vocalization: The advertisement call of *A. passarellii* consists of a stereotyped, single, tonal note (non-pulsed), with a duration of 187–290 ms, emitted sporadically at intervals of 1–25 s. Calls are produced at a rate of 5 per minute and show a pronounced gradual increase in amplitude toward the end of the note, reaching a peak at 82 ± 2 % (77–87%) of its duration, followed by a decrease in amplitude (Fig. 4). Dominant frequency peaks range from 3,000 to 3,656 Hz, with minimum and maximum frequencies ranging from 2,812 to 3,562 Hz and 3,187 to 3,844 Hz, respectively (Fig. 4). Notes exhibit a slight frequency rise throughout their duration, averaging an increase of 129 Hz between the first and third quartiles of frequency. The dominant frequency corresponds to the fundamental harmonic (Fig. 4). Air temperature during recordings ranged from 22 to 24 °C. Quantitative call traits are summarized in Table 3.

Distribution: *Arcovomer passarellii* has historically been reported from southern Espírito Santo, coastal Rio de Janeiro, and southeastern coastal São Paulo. However, based on our molecular data and integrative taxonomic analyses, we restrict *A. passarellii* to the state of Rio de Janeiro (Fig. 1). Populations previously assigned to this species in Espírito Santo and São Paulo are herein recognised as distinct species. Of the seven historically reported populations, three are currently considered missing according to the criteria of Toledo et al. (2023), including the type locality in Duque de Caxias, RJ (Table 4).

Table 3. Advertisement call traits of the three known cryptic species of *Arcovomer*. Values are presented as mean ± standard deviation (minimum–maximum).

	A. moqueca sp. n. Aracruz, ES	*A. ubatuba* sp. n. Ubatuba, SP	*A. passarellii* Seropédica, RJ
Traits	2 males (7 calls)	6 males (18 calls)	2 males (12 calls)
Call duration (ms)	215.4±18.7 (192.6–238.8)	277.1±36.0 (194.3–332.9)	241.2±37.4 (187.1–289.9)
Inter-call interval (s)	9.9±4.7 (0.7–14.4)	45.7±17.1 (10.8–68.8)	13.9±2.0 (1.3–25.0)
Calls per minute	9.5±5.0 (5.9–13.0)	2.2±0.7 (1.3–3.6)	5.3±0.2 (5.1–5.4)
Dominant frequency (Hz)	3,515.6±132.6 (3,187.5–3,843.8)	3,138.9±201.1 (2,799.3–3,375.0)	3,375.0±397.7 (3,000.0–3,656.3)
Min. dominant freq. reached (Hz)	3,242.2±209.9 (2,625.0–3,609.4)	2,950.7±157.0 (2,627.1–3,187.5)	3,210.9±397.7 (2,812.5–3,562.5)
Max. dominant freq. reached (Hz)	3,687.5±110.5 (3,375.0–3,984.4)	3,269.1±198.6 (2,971.6–3,562.5)	3,527.3±381.2 (3,187.5–3,843.8)
1st Quartile Frequency	3,390.6±154.7 (3,000.0–3,750.0)	3,036.5±153.1 (2,756.3–3,187.5)	3,304.7±430.9 (3,000.0–3,656.3)
3rd Quartile Frequency	3,570.3±143.6 (3,187.5–3,890.6)	3,175.3±179.9 (2,885.4–3,375.0)	3,433.6±414.3 (3,000.0–3,750.0)
Peak of 2nd harmonic frequency (kHz)	6,890.6±0.0 (6,375.0–7,500.0)	6,408.5±154.5 (6,201.6–6,632.2)	–
Air temperature (°C)	25.0–27.3	13.5–23.4	22.0–24.0
Water temperature (°C)	–	14.5–23.0	21

***Arcovomer moqueca* sp. n.**
(Figs 5–6)

ZooBank LSID: urn:lsid:zoobank.org:act:278F0061-C07A-4471-AAB2-882771EC3AF6

Arcovomer passarellii: Pombal Jr & Bastos (1992); de Sá et al. (2012), Jennings et al. (2016)

Holotype: CFBH 27174, an adult male, collected at microbacia Olho D'Água, municipality of Aracruz, ES, Brazil, collected in September 2009 by C. F. B. Haddad.

Paratypes: Forty-five specimens in total (33 adult males and 12 adult females). Adult males: CFBH 25303, 25305 collected at Barra do Riacho (Portocel), Aracruz, ES between 01–06 December 2009 by J. L. Gasparini, J. Aragon, and R. Zorzal; CFBH 27149 collected at Barra do Riacho (Portocel), Aracruz, ES on 14 December 2008 by J. L. Gasparini; CFBH 27152–8 collected at Barra do Riacho (Portocel), Aracruz, ES on 27 February 2009 by J. L. Gasparini; CFBH 27163–4 collected at type locality in February 2009 by C. F. B. Haddad; CFBH 27166 collected at type locality in August 2009 by C. F. B. Haddad; CFBH 27170–1 collected together with the holotype; CFBH 27175–9, 27182–3, 27185 collected at type locality on 15 November 2010 by C. F. B. Haddad; CFBH 37658–63, 37665 collected at Barra do Riacho, Aracruz, ES on 11 October 2009 by J. L. Gasparini; A. P. Almeida; R. Zorzal, R. B. Ferreira; CFBH 38330 collected at Reserva da Vale, Macanaiba trail, Linhares, ES on 07 February 2015 by C. F. B. Haddad; J. L. Gasparini; A. P. Almeida; C. A. Brasileiro; and ZUEC 9714 collected at Aracruz, ES on 16 August 1996 by J. L. Gasparini. Adult females: CFBH 2182–3 collected at Aracruz, ES between 02–04 January 1994 by R. P. Bastos, J. L. Gasparini; CFBH 25300, 25302 collected at Barra do Riacho (Portocel), Aracruz, ES between 01–06 December 2009 by J. L. Gasparini, J. Aragon, R. Zorzal; CFBH 27160–1 collected at the type locality on 13 December 2008 by C. F. B. Haddad; CFBH 27172 collected at type locality in September 2009 by C. F. B. Haddad; CFBH 27180–1, 27184 collected at type locality on 15 November 2010 by C. F. B. Haddad; CFBH 39234 collected at Barra do Riacho, Aracruz, ES by J. L. Gasparini (undetermined date); ZUEC 11369 collected at type locality on 29 November 1996 by J. L. Gasparini.

Refereed specimens: CFBH 16255 juvenile specimen collected at Aracruz, ES between 25–30 May 2007 by L. F. Toledo; CFBH 25434–5 adult males collected at Barra do Riacho (Portocel), Aracruz, ES between 01–06 December 2009 by J. L. Gasparini, J. Aragon, R. Zorzal; CFBH 25454, 25456–8 adult males collected at Barra do Riacho, Aracruz, ES on 01 September 2009 by J. L. Gasparini; CFBH 27076 juvenile specimen collected at Vale do Rio Doce Company, around Lagoa 7, Vitória, ES on 10 November 2004 by J. L. Gasparini, R. C. Bianchi; CFBH 27151 lot of tadpoles collected at Barra do Riacho (Portocel), Aracruz, ES on 17 January 2009 by J. L. Gasparini; CFBH 27162 lot of tadpoles collected at type locality on 13 December 2008 by C. F. B. Haddad; CFBH 27173 adult male collected together with the holotype; CFBH 29293–4 (undetermined sex) collected at Barra do Riacho, Aracruz, ES in December 2009 by J. L. Gasparini; CFBH 37664 (undetermined sex) at Barra do Riacho, Aracruz, ES on 11 October 2009 by J. L. Gasparini, A. P. Almeida, R. Zorzal, R. B. Ferreira; CFBH 39236 (undetermined sex) collected at Suruaca Lagoa, Linhares, ES by J. L. Gasparini (undetermined date).

Etymology: The specific name is a noun in apposition, derived from the African Bantu language, Kimbundu,

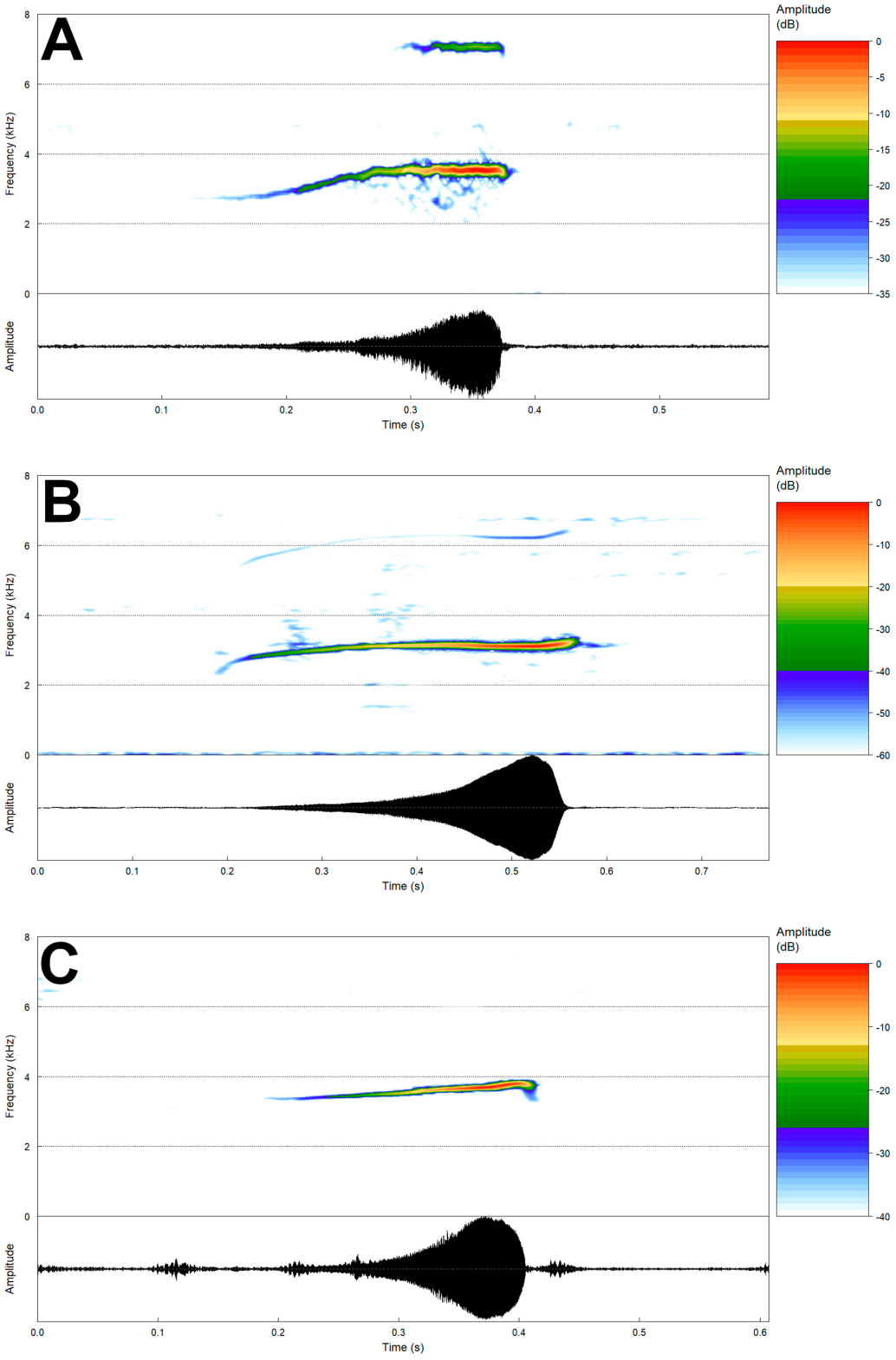

Figure 4. Advertisement calls of the three species of *Arcovomer*. (A) Audiospectrogram (above) and corresponding oscillogram (below) of *A. moqueca* sp. n. from Aracruz, ES, recorded by C. F. B. Haddad on 16 January 1991 at 20:20 h (recording FNJV 32251, unvouchered); (B) *A. ubatuba* sp. n. from its type locality, recorded by A. A. Giaretta on 26 December 2008 at 20:30 h (recording Arcovomer_passarUbatubSP4aAAGmt.wav, unvouchered); (C) *A. passarellii* from FLONA Mário Xavier, Seropédica, RJ, recorded by A. J. Cardoso on 9 December 1982 at 21:00 h (recording FNJV 11692, voucher ZUEC 5206).

Table 4. *Arcovomer* spp. populations, indicating the first and last years of collection, population status (classified as stable when recent samples are available, or missing when no individuals have been recorded since 1990, following Toledo et al. 2023), and populations represented in molecular phylogenies. Type localities are shown in bold. When multiple individuals were sampled in the same year, a single specimen was selected as the reference voucher for that year. For populations with records from only one year, only the last year of collection is presented.

Species	Locality	First year sampled	Voucher	Last year sampled	Voucher	Population status	Represented in phylogenies
A. moqueca	Anchieta, ES	2003	MBML-A 6214	2004	MBML-A 3573	Stable	–
A. moqueca	**Aracruz, ES**	1970	MBML-A 593	2019	MBML-A 12014	Stable	Present study; Jennings et al. 2015
A. moqueca	Linhares, ES	1979	ZUFRJ 13802	2015	CFBH 38330	Stable	Present study
A. moqueca	Mimoso do Sul, ES	–	–	2010	ZUFRJ 12318	Stable	–
A. moqueca	Serra, ES	–	–	2009	UFES-CTA 3294	Stable	–
A. moqueca	Sooretama, ES	1997	MNRJ 22834	2004	CFBH 33178	Stable	–
A. moqueca	Vitória, ES	–	–	2004	CFBH 27076	Stable	Present study
A. passarellii	Armação dos Búzios, RJ	–	–	2012	MNRJ 82111	Stable	Present study; Jennings et al. 2015
A. passarellii	Arraial do Cabo, RJ	–	–	2000	MNRJ 43399	Stable	–
A. passarellii	**Duque de Caxias, RJ**	–	–	1944	MNRJ 1012	Missing	–
A. passarellii	Maricá, RJ	–	–	2012	MNRJ 80702	Stable	Present study; Jennings et al. 2015
A. passarellii	Rio das Ostras, RJ	2003	MNRJ 34590	2025	ZUEC-AMP 26936	Stable	Present study
A. passarellii	Rio de Janeiro, RJ	–	–	1980	ZUFRJ 1067	Missing	–
A. passarellii	Seropédica, RJ	1964	HU-ZOO A 64339	1982	ZUEC-AMP 5206	Missing	–
A. ubatuba	Bertioga, SP	–	–	2014	CFBH 39737	Stable	Present study
A. ubatuba	Santos, SP	–	–	2007	CFBH 19359	Stable	Present study
A. ubatuba	**Ubatuba, SP**	1991	ZUEC-AMP 9175	2020	FNJV 45584	Stable	Present study

'mu'keka', meaning fish stew, or from the indigenous Tupi, 'pokeka' and 'moquem', meaning wrapped and grilled respectively. The word 'moqueca' subsequently emerged from the Portuguese. It is a Brazilian seafood stew, traditional from the state of Espírito Santo, and declared as a cultural asset, intangible heritage, of the state (Brazil 2015, Law No. 10.463). It honors the state of Espírito Santo, celebrating its traditions, peoples, cultural diversity, and rich culinary heritage.

Diagnosis: Assigned to the genus *Arcovomer* based on phylogenetic evidence and the presence of an arched vomer, a diagnostic osteologic synapomorphy of the genus sensu Carvalho (1954). This species is phenotypically cryptic in relation to *A. passarellii*.

Comparisons: Because these taxa are phenotypically cryptic, the following comparisons summarize patterns of divergence rather than diagnostic characters. *Arcovomer moqueca* sp. n. can be differentiated from *A. passarellii* by a combination of morphometric and molecular evidence. Morphometrically, males of *A. moqueca* sp. n. are consistently smaller, with reduced SVL compared to *A. passarellii* (Table 2). Significant differences are also evident in forearm length, arm length, hand length, thigh length, and interorbital distance, all of which are smaller in *A. moqueca* sp. n. than in *A. passarellii* (Fig. S1). Acoustically, *A. moqueca* sp. n. cannot be readily distinguished from *A. passarellii*, as both share similar call rates and inter-call intervals (Table 3). Genetically, uncorrected p-distances in the COI gene between *A. moqueca* sp. n. and *A. passarellii* ranged from 13.1 to 13.2%, and for 16S ranged from 5.4 to 6.5%. These levels of divergence are within the range reported for species-level differentiation in microhylids (Perl et al. 2014), and higher than tentative thresholds for flagging candidate species in Neotropical anurans (Lyra et al. 2017). Phylogenetic inference supports *A. moqueca* sp. n. as sister taxa to a clade containing *A. passarellii* and the lineage from the state of São Paulo.

Description of the holotype: Adult male; body ovoid, robust (Fig. 6A–B). Head triangular, length 33.7% of SVL, wider than long; snout rounded in dorsal and lateral views; canthus rostralis straight, weakly defined; loreal region slightly concave; nostrils protuberant, close to tip of snout, anterolaterally oriented, visible in dorsal view; interorbital region slightly concave, cranial crests absent; occipital and postorbital folds absent; internarial distance 26.2% of head width; eye medium-sized, diameter 22.9% of head length; tympanic annulus and membrane not visible. Upper jaw more projected than the lower (Fig. 6C–D). Tongue large, ovoid, free lateral and posterior borders; vomerine teeth absent; choanae circular, widely separated, anterior to eyes. Arms and forearms slender, without tu-

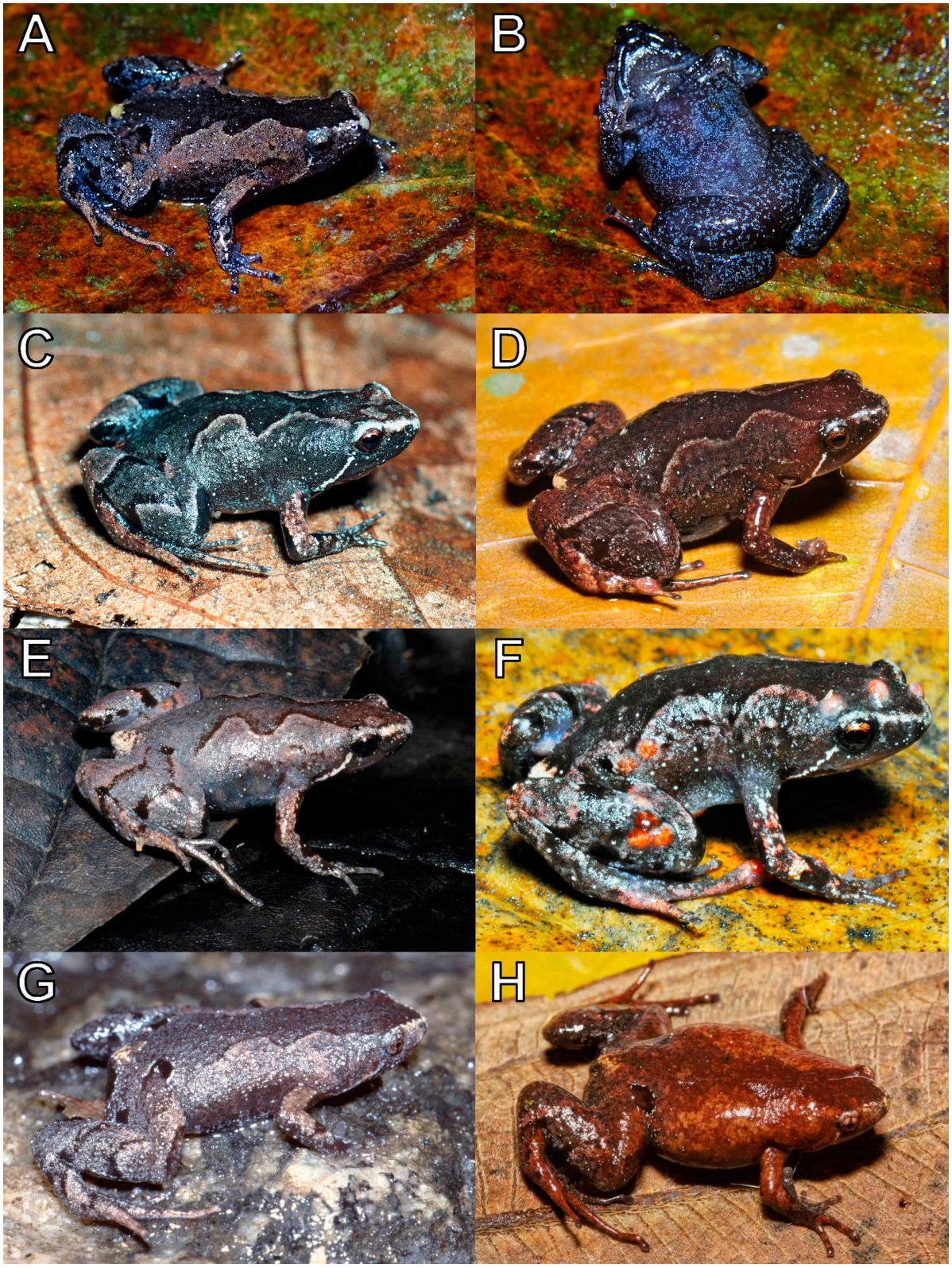

Figure 5. Variation in coloration in life among three species of *Arcovomer*: *A. passarellii* (ZUEC-AMP 26936) from Rio das Ostras, state of Rio de Janeiro (A–B); *A. moqueca* sp. n. from Aracruz (type locality), state of Espírito Santo (C–F); *A. ubatuba* sp. n. from Ubatuba (type locality), state of São Paulo (G–H). Note the differences in color patterns among individuals, with the dorsum and limbs ranging from light grey to dark brown.

bercles or crests. Hands without webbing; fingers slightly fringed, lacking dermal spines or terminal expansions; relative finger length II < III < V < IV; nuptial pads absent (Fig. 6E). Subarticular tubercles rounded, robust; supernumerary tubercles absent; palmar tubercle rounded, undivided; thenar tubercle elliptical. Tibial and tarsal ridges absent. Feet without webbing; toes slightly fringed along both margins of all toes; inner metatarsal tubercle oval, outer absent; single, rounded subarticular tubercles; supernumerary plantar tubercles absent. Relative toe length I < II < V < III < IV (Fig. 6F). Toe IV with dermal spines along lateral margin; Toe V with spines along medial and lateral margins; other toes lacking spines. Tarsus and lateral region of foot beneath Toe V bearing numerous dermal spines (Fig. 6F).

Measurements of holotype (mm): SVL 16.9, HL 5.7, HW 6.1, InD 1.6, IoD 2.1, END 1.3, ESD 1.7, NSD 0.3, ED 1.4, ND 0.1, AL 4.1, FaL 2.5, HaL 4.3, ThL 7.1, TL 7.3, TAL 3.9, FtL 8.7.

Colour pattern of the holotype in preservative: Dorsum brown, with a characteristic dark brown longitudinal blotch extending from the tip of the snout across the top of the head and body to the cloacal region (Fig. 6A–B). Ventral surface whitish, with blotches on the pectoral region. Vocal sac slightly darker than belly, with a higher concentration of melanophores and some small unpigmented patches. Dorsum slightly darker than dorsal surfaces of limbs. A discontinuous whitish line, formed by small blotches, extends from behind the eye to the arm insertion. Small rounded whitish blotches present dorsolaterally between the axilla and inguinal region. Ventral surfaces of arms and legs with numerous melanophores and brown blotches on arms. Palm of hand brown and pigmented; sole of foot brown, pigmented, darker than hand, arm, and hind limb, with coloration similar to that of the dorsal region of hind limbs. Dorsal surface of forearm light brown with several transverse dark brown blotches, including a dark stripe extending from mid-arm to the elbow. Dorsal surfaces of hind limbs light brown with discontinuous transverse dark brown stripes and scattered brown blotches. Dark brown transverse stripes present on forearm (1–2), thigh (2), shank (2), tarsus (2), and foot (1) (Fig. 6A–B). Two conspicuous dark brown blotches on the dorsal surface of the pelvic girdle, extending onto the proximal thighs. Cloacal region with a dark brown blotch. The coloration of the holotype in life is unknown.

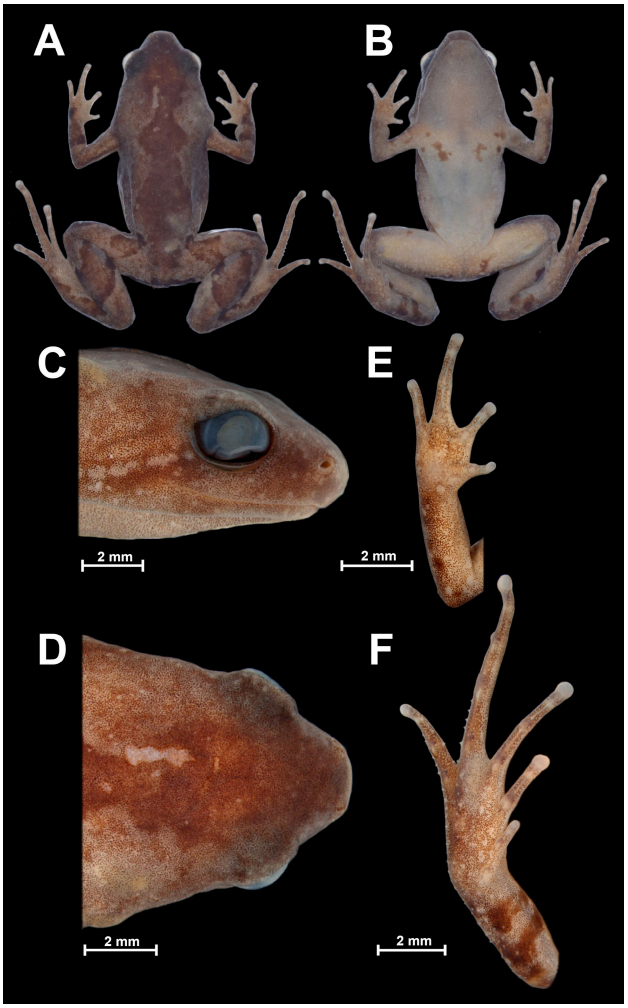

Figure 6. *Arcovomer moqueca* sp. n. holotype, adult male (CFBH 27174, SVL = 16.9 mm). (A) Dorsal view; (B) ventral view; (C) head, lateral view; (D) head, dorsal view; (E) hand, ventral view; (F) foot, ventral view.

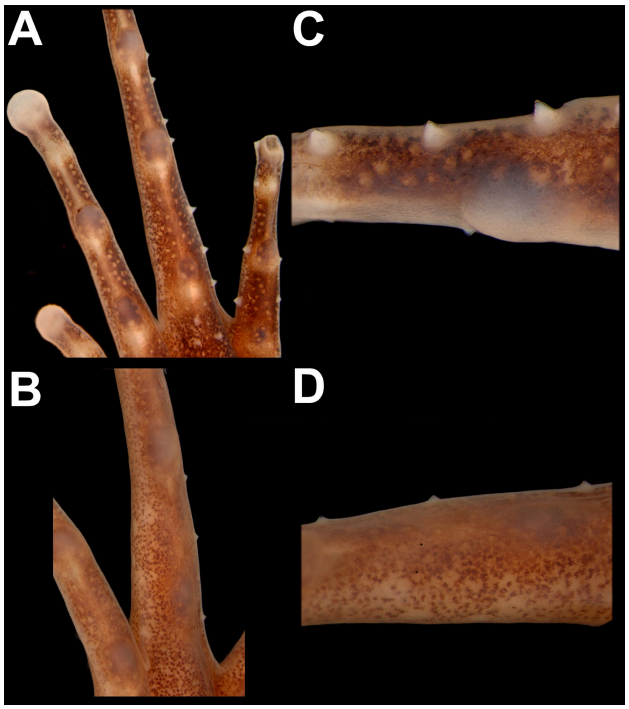

Figure 7. Sexual dimorphism in the dermal spines of toes in *Arcovomer moqueca* sp. n. (A) Adult male CFBH 27171 and (B) adult female CFBH 27184, with close-ups highlighting the difference in spine size on Toe IV of the male (C) and female (D).

Variation in type series (in preservative): Paratypes CFBH 2182–2183, 25300, 25302–25303, 27149, 27152–27158, 27160–27161, 27172, 27178, 27180–27182, 27185, and 39234 exhibit a lighter brown dorsum. Except for CFBH 25302, 27156, 27160, and 27166, all paratypes lack the brown pectoral blotches observed in the holotype. Females are larger than males, and females CFBH 2182, 27161, and 39234 contain mature oocytes. Specimen CFBH 27178 has lost much of its coloration and is noticeably paler, likely because of preservation rather than true chromatic variation. Another character that shows remarkable variation among individuals is the number, distribution, and development of dermal spines on the tarsus, foot, and toes (Fig. 7A–D); these spines are most conspicuous on toes IV and V (Fig 7A, C). In females, however, dermal spines are smaller and fewer than in males (Fig. 7B, D), indicating sexual dimorphism in this structure.

Coloration in life: Specimens show considerable intraspecific variation in dorsal and limb coloration. The dorsum may be greyish with a prominent black dorsal blotch bordered by a white stripe, sharply separating darker and lighter areas, forming the genus-typical conspicuous pattern (Fig. 5A–H). Numerous white dorsal spots occur. A thick white stripe runs from the snout tip across the nostril–eye–eyelid region, while a thinner white line extends posteriorly from behind the eye to the forelimb insertion. Reddish stripes occur on the arms, tarsus, and fifth toe. Transverse stripes present on forearms, thighs, shanks, tarsus, and foot. Dorsal coloration can range from dark grey to light brown, and marking intensity varies (see Fig. 5 for further details).

Vocalization: The advertisement call of *Arcovomer moqueca* sp. n. consists of a stereotyped, single, tonal note (non-pulsed) lasting 193–239 ms, which is emitted sporadically at intervals of 0.7–14 s. Calls are produced at a rate of 6–13 per minute and exhibit a pronounced gradual increase in amplitude toward the end of the note, reaching a peak at 83 ± 4% (74–88%) of its duration, followed by a decrease in amplitude (Fig. 4A). Dominant frequency peaks range from 3,187 to 3,844 Hz, with minimum and maximum frequencies ranging from 2,605 to 3,609 Hz and 3,375 to 3,984 Hz, respectively (Fig. 4A). Notes show a slight frequency rise throughout their duration, averaging an in-

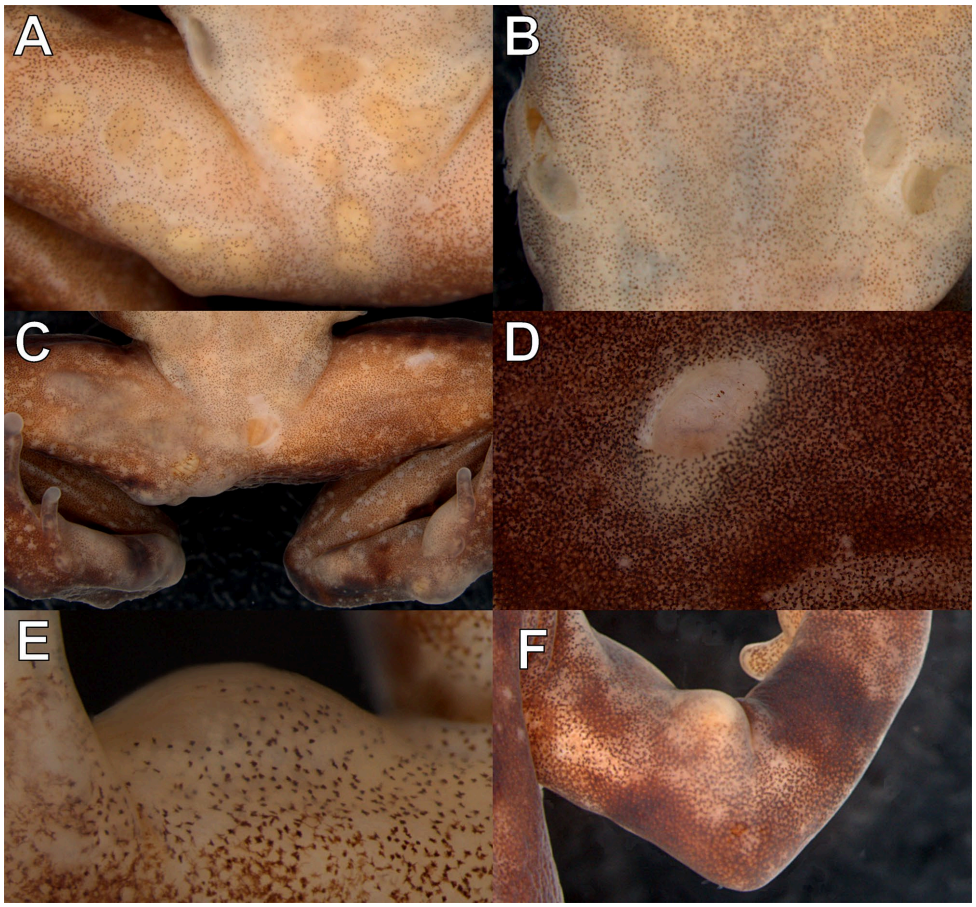

Figure 8. Host-parasite relationship between orange-colored chiggers (mite larvae) and *Arcovomer moqueca* sp. n., highlighting the regions where lesions were observed in the type series. (A) Ventral surface of thigh, belly, and cloacal region of CFBH 27164. (B) Belly of CFBH 27173. (C) Ventral surface of hindlimbs of CFBH 21176. (D) Dorsal surface of body of CFBH 27173. (E) Arm of the holotype CFBH 27174. (F) Arm of CFBH 27176.

crease of 180 Hz between the first and third quartiles of frequency. The dominant frequency corresponds to the fundamental harmonic, and the second harmonic ranges from 6,375 to 7,500 Hz (Fig. 4A), with up to four harmonics observed. Air temperature during recordings ranged from 25.0 to 27.3 °C. Traits considered static (between-male CV < 11%) for *A. moqueca* sp. n. included call duration (8.7%) and all spectral traits (3.8–6.5%), whereas inter-call interval (47.5%) and calls per minute (53.3%) were classified as dynamic. Quantitative call traits are summarized in Table 3.

Geographic distribution: *Arcovomer moqueca* sp. n. is currently known from localities in the municipalities of Anchieta, Aracruz, Linhares, Mimoso do Sul, Serra, Sooretama, and Vitória, all in the state of Espírito Santo, Brazil (Fig. 1; Table 4).

Parasitism: Subcutaneous mites (Acari, Trombidiformes) were abundant in *Arcovomer moqueca* sp. n., as 15 out of the 33 males of the type series (45%) exhibited mite infections (Fig. 8). These lesions were mostly concentrated on the ventral surfaces of the thighs, belly, hind limbs, and feet (Fig. 8A–C), but were also present on various ventral or dorsal regions of the body and forelimbs (Fig. 8D–F). We were unable to identify the mite species, as multiple species of distinct genera were already reported infesting Brazilian anurans (Mendoza-Roldan 2020). In Brazil, studies on parasitic mite larvae in anurans remain scarce, yet species from the families Bufonidae, Cycloramphidae, Hylidae, and Leptodactylidae are known hosts (Hatano et al. 2007, Rodrigues et al. 2018, Mendoza-Roldán et al. 2020). Our record represents the first documented case of mite parasitism in the family Microhylidae, occurring in *A. moqueca* sp. n. from Espírito Santo. This finding expands the known host range of parasitic mites in Brazilian amphibians and highlights the potential for overlooked host–parasite associations, particularly in less-studied taxa.

***Arcovomer ubatuba* sp. n.**
(Figs 5, 9)

ZooBank LSID: urn:lsid:zoobank.org:act:
2C27BE85-E41D-4C61-9A80-41CAEC5974CD
Arcovomer passarelli: Giaretta & Martins (2009), Malagoli et al. (2012)

Holotype: CFBH 35193, an adult male, collected at Picinguaba, municipality of Ubatuba, São Paulo, Brazil, collected between 01–03 November 1991 by J. P. Pombal Jr., E. C. P. Pombal, and R. P. Bastos.

Paratypes: Eight specimens (six adult males and two adult females). Adult males: CFBH 1422 collected at type locality between 16–19 January 1992 by R. P. Bastos and A. R. Silveira; CFBH 1572 collected at type locality on 02 February 1992 by C. F. B. Haddad, R. P. Bastos and M. C. Morellato; CFBH 6399 collected at type locality on 16 October 2003 by C. F. B. Haddad and A. M. Haddad; CFBH 35149 and 35194 collected together with the holotype; and ZUEC 9174–5 collected at type locality on 03 November 1991 by J. P. Pombal Jr and E. C. P. Pombal. Adult females: CFBH 3928 collected at type locality between 14–24 January 2002 by M. Hartmann, P. A. Hartmann, and L. O. M. Giasson; and CFBH 35195 collected together with the holotype.

Refereed specimens: CFBH 19359 adult female collected at Sítio São José, Santos, SP on 06 December 2007 by L. Malagoli; CFBH 1573 adult male collected at type locality on 02 January 1992 by C. F. B. Haddad, R. P. Bastos and M. C. Morellato.

Etymology: The specific epithet 'ubatuba' honours the municipality of Ubatuba, state of São Paulo, Brazil, where this species was first collected and which is its type locality. The name is derived from the indigenous Tupi language, 'uba' meaning "place" and 'tuba' meaning "canoes", referring to the traditional gathering point of canoes during the Tamoio Confederation. The epithet is treated as a noun in apposition. This name relates to the species type locality and evokes the cultural and historical heritage of the people from Atlantic Forest coastal region.

Diagnosis: Placed in the genus *Arcovomer* based on phylogenetic position. Externally cryptic relative to *A. passarellii* and *A. moqueca* sp. n., *A. ubatuba* sp. n. is reliably differentiated only through an integrative approach combining morphology, bioacoustics, and genetics.

Comparisons: *Arcovomer ubatuba* sp. n. can be differentiated from *A. passarellii* and *A. moqueca* sp. n. by a combination of acoustic, morphometric, and molecular evidence. Males are generally larger than those of *A. moqueca* sp. n., with significant differences in 11 of 17 measured variables (Fig. S1). Compared to *A. passarellii*, the only significant morphometric difference is foot length. The advertisement call of *A. ubatuba* sp. n. is the most distinctive among the three species, particularly in calls per minute and inter-call interval, being the phenotypic traits that most clearly differentiate it from the two congeners (Table 3; Figs. 4A–C, S3). Phylogenetic analyses support *Arcovomer ubatuba* sp. n. as sister taxa to *A. passarellii*. The uncorrected p-distances for the COI between *A. ubatuba* sp. n. and *A. passarellii* ranged from 9.3 to 10.4%, and between *A. ubatuba* sp. n. and *A. moqueca* sp. n. ranged from 13.9 to 15.1%. The uncorrected p-distances for 16S between *A. ubatuba* sp. n. and *A. passarellii* ranged from 4.8 to 7.2%, and *A. ubatuba* sp. n. and *A. moqueca* sp. n. ranged from 5.8 to 9.0%.

Description of the holotype: Adult male with robust, ovoid body (Fig. 9A–B). Head triangular, its length 35.1% of SVL; head wider than long; snout rounded in both dorsal and lateral views; canthus rostralis straight, weakly defined (Fig. 9C–D); loreal region shallowly concave; nostrils protuberant, close to the snout tip, anterolaterally directed, visible from above; interorbital area slightly depressed, with-

out cranial crests; occipital and postorbital folds absent. Internarial distance 24.3% of head width. Eyes of moderate size, eye diameter 20.3% of head length; tympanic annulus and membrane not externally visible. Upper jaw projecting beyond lower (Fig. 9C–D). Tongue large, ovoid, with free lateral and posterior margins; vomerine teeth absent; choanae circular, widely spaced, positioned anterior to eyes. Arms and forearms slender, with smooth skin. Hands without interdigital webbing; fingers slightly fringed, without dermal spines or terminal expansions; relative finger lengths II < III < V < IV. Nuptial pads absent. Subarticular tubercles rounded and well-developed; supernumerary tubercles lacking; palmar tubercle rounded, undivided; thenar tubercle elliptical (Fig. 9E). A few small dermal spines present near palmar tubercle and wrist. Tibial and tarsal ridges absent. Feet without webbing, toes slightly fringed on all toes, on both sides; inner metatarsal tubercle oval, outer metatarsal tubercle absent; subarticular tubercles single and rounded; supernumerary plantar tubercles absent. Dermal spines abundant on all toes, most conspicuous on toes IV and V. Numerous dermal spines also along tarsus and lateral surface of foot beneath toe V (Fig. 9F). Cloacal region bearing several dermal spines. Relative toe length I < II < V < III < IV. Ventral surface of thigh with mite-induced injury (Fig. 9B).

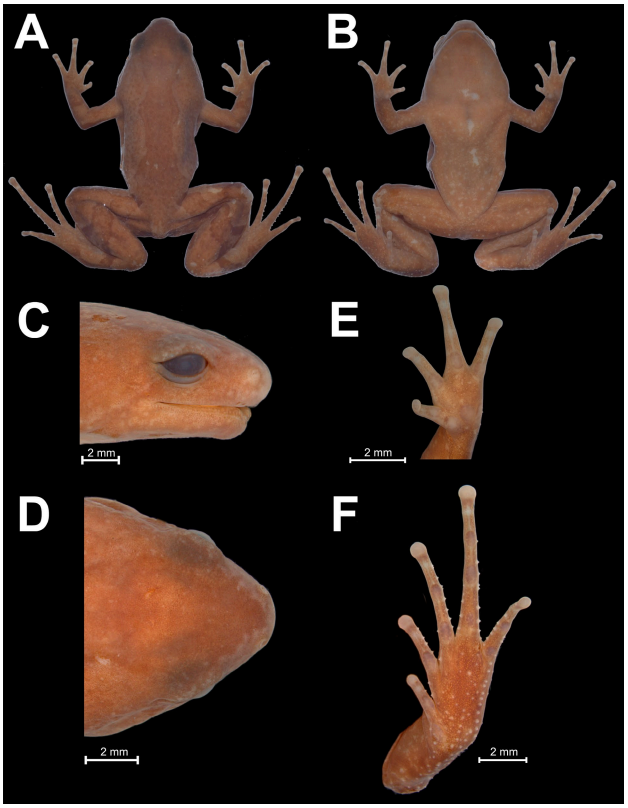

Figure 9. *Arcovomer ubatuba* sp. n. holotype, adult male (CFBH 35193, SVL = 19.1 mm). (A) Dorsal view; (B) ventral view; (C) head, lateral view; (D) head, dorsal view; (E) hand, ventral view; (F) foot, ventral view.

Measurements of holotype (mm): SVL 19.1, HL 6.7, HW 7.4, InD 1.8, IoD 3.0, END 1.3, ESD 1.8, NSD 0.3, ED 1.5, ND 0.2, AL 5.1, FaL 3.1, HaL 5.2, ThL 8.6, TL 8.9, TAL 3.9, FTL 10.8.

Colour pattern of the holotype in preservative: Dorsum light brown with a distinctive dark brown longitudinal blotch extending from the tip of the snout, across the top of the head, and along the body to the cloacal region. Ventral surface brown, covered with numerous small, well-spaced white spots, plus three white blotches on the belly, chest, and vocal sac. Vocal sac similar in colour to belly and chest but lacking the white spots. Dorsum slightly darker than dorsal surfaces of limbs. Small rounded white spots present dorsolaterally between axilla and groin. Ventral surfaces of arms and legs bearing the same small white spots as the belly. Palms light brown, pigmented. Sole of foot brown, pigmented and darker than hand, arm, and hind limb; coloration of the sole similar to that of dorsal hind limbs. Dorsal surface of forearm light brown with transverse dark brown blotches, including a conspicuous dark mark extending from mid-arm to the elbow. Dorsal surfaces of limbs light brown with discontinuous transverse dark brown stripes and scattered brown blotches. Dark brown transverse stripes present on forearm (1), thigh (2), shank (2), and tarsus (1) (Fig. 9A–B). Two conspicuous dark brown blotches on the upper dorsal surface of the pelvic girdle, extending toward mid-thigh. Cloacal region with a dark brown mark (Fig. 9A). The coloration of the holotype in life is unknown.

Variation in type series: Specimens CFBH 1422, 1572, 35149, and 35195 exhibit white blotches on the dorsum, including the body, head, elbow, or thigh. Specimen CFBH 9174 bears a large white blotch on the ventrolateral region of the belly. The number and size of dermal spines on the toes vary considerably, being much less developed in females. In addition to this sexual dimorphism, females are clearly larger than males in overall body size.

Vocalization: The advertisement call of *Arcovomer ubatuba* sp. n. is composed of a stereotyped single note with a clear tonal structure (non-pulsed), lasting 194–333 ms. Calls are emitted irregularly, with inter-call intervals ranging from 10.8 to 68.8 s, and occur at a rate of 1.3–3.6 calls per minute. Each note shows a gradual increase in amplitude, typically peaking at 84 ± 3% (68–95%) of its total duration, and then decreasing toward the end (Fig. 4B). The dominant frequency varies between 2,799 and 3,375 Hz, while the minimum and maximum frequencies range from 2,627 to 3,187 Hz and 2,972 to 3,563 Hz, respectively (Fig. 4B). A slight upward modulation in frequency is present throughout the note, with an average increase of about 140 Hz between the first and third quartiles. The dominant frequency corresponds to the fundamental harmonic, and a second harmonic is consistently observed between 6,202 and 6,632 Hz, with up to four harmonics detected (Fig. 4B). Recordings were made at air temperatures between 13.5 and 23.4 °C. Among measured traits, spectral parameters were

classified as static (between-male CV = 3.2–6.4%), whereas inter-call interval (37.4%) and call rate (31.0%) were dynamic. Call duration (CV = 13%) was considered intermediate. A summary of quantitative call variables is presented in Table 3.

Geographic distribution: *Arcovomer ubatuba* sp. n. is currently known from its type locality, Ubatuba, as well as from the municipalities of Santos and Bertioga, all in the state of São Paulo, Brazil (Fig. 1; Table 4). Most specimens were collected in Ubatuba (Table S1).

Discussion

Our study demonstrates that the genus *Arcovomer*, previously considered monotypic, comprises three distinct cryptic species. By integrating morphological, molecular, and acoustic data, we were able to delineate species boundaries and reveal previously hidden diversity. These findings reinforce that the Atlantic Forest, despite being extensively studied, still harbours considerable undiscovered biodiversity (Lima et al. 2020, Anunciação et al. 2023). By uncovering hidden diversity within *Arcovomer*, our work contributes to a more complete understanding of speciation patterns and emphasizes the continued need for comprehensive surveys in this globally important biome. Moreover, recognizing cryptic species complexes or highly genetically divergent populations can improve conservation planning by allowing management strategies to better reflect underlying evolutionary lineages (Bickford et al. 2007). The descriptions of these two new species thus have direct implications for regional biodiversity management.

The distribution of the three *Arcovomer* species coincides with biogeographic patterns and phylogenetic diversity documented for different Atlantic Forest taxa (e.g., Vasconcelos et al. 2014, Brown et al. 2020, da Silva et al. 2024). Vasconcelos et al. (2014), for example, identified four distinct biogeographic regions for the anuran fauna in the Brazilian costal region, two of which are particularly relevant for *Arcovomer*: the "southeast" and the "north" clusters. *Arcovomer passarellii* from Rio de Janeiro, together with *A. ubatuba* sp. n. from the São Paulo coast, fall within the "southeast" cluster, a region characterized by highly diverse and range-restricted anurans associated with coastal Atlantic Forest, dominated by evergreen rainforest, rugged topography, and pronounced climatic variability, particularly in precipitation and temperature seasonality. In contrast, *A. moqueca* sp. n. from Espírito Santo occurs in the "north" cluster, which comprises semideciduous/deciduous and evergreen forests, also harbouring range-restricted taxa but under warmer conditions with reduced interannual variation in temperature and precipitation. The allopatric distributions of the three species, combined with the restriction of the genus *Arcovomer* to the Atlantic Forest biome, reinforce the hypothesis proposed by several authors (e.g., Carnaval et al. 2009, Vasconcelos et al. 2014, Brown et al. 2020) that diversification of Atlantic Forest anurans has been strongly shaped by historical and geographic factors, including the persistence of Pleistocene refugia and the influence of major mountain chains.

The descriptions of *A. ubatuba* sp. n. and *A. moqueca* sp. n. highlight the invaluable role of decades of specimen collection and the long-term curation of scientific collections and sound archives. The holotype and paratypes of *A. ubatuba* sp. n. were collected in the early 1990s, and some of the sound recordings we analysed, now deposited at FNJV, date back to the 1960s and 1980s. Recordings that remain unarchived face a high risk of loss due to media deterioration or missing metadata, emphasizing the critical need for their proper deposition (Dena et al. 2018, 2019). In Brazil, home to roughly 15% of global anuran diversity (Frost 2026), most collections and archives are not institutionalized, and formal curatorship is often unrecognized by public institutions, putting future biodiversity research at risk. Through careful preservation and broad accessibility of specimens and sound recordings, we not only safeguard irreplaceable scientific resources but also provide a foundation for future studies in taxonomy, ecology, and conservation, demonstrating that the curation of collections and archives is an investment in the continued understanding and preservation of biodiversity.

By describing these two additional species and reviewing museum collections encompassing all populations of *Arcovomer* spp., it is now possible to improve assessments of conservation status and population trends across the three recognized lineages. For instance, *A. passarellii*, under the revised taxonomy, was previously classified as Endangered (EN) in the Espírito Santo state red list (Fraga et al. 2019) and as Data Deficient (DD) in an earlier red list for the state of São Paulo (Garcia et al. 2010), yet it is currently listed as Least Concern (LC) in the Brazilian national red list (Brazil 2022). These discrepancies underscore the critical role of taxonomic resolution in species conservation assessments. Notably, three out of seven known populations of *A. passarellii* are currently missing in the state of Rio de Janeiro (including *A. passarellii* type locality). This condition should be considered in future revisions of threatened species lists at both state and national levels.

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Supplementary data

The following data are available online:

Supplementary Figure S1. Haplotype networks for the nuclear loci analysed.

Supplementary Figure S2. Heatmap of pairwise permutation tests (p-values) across 17 morphometric variables.

Supplementary Figure S3. Multidimensional scaling (MDS) of acoustic traits and Random Forest variable importance plot.

Supplementary Table S1. *Arcovomer* spp. records deposited in scientific collections.

Supplementary Table S2. Primers used, PCR reaction temperatures and references for primer sequences.

Supplementary Table S3. GenBank accession codes.

Supplementary Table S4. Raw morphometric data.

Supplementary Table S5. Sound files analysed.