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Paraphyly in the giant torrent-frogs (Anura: Hylodidae: *Megaelosia*) and the description of a new genus

Stenio Eder Vittorazzi[1,2,*], Guilherme Augusto-Alves[3,4,*], Dener das Neves-da-Silva[5], Ana Maria Paulino Telles de Carvalho-e-Silva[5], Shirlei Maria Recco-Pimentel[6], Luís Felipe Toledo[3], Luciana Bolsoni Lourenço[6] & Daniel Pacheco Bruschi[1]

[1)] Graduate Program in Genetics, Departamento de Genética, Universidade Federal do Paraná (UFPR), Curitiba, Paraná, 81531-980, Brazil

[2)] Departamento de Ciências Biológicas, Faculdade de Ciências Biológicas, Agrárias e da Saúde, Universidade do Estado de Mato Grosso (UNEMAT), Tangará da Serra, Mato Grosso, 78300-000, Brazil

[3)] Laboratory of the Natural History of Brazilian Amphibians (LaHNAB), Departamento de Biologia Animal, Instituto de Biologia,Universidade Estadual de Campinas (Unicamp), Campinas, São Paulo, 13083-862, Brazil

[4)] Graduate Program in Ecology, Instituto de Biologia, Unicamp, Campinas, São Paulo,13083-862, Brazil

[5)] Laboratory of Amphibian Biosystematics (LABAN), Departamento de Zoologia, Instituto de Biociências, Universidade Federal do Estado do Rio de Janeiro (UNIRIO), Rio de Janeiro, Rio de Janeiro, 22290-240, Brazil

[6)] Laboratory of Chromosomal Studies (LabEsC), Departamento de Biologia Estrutural e Funcional, Instituto de Biologia, Unicamp, Campinas, São Paulo, 13083-862, Brazil

* These authors contributed equally to this study

Corresponding author: Stenio Eder Vittorazzi, e-mail: stenio.vittorazzi@gmail.com

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Abstract. The family Hylodidae is composed of 46 species distributed in three genera: *Crossodactylus* (13 species), *Hylodes* (26 species), and *Megaelosia* (seven species). Although the monophyly of the Hylodidae is supported by previous molecular phylogenetic inferences, the monophyly of *Megaelosia* and/or *Hylodes* has been questioned. *Crossodactylus* and *Hylodes* share the plesiomorphic diploid chromosomal number 2n = 26, whereas in the species of *Megaelosia* as karyotyped up to now, diploid numbers have ranged from 28 to 32. Here, we expand to six (of seven) the number of species of *Megaelosia* sampled in a phylogenetic approach based on mitochondrial and nuclear DNA sequences and describe the 26-chromosome karyotype of *M. goeldii* for the first time. Our results provide an improved perspective on the relationships among the frogs described previously in the genus *Megaelosia*, and we propose a new taxonomic arrangement, in which *Megaelosia* is a monotypic genus, with a new genus being described to accommodate the remaining species allocated previously to *Megaelosia*. We also confirm that 2n = 26 is the plesiomorphic diploid number in the Hylodidae, and conclude that an increased diploid chromosome number, together with two morphological traits and one ethological characteristic, are potential synapomorphies of the new genus.

Key words. Amphibia, cytogenetic, *Hylodes*, new genus, phylogenetic inferences, systematics, taxonomy.

Introduction

The family Hylodidae is currently composed of 46 species distributed in three genera: *Crossodactylus* Dúmeril & Bibron, 1841 (13 species), *Hylodes* Fitzinger, 1826 (26 species), and *Megaelosia* Miranda-Ribeiro, 1923, with seven species (Frost 2021, Segalla et al. 2021). The genus *Megaelosia* is currently composed of seven species – *Megaelosia apuana* Pombal, Prado & Canedo, 2003, *M. bocainensis* Giaretta, Bokermann & Haddad, 1993, *M. boticariana* Giaretta & Aguiar-Jr., 1998, *M. goeldii* (Baumann, 1912), *M. jordanensis* (Heyer, 1983), *M. lutzae* Izecksohn & Gouvêa, 1987, and *M. massarti* (De Witte, 1930) (Frost 2021). In addition to the overall microendemism of most *Megaelosia* species, they typically occur at low population densities (Toledo et al. 2014) and are known only from their type localities or extremely limited areas of occurrence (Giaretta et al. 1993, Santos et al. 2011, Muscat et al. 2020a), located primarily within the Serra da Mantiqueira and Serra do Mar mountain ranges, in southeastern Brazil [in the states of São Paulo (SP), Rio de Janeiro (RJ), Espírito Santo (ES), and Minas Gerais (MG)]. By contrast, a number of populations of *M. goeldii* have been found at multiple localities of the Brazilian

states of São Paulo and Rio de Janeiro (Haddad et al. 2013, Frost 2021).

The phylogenetic inferences published on the Hylodidae up to now have included a reduced number of species, and have not supported the monophyly of either *Hylodes* (Pyron & Wiens 2011) or *Megaelosia* (Grant et al. 2017) due to the relationships of *M. goeldii*. In the phylogenetic tree provided by Pyron & Wiens (2011), the genus *Hylodes* was recovered as paraphyletic with respect to *M. goeldii*, which was the only *Megaelosia* species in the analysis. In the phylogenetic proposal by Grant et al. (2017), which included three *Megaelosia* species, *M. goeldii* was closely related to the species of the genus *Hylodes*, rendering *Megaelosia* a paraphyletic genus. More recently, the phylogenetic analysis by Dubois et al. (2021) also included *M. goeldii* as the only representative species of its genus, and proposed that *Megaelosia* is a junior synonym of *Hylodes*. This synonymization was reversed by Segalla et al. (2021), who revalidated the genus *Megaelosia* until further and more conclusive analyses were available. Given this scenario, a phylogenetic analysis based on a more representative sample of the genus *Megaelosia* will be needed to better evaluate the interspecific and intergeneric relationships within the Hylodidae.

Hylodes and *Crossodactylus* are cytogenetically similar. Except for *Hylodes nasus*, which has a diploid number of 2n = 24 (Bogart 1991), the karyotypes of the *Hylodes* and *Crossodactylus* species all have the same diploid number (2n = 26) and a highly similar chromosome morphology (Beçak 1968, Brum-Zorrila & Saez 1968, Bogart 1970, Denaro 1972, De Lucca & Jim 1974, Aguiar-Jr. et al. 2004, Amaro 2005). In the genus *Megaelosia*, on the other hand, diploid numbers range from 2n = 28 to 2n = 32 chromosomes (Melo et al. 1995, Giaretta & Aguiar-Jr. 1998, Rosa et al. 2003). That is, while *M. lutzae* has a diploid number of 2n = 32 (Rosa et al. 2003), and *M. boticariana* is 2n = 30 (Giaretta & Aguiar-Jr. 1998, Rosa et al. 2003), *M. massarti* has 2n = 28 or 29, due to the presence of B chromosomes, that is, B1 or B2 (Melo et al. 1995, Rosa et al. 2003). The karyotype of *M. goeldii*, which is the species that has represented its genus in many of the published phylogenies, was unknown prior to the present study.

In order to advance our knowledge on the genus *Megaelosia*, in the present study, we (i) describe the karyotype of *M. goeldii* and (ii) verify the monophyly of this genus based on mitochondrial and nuclear DNA sequences. Finally, as a result of our analyses we describe a new genus, to avoid paraphyly in the genus *Megaelosia*.

Material and methods

Specimens analyzed

For our cytogenetic analysis, we sampled four *Megaelosia goeldii* tadpoles, which had been collected at the type locality (Table 1). The samples used for the phylogenetic inferences were obtained from tadpoles or adults of *M. goeldii*, *M. apuana*, *M. boticariana*, *M. massarti*, *M. jordanensis*, and *M. lutzae*, which were collected, as far as possible, at their respective type localities (see Table 1, Fig. 1). We also included individuals collected from Pindamonhangaba (Fazenda Votorantin Celulose) in São Paulo state (SMRP 81.3, SMRP 81.6, and SMRP 81.8 – see Supplementary Supplementary Table S1), which are the same samples used by Rosa et al. (2003), who identified the species as *M. lutzae*. As Pindamonhangaba is about 100 km (in a straight-line) from Itatiaia National Park, RJ, the type locality of *M. lutzae* (Izecksohn & Gouvêa 1987), and said authors provided no information on the criteria they used to identify the species as *M. lutzae*, we conservatively treated these specimens as *Megaelosia* sp.

The ingroup in the phylogenetic analyses also included samples of two adults of *Hylodes asper* from Santo André, two adults of *H. japi* from Jundiaí, and one adult of *H. phyllodes* from Santo André, all in the state of São Paulo (SP); one adult of *H. heyeri* from Morretes, in the state of Paraná (PR); and two adults of *Crossodactylus gaudichaudii* from Rio de Janeiro (RJ). For information on museum catalogue numbers, collecting localities, and the GenBank accession numbers of the nucleotide sequences, see Supplementary Table S1.

All our samples of cells and tissues were deposited in the Shirlei Maria Recco-Pimentel (SMRP) collection of the Laboratory of Chromosomal Studies (LabEsC) and the Luís Felipe Toledo tissue collection (TLFT), both in the Institute of Biology at Campinas State University (UNICAMP), Brazil. The collection of specimens was authorized by the Brazilian Institute for the Environment and Renewable Natural Resources (IBAMA #02027.011919–95), through the Chico Mendes Institute for the Conservation of Biodiversity (ICMBio/SISBIO #17914-2/21619-1, #52352-2 and #75770-1), and were registered in the National System for the Management of Genetic Patrimony and Associated Traditional Knowledge (SisGen #ACD362E). For tissue extraction, the specimens were euthanized through the application of anaesthetic (10% Lidocaine) to the skin, following the recommendations of the Herpetological Animal Care and Use Committee (HACC) of the American Society of Ichthyologists and Herpetologists (available at http://www.asih.org).

To complement our matrix, we downloaded GenBank sequences of *M. goeldii*, *M. jordanensis*, *M. boticariana*, *H. phyllodes*, *H. nasus*, *H. meridionalis*, *H. caete*, *H. sazimai*, *H. perere*, *H. ornatus*, *H. amnicola*, *H. lateristrigatus*, *Crossodactylus schmidti*, and *C. caramaschii*. The outgroup included species representative of the family Alsodidae, which has been inferred to be the sister taxon to Hylodidae (Pyron & Wiens 2011, Grant et al. 2017; for details, see Supplementary Table S1).

Description of the karyotype

Mitotic metaphases were obtained from cell suspensions of the intestinal epithelium of four specimens of *M. goeldii*

Table 1. Species of *Megaelosia* analysed in the present study and their respective sampling localities in Brazil. The type locality of each species, where pertinent, is indicated by an asterisk.

Species	Specimens analysed	Locality
Megaelosia goeldii	4 tadpoles	Serra dos Órgãos National Park, Teresópolis, RJ*
Megaelosia apuana	3 tadpoles	Caparaó National Park, Alto Caparaó, MG
Megaelosia boticariana	3 tadpoles	Gruta Funda Park, Atibaia, SP* and São Francisco Xavier, São José dos Campos, SP
Megaelosia massarti	2 adults	Paranapiacaba, Santo André, SP*
Megaelosia jordanensis	1 tadpole	Campos do Jordão, SP*
Megaelosia lutzae	5 tadpoles	Itatiaia National Park, Itatiaia, RJ*
Megaelosia sp.	3 tadpoles	Fazenda Votorantin Celulose, Pindamonhangaba, SP

treated previously with colchicine (King & Rofe 1976, with the modifications of Gatto et al. 2018). The chromosomes were stained with Giemsa (10%) and C-banded (King 1980), and then silver-stained using the Ag-NOR method (Howell & Black 1980) to detect the Nucleolus Organizer Regions (NORs). We used 2n as the somatic chromosome number, and FN as the fundamental number or number of chromosome arms, following White (1954). The metaphasic chromosomes were photographed under an Olympus BX-60 microscope (Tokyo, Japan), edited in Adobe Photoshop, and classified according to Green & Sessions (1991).

Extraction of the DNA

Genomic DNA was extracted from liver or muscle tissue, previously stored in ethanol at -80°C. The tissue was lysed in TNES (50 mM Tris pH 7.5, 400 mM NaCl, 20 mM EDTA, and 0.5% SDS) supplemented with proteinase K (100 µg/mL) at 56°C for approximately 4 hours. After lysis, the samples were treated with NaCl to a final concentration of ~1.7 M. The DNA was precipitated in isopropyl alcohol, washed in ethanol (70%), and rehydrated in TE (10 mM Tris-HCl, 1 mM EDTA, pH 8). For quality control, and to quantify the genomic DNA, the samples were electrophoresed in 0.8% agarose gel and analyzed by NanoDrop (Thermo Fisher Scientific).

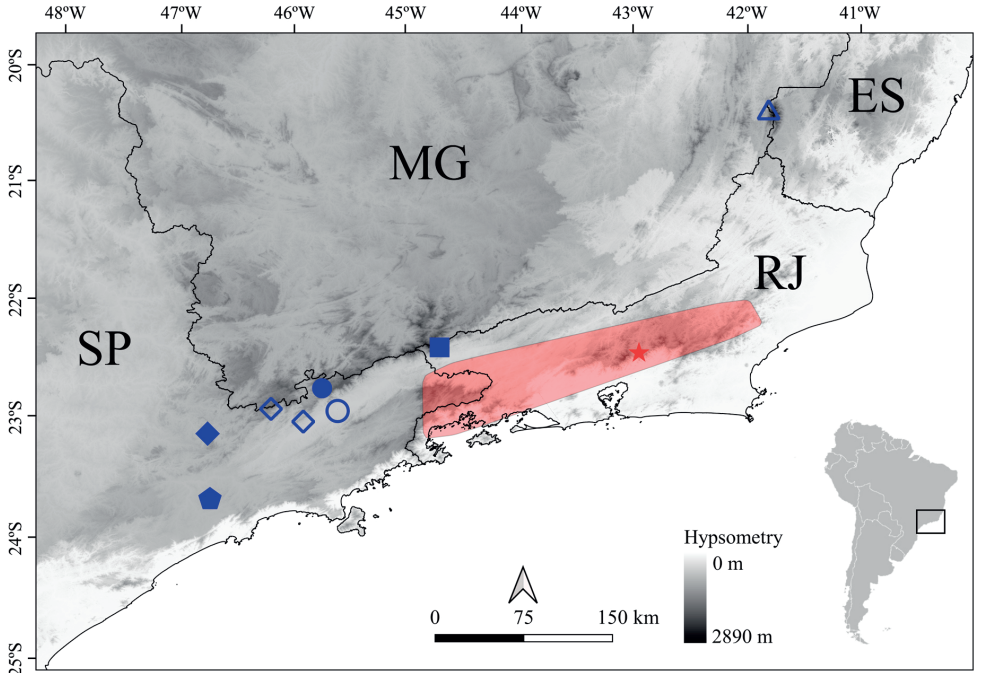

Figure 1. Schematic map showing the geographic distribution of the *Megaelosia* samples analysed in the present study. Red star = *Megaelosia goeldii* (Teresópolis, RJ), with its geographic distribution shaded in red; blue triangle = *Megaelosia apuana* (Alto do Caparaó, MG); blue circle (filled) = *Megaelosia jordanensis* (Campos do Jordão, SP); blue circle (open) = *Megaelosia* sp. (Pindamonhangaba, SP); blue rhombus = *Megaelosia boticariana* (Atibaia, São José dos Campos and Caçapava, SP); blue pentagon = *Megaelosia massarti* (Piracicaba, SP), and blue square = *Megaelosia lutzae*. Filled symbols indicate type localities.

Sequencing of the mitochondrial and nuclear DNA

To generate data for the phylogenetic analysis and genetic distance estimates, a mitochondrial fragment that included the 12S and 16S rRNA genes and the tRNA-Val gene was amplified by PCR using the primer pairs MVZ 59 (Graybeal 1997) and Titus I (Titus 1992) (or MVZ50; Graybeal 1997), and 12SL13 (Feller & Hedges 1998) (or H1KF – Carvalho et al. 2020) and 16Sbr (Palumbi et al. 2002). In some cases, we used the primers 12SKf and H1KR (Carvalho et al. 2020) to amplify a fragment of the 12S rRNA gene. A segment of the nuclear RAG-1 gene was also amplified by PCR, using the primers RAG-1F and RAG-1R (Faivovich et al. 2005). The amplified products were electrophoresed in 1% agarose gel and then purified using the GFX PCR and Gel Band DNA Purification kit (GE Healthcare) according to manufacturer's instructions. The samples were sequenced using the BigDye Terminator kit (Applied Biosystems). In addition to the primers cited above, we used the internal primers 16SL2a, 16H10 (Hedges 1994), and 16Sar (Palumbi et al. 2002) for sequencing.

The products of the sequencing reaction were purified by precipitation in 80% ethanol and centrifuged at 1,200 rpm for 30 minutes, and were then washed in 70% ethanol and centrifuged for 10 minutes. Once dried, the products were resuspended in loading dye (Blue-Dextran-EDTA/Formamide, 1:5), denatured for 3 minutes at 94°C and then transferred to an automatic sequencer. The sequences were edited using the Bioedit software, available at http://www.jwbrown.mbio.ncsu.edu/BioEdit/bioedit.html (Hall 1999).

Analysis of the DNA sequences

The mitochondrial matrix (2,337 bp) was concatenated with that of the Rag1 fragment (428 bp). The sequences were aligned using MAFFT v7 (Katoh et al. 2019; https://mafft.cbrc.jp/alignment/server/), following the G-INS-i strategy, and the resulting matrix had 2,765 bp and 68 terminals.

The phylogenetic analyses were based on Maximum Parsimony (MP) and Bayesian Inference (BI) approaches. The MP analyses were conducted in TNT v1.5 (Goloboff et al. 2016) using the new technology search option (including sectorial searches, ratchet, tree drifting, and tree fusing). Gaps were considered as a fifth state, and node support was evaluated by bootstrap analysis with 1,000 replicates. The BI was run in BEAST v2.6 (Bouckaert et al. 2019), based on the GTR model of DNA evolution, inferred by MrModeltest v2.3 (Nylander 2004). A relaxed clock log normal approach and the Yule process were applied. The final Markov Chain was run twice for 100 million generations. Each tree was sampled every 10,000 generations and the first 25% of the trees were discarded as burn-ins. Tracer v1.5 was used to check the convergence of the chains (Rambaut & Drummond 2009). The trees were summarized with TreeAnnotator v6.0 and visualized in FigTree v1.4.2 (Rambaut & Drummond 2019).

Confidence intervals of the support values were calculated following Huelsenbeck & Hillis (1993) and Felsenstein (2004), and the nodes with a posterior probability (PP) of > 0.95 and bootstrap (BS) values of at least 75% were considered to be sufficiently resolved, whereas those with a PP of 0.90–0.95 and BS values of 50–75% were considered to indicate a tendency, and those with a PP of less than 0.90 or BS below 50% were considered to be unresolved.

The genetic distances (p-distance) between species were calculated using a 1,402-bp fragment of the mitochondrial 16S gene (delimited by the primers 12SL13 and 16SBr) and a 538-bp fragment of the 16S gene (the 16Sar-16SBr fragment). All ambiguous positions were removed from each pair (pairwise deletion option) and the analyses were run in MEGA X (Kumar et al. 2018).

Inference of ancestral diploid numbers

To infer the ancestral chromosome number in *Megaelosia* we used the ChromEvol server (Glick & Mayrose 2014, Rice & Mayrose 2020) and the Bayesian Inference topology from this work as the input. To find the model that best fits our data, we first performed a test using all models available on ChromEvol and selected the model with the lowest Akaike Information Criterion (AIC) value, which was "BASE_NUM". A second test was performed with optimized parameters in the best model. The haploid chromosome numbers of *M. goeldii* (from this work), *M. boticariana, M. lutzae*, *M. massarti*, three *Crossodactylus* species, and five *Hylodes* species (as previously cited) were plotted into the analysis. Unknown chromosome numbers were coded "x".

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 of the ICZN. The LSID (Life Science Identifier) for this publication is: urn:lsid:zoobank.org:pub: 7FF6FDDC-4869-4C87-9FC3-26359042BEE6.

Results

Megaelosia goeldii karyotype description

The karyotype of *M. goeldii* has a diploid number of 2n = 26 and a fundamental number of FN = 52, being composed of one metacentric chromosome pair (pair 4) and 12 submetacentric pairs (pairs 1–3 and 5–13) (Fig. 2a). In the Giemsa-stained metaphases, secondary constrictions

Table 2. Uncorrected *p*-distances (in percentages) between *M. goeldii* and the *Phantasmarana* species based on the 1,402-bp (below diagonal) and 538-bp fragments (above diagonal) of the mitochondrial 16S gene. The grey cells in the diagonal show intraspecific distances (larger fragment/smaller fragment).

Taxon	1	2	3	4	5	6	7
1. *Megaelosia goeldii*	0.1/0.2	8.2	7.3	7.2	7.5	6.9	6.7
2. *Phantasmarana apuana*	13.4	0/0	1.7	1.6	1.6	3.2	3.5
3. Phantasmarana sp.	13.1	1.9	0.2/0.2	0.2	1.2	2.6	2.7
4. *Phantasmarana jordanensis*	13.0	1.7	0.3	0.3/0.2	1.1	2.5	2.6
5. Phantasmarana lutzae	13.1	2.1	1.7	1.6	0.3/0.4	2.8	2.8
6. *Phantasmarana massarti*	12.6	4.5	4.0	3.9	4.0	0.2/0	0.9
7. *Phantasmarana boticariana*	12.5	4.3	3.9	3.8	4.0	2.3	0.9/0.1

were observed in the pericentromeric region of the long arm of the homologs of pair 3 (Fig. 2a), which coincided with the NORs detected by silver-staining (Fig. 2b). Size heteromorphy of the homologous NORs was observed in two of the four individuals analyzed. The C-bands were detected in the centromeric region of all the chromosomes and pericentromerically in the long arm of the homologs of pair 3 (coinciding with the NORs) and in the short arm of pair 12 (Fig. 2c).

Phylogenetic inferences on the Hylodidae

In both the BI and the MP analyses, the internal relationships in the monophyletic family Hylodidae recovered paraphyly of *Megaelosia* in relation to *Hylodes* (Fig. 3), with *M. goeldii* being the sister taxon of the clade composed of the 12 *Hylodes* species included in our dataset. The *M. goeldii*+*Hylodes* clade had moderate support (posterior probability = 0.9, bootstrap = 68%). All the remaining species of *Megaelosia* analyzed here (i.e., *M. massarti*, *M. boticariana, M. apuana, M. jordanensis, M. lutzae*, and *Megaelosia* sp.) were recovered in a highly supported clade (posterior probability = 1.0, bootstrap = 100%), which was the sister group of the *M. goeldii*+*Hylodes* clade (Fig. 3). This topology indicates the need for a taxonomic rearrangement to avoid paraphyly in *Megaelosia* and to this end, we propose a new genus, which is described below.

The new genus presented well-supported internal relationships in our phylogenetic analyses, with *M. massarti* as the sister species of *M. boticariana*, and *M. jordanensis* as the sister species of *M. apuana* and *M. lutzae* (Fig. 3).

The specimens SMRP 81.3, SMRP 81.6, and SMRP 81.8, identified a priori as *Megaelosia* sp., and analyzed previously by Rosa et al. (2003), were nested within our sample of *M. jordanensis*, rather than the topotypes of *M. lutzae*, in both topologies (Fig. 3). The genetic distances between these three *Megaelosia* sp. individuals and the *M. jordanensis* specimens, including a *M. jordanensis* topotype (CFBH 28578), estimated from a 1,402-bp fragment of the 16S rRNA gene, were very low (approximately 0.3%; Table 2). We thus conclude that the specimens collected by Rosa et al. (2003) are, in fact, individuals of *M. jordanensis*.

Ancestral diploid numbers

The ancestral chromosomal number analysis recovered the node that includes *Hylodes*+*M. goeldii* with 2n = 26 (posterior probability = 0.98). The node that includes the

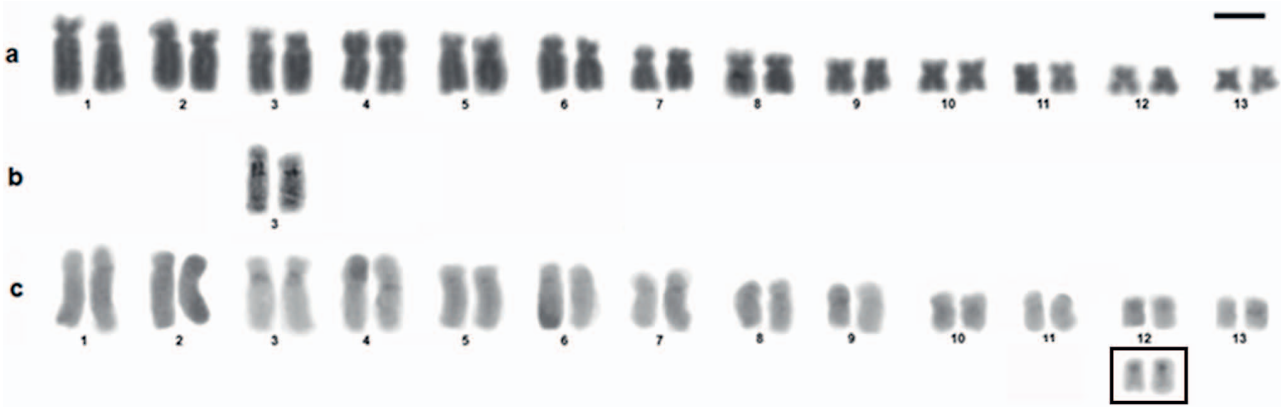

Figure 2. Karyotype of *Megaelosia goeldii*. (a) Giemsa staining, (b) Chromosome pair 3 showing the nucleolus organizer regions (NORs) detected by silver-staining, (c) C-banding. Scale bar = 5 μm.

other *Megaelosia* species (i.e., *M. massarti*, *M. boticariana*, *M. apuana*, *M. jordanensis*, *M. lutzae*, and *Megaelosia* sp.) was recovered with 2n = 28 as the most likely ancestral diploid number (posterior probability = 0.75) for these species.

Phantasmarana gen. nov.

ZooBank: LSID urn:lsid:zoobank.org:pub:E26B7159-0F73-4D09-AB4E-E39E24F0B23F

Type species: *Elosia massarti* de Witte, 1930

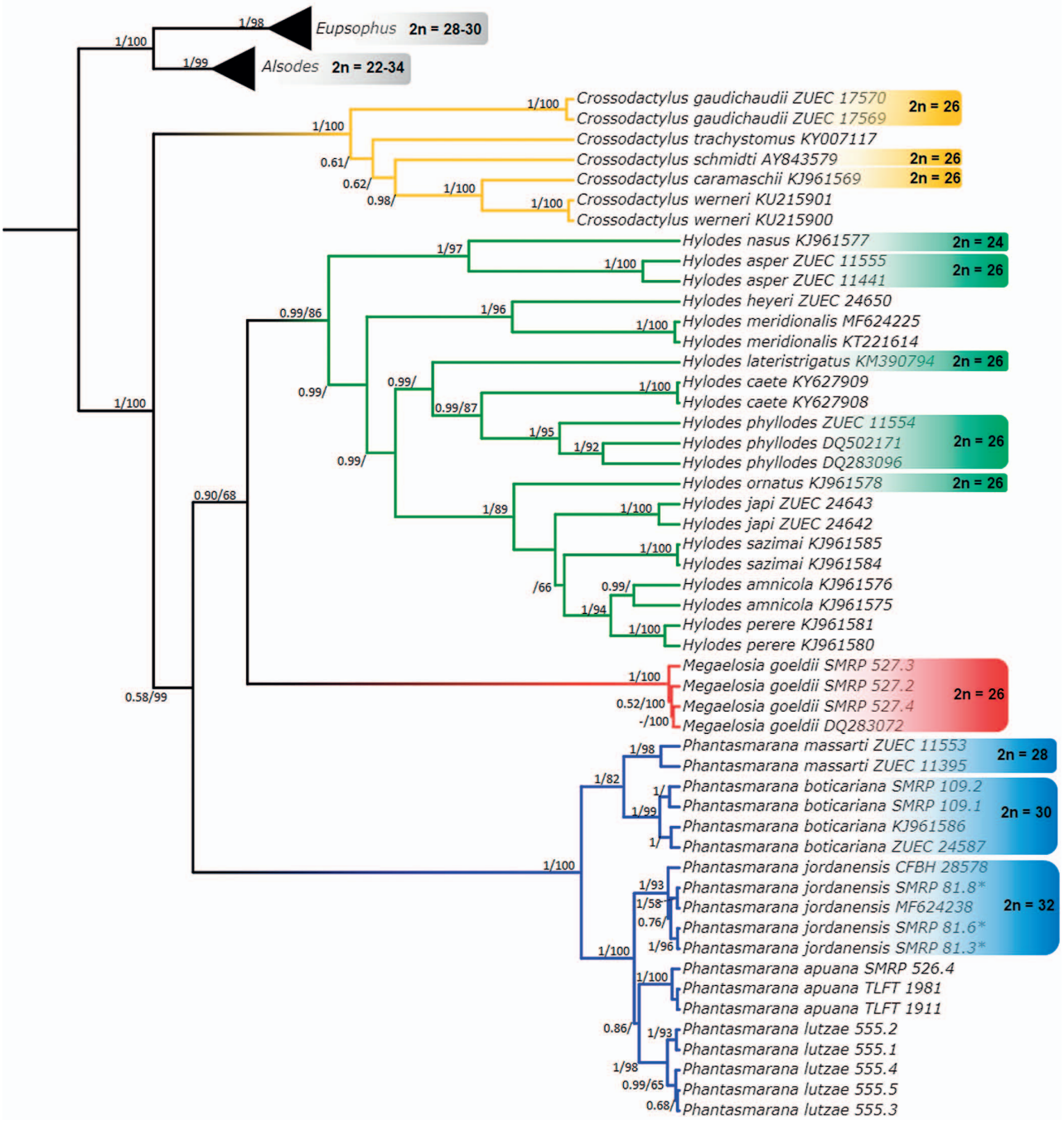

Figure 3. Phylogenetic relationships of the family Hylodidae inferred by Bayesian analysis of the mitochondrial sequence H1 and the RAG-1 fragment. The numbers at the nodes are posterior probabilities (left) and bootstrap values in percentage (right), inferred from Bayesian and Maximum Parsimony analyses, respectively (values of less than 50% have been omitted). The asterisk indicates *Phantasmarana* sp., which we assigned to *P. jordanensis*. Diploid numbers (2n) were obtained in previous studies (cited in the text; Blotto et al. 2012, Quercia et al. 2020), except for *M. goeldii*, which is described in the present study.

Diagnosis. This genus was recognized as monophyletic in our phylogenetic analyses based on mitochondrial and nuclear DNA sequences and can also be diagnosed by the combination of the following phenotypic traits: (i) presence of paired lateral white vocal sacs (vs. absent in *Megaelosia*, completely translucent in *Hylodes*, and partially translucent in *Crossodactylus*: Fig. 4), (ii) presence of a vocal slit (vs. absent in *Megaelosia*), (iii) fold of fifth toe does not reach outer metatarsal tubercle (vs. reaching this tubercle in *Megaelosia*, not extending much beyond the base of the proximal subarticular tubercle in *Crossodactylus* and *Hylodes*), (iv) snout rounded or slightly protruding in lateral view (vs. clearly protruding in lateral view in *Megaelosia*), (v) finger discs more than 30.5 times the SVL (vs. less than 30.5 times in *Megaelosia*), (vi) scutes on finger discs absent or weakly developed (vs. well developed in *Megaelosia*), (vii) adult snout–vent length exceeding 6 cm (vs. less than 5 cm in *Hylodes* and *Crossodactylus*), (viii) absence of advertisement call (vs. present in *Crossodactylus* and *Hylodes*, except for *H. vanzolinii*; but absent in *Megaelosia*), and (ix) diploid numbers with 28 or more chromosomes (vs. 26 in *Megaelosia*, *Hylodes* and *Crossodactylus*). Traits i, iii, and ix represent putative synapomorphies for this new genus.

Content. Six species. *Phantasmarana apuana* (Pombal, Prado & Canedo, 2003) comb. nov.; *P. bocainensis* (Giaretta, Bokermann & Haddad, 1993) comb. nov.; *P. boticariana* (Giaretta & Aguiar-Jr., 1998) comb. nov.; *P. jordanensis* (Heyer, 1983) comb. nov.; *P. lutzae* (Izecksohn & Gouvêa, 1987) comb. nov.; and *P. massarti* (De Witte, 1930) comb. nov.

Sister taxon. The clade including *Hylodes* and *Megaelosia*.

Description. Adult frogs of this genus are large, with SVL ranging from 6 to 12 cm in adult males and females. Tadpoles are giant, reaching 13 cm or more in total length (Supplementary Table S2 and Appendix S1) and may remain in the larval stage for more than one year (Augusto-Alves et al. unpublished data). Adults and larvae are likely nocturnal, inhabit forest streams, and adults are generalist, feeding on invertebrates, fish, and other amphibians (Peloso & Pavan 2007, Alves & Toledo 2017, Augusto-Alves et al. 2018). Although they lack an advertisement call, a mysterious type of call has been reported in *P. boticariana* (Muscat et al. 2020b), and adult males may use their contrasting white vocal sac for visual signalling in intraspecific nocturnal communication (Augusto-Alves et al. 2018).

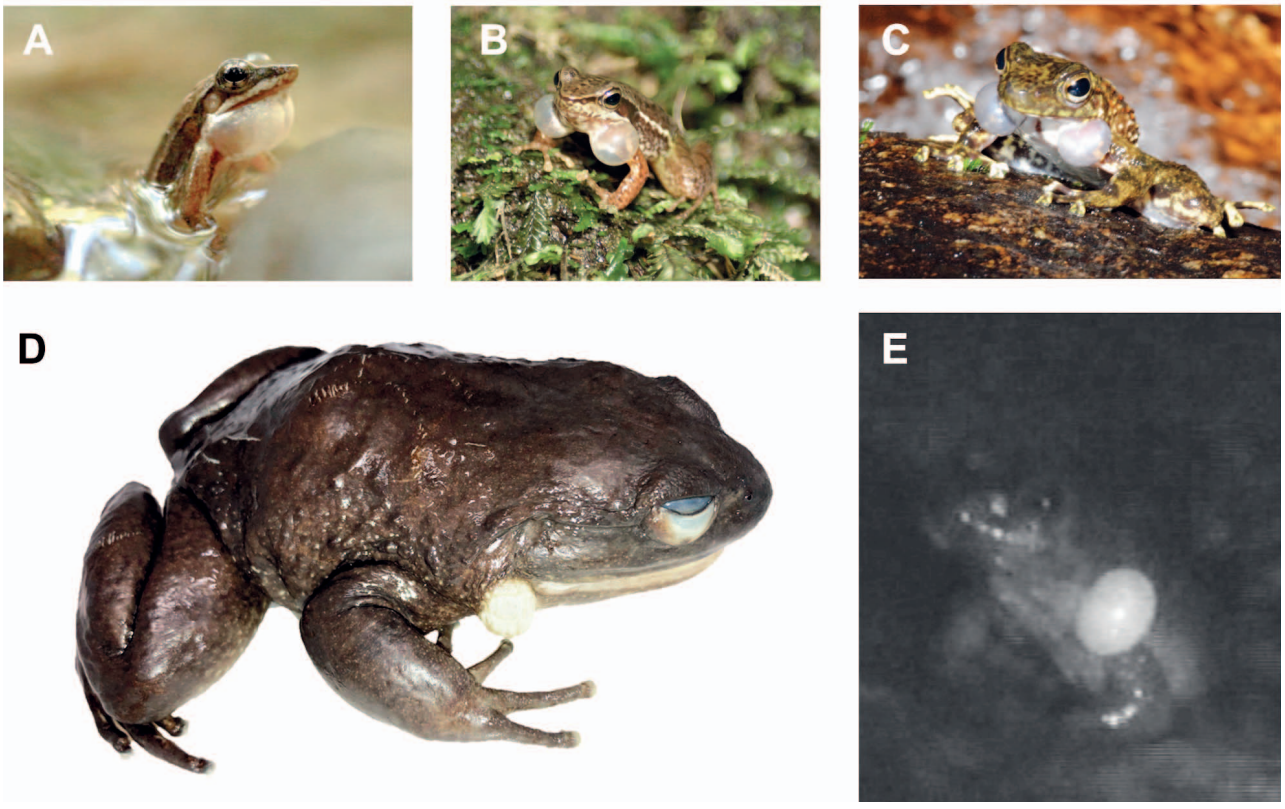

Figure 4. Adult male *Crossodactylus caramaschii* (frame extracted from a video by D. Perrella: ZUEC-VID 800) highlighting its partially translucent vocal sac (A); Adult male *Hylodes phyllodes* (photograph by L. F. Toledo) and *Hylodes asper* (photograph by M. Martins) with completely translucent vocal sacs (B-C); preserved male *P. boticariana* (D: photograph by L. F. Toledo: ZUEC 11843) and live male *P. apuana* (E: frame extracted from video by G. Augusto-Alves: ZUEC-VID 402; Augusto-Alves et al. 2018) with contrasting white vocal sacs.

Etymology. The name *Phantasmarana* is derived from the combination of Latin words *phantasma* (= phantom) + *rana* (frog), meaning "phantom frogs" in reference to the extraordinary rarity of frogs of this genus in the wild (only few herpetologists have seen these frogs in their natural habitats), the lack of an advertisement call, and the fact that some enigmatic sounds have been reported. The gender is feminine.

Distribution. The genus *Phantasmarana* occurs in forested areas of the Atlantic rainforest biome, ranging from the state of São Paulo (southern limit) to the states of Minas Gerais and Espírito Santo (northern limit). Endemic to Brazil.

Megaelosia Miranda-Ribeiro, 1923

Type species: *Hylodes goeldii* Baumann, 1912

Diagnosis. This genus is monotypic and can be diagnosed by the combination of the following traits: (i) males lacking vocal sacs and vocal slits (vs. present in *Hylodes* and *Phantasmarana*); (ii) fold of fifth toe reaching outer metatarsal tubercle (vs. not reaching this tubercle in *Phantasmarana*); (iii) snout subacuminate in dorsal view and protruding in profile (vs. rounded or slightly protruding in lateral view in *Phantasmarana*); (iv) tips of fingers and toes with a pair of scutes free from subunguis (vs. absent or weakly developed in *Phantasmarana*); (v) finger discs less than 30.5 times the SVL (vs. more than 30.5 times in *Phantasmarana*); (vi) prootic not fused with the frontoparietals (vs. fused in *Hylodes*, unknown in *Phantasmarana*); (vii) alary process of the hyoid absent (vs. rudimentary in *Hylodes*, unknown in *Phantasmarana*); (viii) mesosternum composed of cartilaginous plaques (vs. bony, broad, and bifurcated posteriorly in *Hylodes*, unknown in *Phantasmarana*); (ix) absence of apical supplementary elements of the m. intermandibularis (vs. present in *Hylodes* and *Phantasmarana*); (x) zygomatic ramus larger than optic ramus (vs. absent in *Hylodes*, unknown in *Phantasmarana*); (xi) absence of advertisement call (vs. present in *Hylodes* spp., except in *H. vanzolinii*), and (xii) diploid number = 26 chromosomes (vs. 28 or more in *Phantasmarana*). Traits i and ix (lack of vocal slit, sac, and supplementary elements of the m. intermandibularis) are autapomorphies of this genus.

Content. One species: *Megaelosia goeldii* (Baumann, 1912).

Sister taxon. The genus *Hylodes.*

Description. Adult frogs of this genus are large, with SVL varying between 8.2 and 9.5 cm SVL in both adult males and females (Supplementary Table S2). Tadpoles are large, one was measured, and found to be 11.7 cm in total length (Nuin 2003). Adults and larvae are thought to be diurnal (Giaretta et al. 1993, Nuin 2003) and adults are generalists, feeding on invertebrates and other amphibians (Giaretta et al. 1993). Although there is no advertisement call, an unidentified type of call was reported in *M. goeldii* (Muscat et al. 2020b).

Distribution. The single species of the genus occurs in forested areas of the Atlantic rainforest biome, from the north of the state of São Paulo (Serra da Bocaina) to central Rio de Janeiro state (Desengano State Park). Endemic to Brazil.

Discussion

Intrageneric relationships in the family Hylodidae

The family Hylodidae now consists of four genera, *Crossodactylus*, *Hylodes*, *Megaelosia*, and *Phantasmarana*, with the latter being proposed here to accommodate six species assigned previously to the genus *Megaelosia*, which is now a monotypic genus.

The inclusion in our phylogenetic analyses of six of the seven species previously assigned to *Megaelosia* allowed us to revisit the systematics of this genus and elucidate the interspecific relationships of *M. goeldii*. Phylogenetic and morphological evidence supports the definition of *M. goeldii* as the only species of the genus *Megaelosia*, while the remaining species assigned historically to this genus in fact form a distinct clade. The monotypic *Megaelosia* is now demonstrated to be a sister group of the genus *Hylodes*, as inferred from our two phylogenetic analyses.

In addition to molecular evidence, a number of morphological characters distinguish *Phantasmarana* from *Hylodes* and *Megaelosia*. As proposed by Nuin & Val (2005), two synapomorphies of *Hylodes* may be recognized – the fusion of the prootic and the frontoparietals, and the rudimentary alary process of the hyoid. *Megaelosia goeldii* has two autapomorphic conditions, as described by Elias-Costa et al. (2017), that is, the absence of a vocal sac and the apical supplementary elements of the m. intermandibularis. Furthermore, Nuin & Val (2005) described the zygomatic ramus as being larger than the optic ramus in *M. goeldii*, a trait that is absent in *Hylodes*. *Phantasmarana bocainensis* (missing from our molecular analysis) was included in the new genus due to the fact that the fold of the fifth toe does not reach the outer metatarsal tubercle (see Giaretta et al. 1993), one of the putative synapomorphies of *Phantasmarana.*

From a biogeographic perspective, the species of *Phantasmarana* occur in the Serra da Mantiqueira and Serra do Mar mountain ranges (in SP, RJ, and MG). The Serra da Mantiqueira hosts several endemic anuran taxa (Cruz & Feio 2007, Haddad et al. 2013, Silva et al. 2018), and phylogeographic evidence supports the historical role of geomorphological changes as a source of the vicariant processes that likely resulted in the high level of interspecific diversification observed in this region (Chaves et al. 2014). The *Phantasmarana* populations, which are associated with high altitude, appear to represent a promising model for phylogeographic studies, which may further corroborate evidence of ongoing evolutionary processes in eastern Brazil.

No evidence of exclusive morphological characteristics of the tadpoles or adults have been identified in either of the well-supported clades recovered in *Phantasmarana* (*P. massarti* + *P. boticariana*) and (*P. jordanensis* + (*P. apuana* + *P. lutzae*)) in previous studies (Giaretta et al. 1993, Pombal et al. 2003, Nogueira-Costa et al. 2012, Sichieri et al. 2020). Although *P. massarti* and *P. boticariana* occur at geographically proximal sites, the phylogeographic relationship between them is unclear, given that they are found in distinct geological formations, with *P. massarti* occurring in the Serra do Mar range (Giaretta et al. 1993, Frost 2021) and *P. boticariana* in the Serra da Mantiqueira (Muscat et al. 2020a). The phylogeographic relationships of the clade composed of *P. jordanensis*, *P. lutzae*, and *P. apuana* are also unclear, although *P. jordanensis* and *P. lutzae* are both found in the Serra da Mantiqueira (Nogueira-Costa et al. 2012, Izecksohn & Gouvêa 1987), while *P. apuana* occurs in the Serra do Caparaó (an extension of the Serra da Mantiqueira, but approximately 460 km from the topotype of *P. jordanensis* and 360 km from that of *P. lutzae*; Santos et al. 2011, Zornosa-Torres et al. 2020). Further research is needed to elucidate the phylogenetic relationships between *P. bocainensis* and the other *Phantasmarana* species.

The diploid number of 2n = 26 is a putative plesiomorphic condition of the family Hylodidae, as it is found in the genera *Crossodactylus*, *Hylodes*, and *Megaelosia*. In the most parsimonious scenario, the most recent common ancestor of *Phantasmarana* would have had 2n = 28 chromosomes, derived from a rearrangement of the 2n = 26 karyotype. Subsequent rearrangements would have led to further increases in diploid numbers, as observed in *P. boticariana*, with 2n = 30 chromosomes (Giaretta & Aguiar-Jr. 1998, Rosa et al. 2003) and, in the other clade, *P. jordanensis*, with 2n = 32 (Rosa et al. 2003). However, a more conclusive interpretation of the evolutionary changes in *Phantasmarana* would require chromosomal data from *P. apuana*, *P. lutzae*, and *P. bocainensis*.

The findings of the present study provide a better understanding of the phylogenetic relationships in the Hylodidae, in addition to unravelling the historical taxonomic uncertainties of *Megaelosia*, with most species now being assigned to the new genus, *Phantasmarana*. The present study thus contributes to a more comprehensive viewpoint on the evolutionary processes that have formed this fascinating and diverse anuran family, and it will provide an essential database for future studies in the mountains of eastern Brazil.

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Supplementary data

The following data are available online:

Supplementary Table S1. Specimens included in our analysis with their localities and identification (ID) numbers in scientific collections and the GenBank accession numbers of the nucleotide sequences, with respective bibliographic citations.

Supplementary Table S2. Measurements of analyzed individuals, including information available on literature. Abbreviations: SVL = snout–vent length; TL = total length; HW = head width; HL = head length; FDW = finger discs width.

Appendix S1. Additional hylodid specimens or recordings examined, to complement the information available in the literature, which are deposited in the following Brazilian collections.