

Research Paper

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A new species of lungworm from the Atlantic Forest: *Rhabdias megacephala* n. sp. parasite of the endemic anuran *Proceratophrys boiei*

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Abstract

Rhabdias are lung-dwelling parasites of anurans and some reptiles. Currently, 93 species are known to exist worldwide. The identification of *Rhabdias* species is based mainly on morphological traits of hermaphroditic females that generally have a very conserved morphology. However, different approaches, such as the combination of morphological, molecular, and ecological data, have provided advances in identifying and delimiting rhabdiasid species. Here, we describe a new species of *Rhabdias* from the south of Brazil, with morphological and molecular data. The new species is distinguished from its congeners by having an elongated body, evident cephalic dilation, larger buccal capsule, and large esophagus. In addition to morphological characteristics, we observed significant genetic divergence among the cytochrome oxidase subunit I (COI) sequence of the new species and the closest available sequence, *Rhabdias fuelleborni* (10.24%–10.87%). Furthermore, phylogenetic reconstructions based on the COI gene indicated that the new species represents a different lineage, constituting an outgroup of the species complexes *Rhabdias* cf. *stenocephala* and *Rhabdias fuelleborni* with *Rhabdias* sp. 4. Thus, *Rhabdias megacephala* is the 24th nominal species of the Neotropical region, the 14th Brazilian, and the fourth species described from south of Brazil.

Introduction

Rhabdias Stiles and Hassall, 1905 are lung-dwelling parasites of amphibians and some reptiles (Kuzmin *et al.* 2007, 2016), distributed worldwide, except for Antarctica (Kuzmin *et al.* 2003, 2022). The genus is composed of 93 species, with 23 found parasitising Neotropical hosts (Marcaida *et al.* 2022; Müller *et al.* 2023; Alcantara *et al.* 2023). In Brazil, 13 species of *Rhabdias* have been reported (da Silva *et al.* 2013; Alcantara *et al.* 2023), which is a low number when compared with the diversity of known anurans for the region (Santos-Pereira *et al.* 2018).

Rhabdias species are very similar morphologically, and most species' descriptions are based mainly on morphological characteristics. Thus, the current identification and delimitation of species is still an issue, making taxonomic resolution difficult in most species inventories and ecological studies (Kuzmin 2013; Tavares-Costa *et al.* 2022). However, different approaches, combining morphological and molecular analyses, have contributed to the recognition and identification of new species (Langford & Janovy 2013; Tkach *et al.* 2014; Müller *et al.* 2018). These studies point to the taxonomy of *Rhabdias* lungworms as a rising venue for research that may preclude understanding of the diversification and phylogeography of such a fascinating group of organisms.

Proceratophrys boiei Wied-Neuwied, 1824 is a medium-sized, nocturnal, and terrestrial anuran, found in the leaf litter of primary and secondary forests and also in degraded areas (Prado & Pombal Jr. 2008). This species is endemic in the Brazilian Atlantic Forest, occurring from southern Espírito Santo, southern and western Rio de Janeiro state into south São Paulo, and eastern Paraná to eastern Santa Catarina (Frost 2024). Until now, six species of helminth parasites are known for *Proceratophrys boiei*: *Aplectana delirae* (Fabio, 1971); *Cosmocerca parva* Travassos 1925; *Oxyascaris oxyascaris* Travassos, 1920; *Physaloptera* sp.; Centrorhynchidae gen. sp.; and Physalopteridae gen. sp.1 (Klaion *et al.* 2011; Campião *et al.* 2014; Euclides *et al.* 2022).

Euclides *et al.* (2021) reported a species of *Rhabdias* parasitising *P. boiei* that did not correspond to any known congeneric species. Thus, based on this material, we describe a new species of *Rhabdias* found in *Proceratophrys boiei* based on morphological, molecular, and phylogenetic data using the cytochrome oxidase subunit I (COI) DNA sequences of the mitochondrial DNA.

Material and methods

Host sampling, parasite collection, and identification

Between October 2018 and January 2019, 27 individuals of *Proceratophrys boiei* were collected in Marumbi State Park, municipality of Piraquara, Paraná State, Brazil (Mananciais da Serra - 25°30'22"S; 45°01'41"W) (Euclides *et al.* 2021). The Marumbi State Park features a subtropical climate composed of forests with typical Atlantic Forest formations, consisting mainly of *Araucaria angustifolia*, the dominant tree species that characterises this type of forest (Reginato & Goldenberg 2007; Bergamini & Thomas 2011). The anurans were euthanised with lidocaine (4%) topically applied, and then the specimens were necropsied by a longitudinal incision in the anteroposterior axis on the ventral region of the body.

The hosts' lungs were examined, and nematodes found were rinsed in saline solution, heat-killed, and stored in 70% alcohol for morphological identification. Some specimens were preserved in 100% alcohol for molecular analysis. For morphological analysis, the nematodes were rehydrated in distilled water, cleared in lactophenol, mounted on temporary slides, and examined under an Olympus BX41 microscope coupled with a drawing tube. We also analysed the morphology of the apical region of the anterior end, by manual sections using a razor blade, and posterior end face observations. Taxonomic illustrations were made using line drawings handmade and posteriorly prepared in CorelDraw 2018 (Corel Corporation, Ottawa, Ontario, Canada) and processed with Photoshop Version 21.0.2 (Adobe Systems Incorporated, San Jose, California, USA).

Specimen measurements are presented as the values of the holotype followed by the mean and range for the entire type series, with both values in parentheses (reported in micrometers unless otherwise indicated). We deposited the type series of the new species in the invertebrate collection of the Federal University of Paraná (Accession numbers: DZUP 541909–541913).

Molecular and phylogenetic analysis

The specimens preserved in absolute alcohol were sectioned in the anterior end (close to the esophagus–intestinal junction) and just after the posterior portion of the female reproductive system. The anterior and posterior parts were stored in absolute alcohol and deposited as the hologenophore (see Pleijel *et al.* 2008) in the parasitological section of the invertebrate collection of the Federal University of Paraná.

We performed DNA extraction from the middle part of the parasite body using the Wizard® Genomic DNA extraction kit (Promega, Madison, Wisconsin, USA), following the manufacturer's instructions. Then, the extracted DNA was submitted to a conventional polymer chain reaction (PCR) with previously designed primers (LCO1490/Foward - 5' GGTCAACAAATCA-TAAAGATATTGG 3' and HC02198/Reverse - 5' TAAACTT-CAGGGTGACCAAAA 3') (described by Folmer *et al.* 1994), and analysis of these amplified fragments was performed using 0.75% agarose gel electrophoresis. The fragments were visualised with ultraviolet light, and the corresponding fragments were cut and purified with the PureLink™ Quick Gel Extraction and PCR Purification Combo Kit (ThermoFisher, Waltham, Massachusetts, USA). After purification, the samples were submitted to PCR for sequencing, using BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Warrington, UK). These samples were

analysed by an ABI PRISM 3500 Genetic Analyser sequencer (Thermo Fisher Scientific [Applied Biosystems], Waltham, Massachusetts, USA).

The obtained sequences were analysed and assembled in FinchTV Version 1.4.0 software (Geospiza [agora parte da PerkinElmer], Waltham, Massachusetts, USA). A search was carried out in Nucleotide Basic Local Alignment Search Tool (BLASTn) using the sequence obtained to investigate the existence of similar sequences in the National Center for Biotechnology Information (NCBI) database, considering only sequences with more than 90% similarity. Using the default parameters, we aligned the sequences in MAFFT v. 7 (Katoh & Standley 2013). We cut the alignment ends and checked the stop codons in Geneious V.4.7 (Biomatters Ltd. [agora parte da Dotmatics], Auckland, New Zealand). We evaluated the substitution saturation with the Iss index by testing the alignment data in DAMBE 7.3.32 software (Xia 2018). We calculated the number of base substitutions between sequences. For analyses calculating estimates and standard errors, we used the Kimura 2-parameter model with the MEGA 11 software package (Kimura 1980; Tamura *et al.* 2021).

For phylogenetic analysis, we ran our aligned matrix using jModelTest 2.1.10 software (Posada 2008). The evolution model selected was GTR + I + G according to the Akaike information criterion (AIC). We performed phylogenetic reconstructions using maximum likelihood (ML) in IQ-Tree software (Minh *et al.* 2020) and Bayesian inference (BI) using MrBayes 3.2.7 (Ronquist *et al.* 2003).

The ML inference used support values of 1000 repetitions (bootstrap), and only nodes with bootstrap values greater than 70% were considered supported. BI was performed using the Markov chain Monte Carlo (MCMC) search, with the following parameters: lset nst = 6, rate = invgamma, ngammacat = 4. Chains with 50,000,000 generations were executed, saving only 1,000 generations. On the burn-in of the first 25% of the generations, only nodes with posterior probability greater than 90% were considered well supported. As an outgroup, we chose *Serpentirhabdias viperidicus* Morais, Aguiar, Muller, Narciso, Silva and Silva, 2017 (KX350054), as has been used in other studies (Müller *et al.* 2018; Willkens *et al.* 2020; Alcantara *et al.* 2023). We used FigTree v1.4.4 (Rambaut 2009) and Adobe Illustrator (Adobe Systems Incorporated) to visualise and edit the trees.

Results

Systematics

Family: Rhabdiasidae Railliet, 1915

Genus: *Rhabdias* Stiles and Hassall, 1905

Species: *Rhabdias megacephala* n. sp. Euclides, Melo & Campião, 2024

Taxonomic Summary

Type host: *Proceratophrys boiei* (Wied-Neuwied, 1824) (Amphibia: Odontophrynidae).

Type locality: Pico Marumbi State Park (Mananciais da Serra), Piraquara, Paraná, Brazil (25°29'23" S; 48°58'37" W).

Site of infection: Lungs

Numbers of specimens/hosts, prevalence, mean infection intensity and range: 87 nematodes were found in 27 frogs, P = 66.6%; 3.2 (1–14).

Type material: Holotype (DZUP: 541909) and seven paratypes (DZUP: 541910–541913) were deposited in the invertebrate collection of the Federal University of Paraná.

GenBank, accession numbers: PP291576

ZooBank Registration: The Life Science Identifier for *R. megacephala* n. sp. is urn:lsid:zoobank.org:pub:C18FED99-0255-412A-AE0C-4A308282D919.

Etymology

The specific name *megacephala* refers to the new species' highly distinguishing morphological feature, namely the prominent cuticular inflation around the cephalic end.

Description

See Figure 1 and Figure 2 (based on holotype and seven paratypes, all gravid hermaphrodites). Body slender, long, 9.7 (9.1; 8.1–9.9) mm in length. Body surface covered by apparent cuticular inflation in anterior and posterior regions, discrete along entire body. Very prominent cuticular inflation in cephalic region 503 (508; 466–570) in width. Cephalic inflation rounded, terminating at its connection

to body wall at level of shoulder-like broadening of the body. Cuticular inflation close to shoulder-like broadening with two to three cuticular folds. Lateral pores arranged in two lines along cuticular inflation, connected by ducts with body wall. Cuticular inflation of tail prominent, with one large fold posterior to anus aperture, with second large fold reaching mid-length of tail and followed by minor folds decreasing in size. Body width at vulva 502 (513.8; 416–623), at esophagus–intestine junction 355 (359.8; 280–465). Oral opening with four enlarged and equidistant lips, situated very close to oral opening; each lip with terminal papilla on its inner edge; two amphids located laterally at some distance from oral opening. Vestibule circular in apical view, with narrow lumen. Buccal capsule cup-shaped 23 (15.2; 11–23) deep and 22 (26.3; 22–34) wide, with 1.04 (0.58; 0.46–1.04) depth/width ratio. Buccal capsule walls consisting of anterior part, with irregular internal surface and posterior one with smooth internal wall. Buccal capsule close to entrance of esophageal lumen with serrated surface. Entrance of esophagus lumen triangular, with serrated edges and esophageal gland located in dorsal region. Esophagus length 683 (722; 630–799), representing 7% (7.9%; 7–9%) of total body length; claviform, rounded apex, and dilation at anterior muscular region. Width of anterior end of esophagus 78 (73.7; 60–88), width

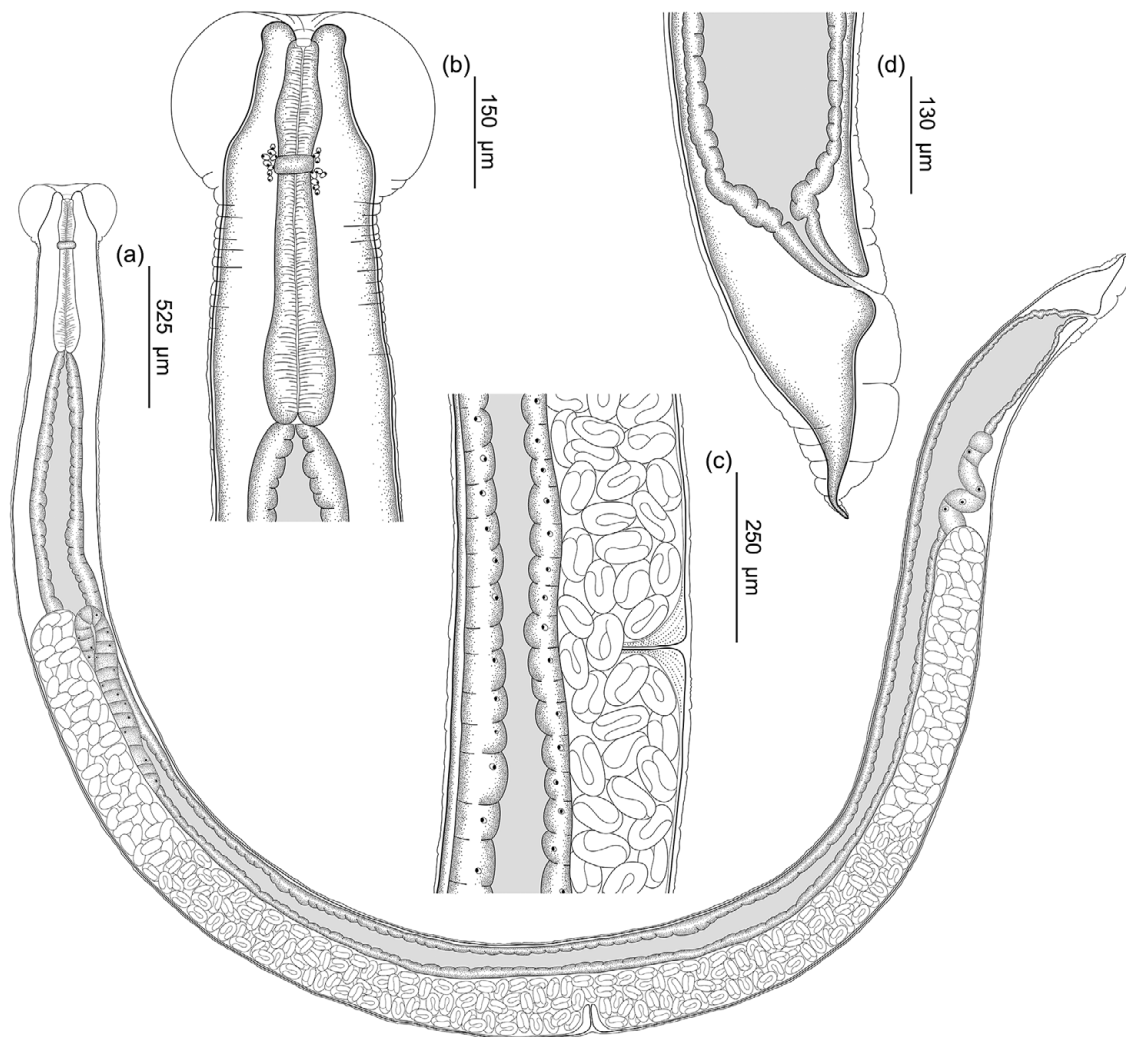


Figure 1. Line drawings of *Rhabdias megacephala* n. sp. from *Proceratophrys boiei*. A) Entire body, lateral view; B) Anterior end of the body, lateral view; C) Vulva region, lateral view; D) Caudal end, lateral view.

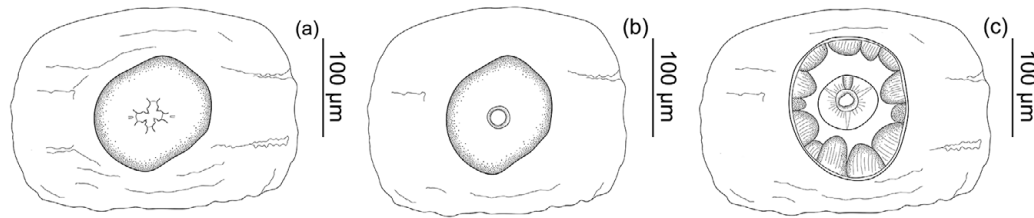


Figure 2. Line drawings of cross sections of anterior end and face view of *Rhabdias megacephala* n. sp. from *Proceratophrys boiei*. A) Anterior extremity end face view; B) Optical section through anterior part of buccal capsule; C) Optical section through posterior part of buccal capsule.

of anterior dilatation of esophagus 75 (75.7; 60–91), width posterior dilatation of the esophagus 98 (98; 83–112), width of bulb 162 (154.8; 124–176). Nerve ring around esophagus after anterior dilatation at 152 (297.7; 152–503) from anterior region. Excretory pore not observed. Intestine thick-walled. Rectum short, funnel-shaped, lined with cuticle. Contents of intestine brown throughout length. Genital system amphidelphic, transverse vagina, slightly post-equatorial vulva located 5.1 (4.7; 4.2–5.2) mm from anterior end, representing 52.2% (51.2%; 45.2–53.6%) of total body length. Uteri with thin walls and numerous eggs (>100), with larvated eggs close to the vulva. Egg size 103 (104.2; 86–114) x 51 (52.8; 40–61) (total of 24 eggs; three eggs measured in each holotype and paratypes). Tail 293 (287.6; 244–338), representing 3% (3.1%; 2.6–3.8%) of body. Phasmid were not observed.

Remarks

Rhabdias megacephala n. sp. belongs to the genus *Rhabdias* because it has the following morphological characteristics: buccal capsule, inflated external cuticle, amphidelphic genital system, conical tail, and it is a parasite of the lungs of an anuran (Kuzmin et al. 2007; Müller et al. 2018; Tavares-Costa et al. 2022). *Rhabdias megacephala* n. sp. has a unique set of morphological characters: a prominent cephalic cuticular dilatation, distinct from the other species, position of the nerve ring located at the anterior end of the esophagus, depth and diameter of the buccal capsule, and length of the esophagus.

The morphology of the apical region of *Rhabdias* spp. and the host biogeographical distribution are helpful in species differentiation. *Rhabdias megacephala* presents an oral opening surrounded by four lips, similar to *Rhabdias leonae* Martínez-Salazar, 2006 and *Rhabdias savagei* Bursley & Goldberg, 2005 found in the Neotropical region. *Rhabdias leonae* is found in the lizard *Anolis megapholidotus* Smyth, 1933 from Mexico and differs from *R. megacephala* n. sp. by having a deeper buccal capsule (*R. leonae* 23–34 vs. *R. megacephala* n. sp. 11–23) and smaller width (*R. leonae* 11–19 vs. *R. megacephala* n. sp. 22–34) (Martínez-Salazar 2006).

Rhabdias savagei described in *Rana cf. forreri* collected in Costa Rica (Boulenger, 1883), despite having four lips like *R. megacephala* n. sp., differs by having a much smaller body (*R. savagei* 4.2–5.3 mm vs. *R. megacephala* n. sp. 8.1–9.9 mm). Additionally, its buccal capsule is also smaller (*R. savagei* 18–24 x 12–18 vs. *R. megacephala* n. sp. 22–34 x 11–23) (Bursley & Goldberg 2005).

Rhabdias hermaphrodita Kloss, 1971, is a Neotropical species that has no information regarding its oral structures arrangement, with scarce morphological information, mainly concerning the presence/absence and arrangement of lips or pseudolabia. *Rhabdias hermaphrodita* was described in *Rhinella crucifer* (Wied-Neuwied, 1821). Some morphological measurements can be compared to differentiate it from *R. megacephala* n. sp. For example, the body

can measure up to 12 mm, which is larger than that of *R. megacephala* n. sp. (8.1–9.9 mm). The tail of *R. hermaphrodita* is also larger, measuring up to 524, whereas *R. megacephala* n. sp. is between 244–338. *Rhabdias hermaphrodita* has no evident dilatation like *R. megacephala* n. sp. (Kloss 1971).

The most important characteristic of *R. megacephala* regarding its prominent cephalic expansion is similar to *R. androgyna* (*R. megacephala* n. sp. 466–570 vs. *R. androgyna* 273–706). *Rhabdias androgyna* Kloss, 1971 described in *Rhinella gr. margaritifera* (Laurenti, 1768) is similar to *R. megacephala* n. sp. in the shape of the cuticle dilatation around the cephalic region. However, *R. androgyna* differs from *R. megacephala* n. sp. in having a cephalic dilatation that divides into outer and inner layers, whereas *R. megacephala* n. sp. lacks this division and is formed by a single layer. Additionally, *R. androgyna* exhibits a cephalic dilatation more oriented towards the apical region, while *R. megacephala* n. sp. presents a distinctly rounded cephalic dilatation. Moreover, for species differentiation, we considered molecular data to make the distinction more integrative, given the well-conserved morphology of *Rhabdias*. (Kloss 1971).

Molecular analysis and phylogenetic study

We obtained a sequence of 630 base pairs from the COI of *R. megacephala* n. sp. After comparing the new sequence with that previously deposited in GenBank using BLASTn (available at NCBI), we found no other sequence with 100% similarity. Genetic distances indicated that *R. megacephala* n. sp. is closest to the species *Rhabdias fuelleborni* (OP651882, OP651884) from *Rhinella diptycha* and *Rhinella icterica*, Paraty, Brazil (Müller et al. 2023), with a genetic divergence of 10.24% (see Supplementary Table S1).

After aligning our sequence and GenBank sequences, we obtained a database with 52 sequences of 380 base pairs in length (supplementary material). The I_{ss} index indicated no saturation in transitions or transversions and I_{ss.c} values were greater than I_{ss} values. The phylogenetic inferences of maximum likelihood and Bayesian inference showed similar topologies, as did the bootstrap support values (B) and posterior probability (PP). The *R. megacephala* n. sp. sequence is grouped with species complex *Rhabdias cf. stenocephala* (MH548271–MH548277) and the species complex formed by *R. fuelleborni* (OP651882–OP651884, OP654198) and *Rhabdias* sp. 4 (MH548291–MH548292), representing an outgroup of this clade formed by the two species complex (supplementary Figure S1). The phylogenetic position of *R. megacephala* n. sp. was not well supported by the values of B (58) and PP (56). However, we found other well-supported lineages: *R. matogrossensis* + *R. breviensis* species complex, *R. fuelleborni* + *Rhabdias* sp. 4, *R. waiapi* + *Rhabdias* sp. 5 and *R. pseudosphaerocephala* species complex, which indicates that more data are needed for a robust phylogenetic hypothesis.

Discussion

Rhabdias megacephala n. sp. is the 24th species of *Rhabdias* from the Neotropical region. The new species is distinguished mainly by evident cephalic dilatation and size of the buccal capsule. Characteristics of apical structures arrangement of *Rhabdias* are important for species differentiation, such as the presence/absence of lips or pseudolabia, as well as the arrangement of these structures (Travassos 1930; Kuzmin *et al.* 2007; Tkach *et al.* 2014). Species from the Neotropical region can be split into three different groups based on the arrangement of apical structures: without lips or pseudolabia, with four submedial lips and two lateral pseudolabia, and with six lips (Tkach *et al.* 2014; Müller *et al.* 2018).

Burseley and Goldberg (2005) identified a group of *Rhabdias* characterised by having four lips, of which only two species, *Rhabdias leonae* and *Rhabdias savagei*, have been described in the Neotropical region. *Rhabdias megacephala* n. sp. shares this distinctive feature, marking the third Neotropical species exhibiting the presence of these four lips. However, it differs in having a circular shaped vestibule in apical view, which does not have papillae around the oral opening. Another feature used in distinguishing species of the genus is the external cuticular inflation (Müller *et al.* 2023).

The sequence of *R. megacephala* n. sp. showed a high degree of divergence at 13.09% (11.9%) compared with the most divergent species, *R. cf. stenocephala*, and compared with the other 52 sequences (12 species) analysed (see Supplementary Table S1). Available phylogenies of *Rhabdias* have been proposed based on short sequences of the COI gene, and in this paper, we used a similar analysis. (Supplementary Figure S1) (Morais *et al.* 2020; Tavares-Costa *et al.* 2022). In fact, in phylogenetic inferences, *R. megacephala* n. sp. remained, with low support, as an outgroup of two clades formed by the species complex *Rhabdias cf. stenocephala* and the species *R. fuelleborni* and *Rhabdias* sp. 4. This low support might be related to the geographic distance between *Rhabdias* species and could also be a consequence of using short COI sequences.

Willkens *et al.* (2020) pointed out that for species from Brazil, more than geographical distance is needed to explain the high divergence in the sequences and, consequently, in the absence of phylogenetic support. However, one hypothesis for the high divergence of *R. megacephala* n. sp. from the other species from Brazil is the fragmentation of the Pan-Amazonian area in the early Pleistocene (Hoorn *et al.* 2010; Sobral-Souza *et al.* 2015; Tavares-Costa *et al.* 2022). Pan-Amazonian fragmentation may have caused species to suffer different selective pressures according to the biome they were exposed to, with enough time to distance *Rhabdias* species, thereby forming a phylogenetic gap. Additional molecular sequences are necessary for more robust phylogenetic hypotheses, ideally with a larger number of base pairs. Furthermore, increased specimen collections from a broader range of locations are also essential.

The differentiation of species, as observed in *Rhabdias*, is a complex phenomenon that can occur through various processes. These processes involve intrinsic characteristics of the species itself or factors related to the hosts. Numerous studies have been dedicated to investigating these mechanisms, including morphological traits, genetic divergence, ecological adaptations, and functional characteristics of the parasite, providing a comprehensive understanding of parasite diversity and the elements influencing it (Poulin 2011; Kamiya *et al.* 2014). This pursuit is particularly crucial in the context of species descriptions, wherein unraveling

the intricacies of differentiation processes contributes to the broader scientific knowledge of parasitology.

Rhabdias species occur on most continents, except Antarctica, and the distribution of these parasites is limited by the distribution of the hosts, both anurans and reptiles (Tkach *et al.* 2014; Kuzmin *et al.* 2015). In South America, *Rhabdias* species are known for more than 22 host species (Campião *et al.* 2014; Alcantara *et al.* 2023; Müller *et al.* 2023). Among the diversity of anuran parasites, 13 nominal species of the family Rhabdasiidae are known in Brazil (Alcantara *et al.* 2023; Müller *et al.* 2023). However, when we consider the diversity of *Proceratophrys* species (43 species) and of anurans known in Brazil (1,252 species), the diversity of *Rhabdias* is still poorly understood (Frost 2024), for *P. boiei*, in addition to the record of *R. megacephala* n. sp., had been reported as host to an unidentified species of *Rhabdias* (*Rhabdias* sp.) (Aguar *et al.* 2018).

Rhabdias species are also known in other species of *Proceratophrys*: *R. androgyna* has been reported for *Proceratophrys tupinamba* (Boquimpani-Freitas *et al.* 2001; Prado & Pombal Jr. 2008). Other unidentified *Rhabdias* spp. have been reported in *Proceratophrys aridus* and *Proceratophrys mantiqueira* (Almeida-Santos 2017; Teles *et al.* 2017; Müller *et al.* 2018). Given the description of *R. megacephala* n. sp., in comparison with other as yet undescribed species, there arises curiosity in understanding its distribution. Could *R. megacephala* n. sp. be endemic, or might the undescribed species actually be *R. megacephala* n. sp.? Aspects such as phenotypic similarities, habitat sharing, exposure to the same infective stages, and phylogenetically conserved resources among hosts may influence the exchange or sharing of different hosts (Fecchio *et al.* 2019; D’Bastiani *et al.* 2020; Euclides *et al.* 2022).

Knowledge of *Rhabdias* species diversity has increased, especially in recent years with species description, phylogenetic, and geographic studies (Müller *et al.* 2018; Tavares-Costa *et al.* 2022; Alcantara *et al.* 2023). Most species have been observed in the North and Midwest regions of Brazil, mainly in the Amazon region (seven species) (Willkens *et al.* 2020; Tavares-Costa *et al.* 2022; Müller *et al.* 2023;). Knowledge of this group of parasites in these localities may be due to a greater study effort. In the Atlantic Forest, studies show how significant the parasitic diversity of anurans is, but knowledge of the diversity of *Rhabdias* species is still underestimated (Martins-Sobrinho *et al.* 2017; Euclides *et al.* 2021). We describe the 24th species of *Rhabdias*, the first species documented in Paraná. *Rhabdias megacephala* n. sp. is also the first described species in *P. boiei*. Discovering parasites such as *Rhabdias* is fundamental to unveiling ecosystem diversity, species evolution, and host–parasite relationships to understand more about the evolutionary history of this cosmopolitan group prevalent among anurans.

Supplementary material. The supplementary material for this article can be found at <http://doi.org/10.1017/S0022149X24000385>.

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Competing interest. None.

Ethical standard. This study's collections and observations were carried out in accordance with the license 62552-1 (Sistema de Autorização e Informação em Biodiversidade – Instituto Chico Mendes de Conservação da Biodiversidade – SISBIO). The Comitê de Ética para Uso de Animais da Seção de Ciências Biológicas da Universidade Federal do Paraná (Opinion no. 1167) certified that the procedures with the use of animals in this work were approved.

Contribution. Conceptualisation: RE and KMC; methodology: RE, HCJ, LHG, SSV; data analysis: LES, HCJ, RFJ, FTVM, and KMC. Writing: RE, KMC, and FTVM; supervision: KMC.

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