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Rhabdias taquariensis n. sp., a new species of lung parasite of *Physalaemus albonotatus* from the Brazilian Cerrado

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Research Paper

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Abstract

The diversity of *Rhabdias* includes 101 species, 71 of which parasitize the lungs of anurans, caudates, gymnophionans, and some occur in reptiles worldwide. Currently, 26 species are found in the Neotropical region, and in Brazil, there are 16 nominal species, a relatively low number considering the high diversity of potential hosts. Here, we describe a new species of *Rhabdias* found in *Physalaemus albonotatus*, with morphological and molecular data, as well as phylogenetic analyses using sequences of the mitochondrial gene Cytochrome Oxidase Subunit I (COI). *Rhabdias taquariensis* n. sp. differs from other known species by a set of morphometric traits and by presenting a well-defined internal shape of the cephalic dilation. Molecular analyses revealed that *R. taquariensis* n. sp. exhibits a significant divergence of 13.6% in COI compared to the *Rhabdias* cf. *stenocephala* species complex. Additionally, phylogenetic reconstructions indicate that the new species represents a distinct lineage, external to a clade formed by species such as *Rhabdias fuelleborni*, *Rhabdias* cf. *stenocephala*, and *Rhabdias waiapi*. *Rhabdias taquariensis* n. sp. is the 27th species described in the Neotropical region and the 16th in Brazil, the first description of a species of the genus *Rhabdias* for *Physalaemus albonotatus*, and one of the few *Rhabdias* species described for the Cerrado biome.

Introduction

Rhabdias Stiles and Hassall are nematode parasites that infect the lungs of anurans and some reptiles (Kuzmin *et al.* 2007; Müller *et al.* 2018). The current diversity of *Rhabdias* includes approximately 101 species, 94 recognized species worldwide, with 26 species identified in the Neotropical region (Alcantara *et al.* 2023; Kuzmin and Tkach, 2024; Marcaida *et al.* 2022; Müller *et al.* 2018; Tkach *et al.* 2025), and with only 16 nominal species in Brazil (Alcantara *et al.* 2023; Euclydes *et al.* 2024; Silva *et al.* 2019; Tavares-Costa *et al.* 2025). The number of *Rhabdias* identified to species level is very low compared to the diversity of anurans, caudates, and gymnophionans hosts (e.g., 1,189 species in Brazil) (Frost 2025; Segalla *et al.* 2021). Additionally, there are several records of *Rhabdias* species without specific descriptions in distinct hosts (Campião *et al.* 2014; Martins-Sobrinho *et al.* 2017; Müller *et al.* 2018; Prosser *et al.* 2013), which may indicate that these parasites are relatively common.

The identification of *Rhabdias* species is primarily based on the morphological characteristics of adult female hermaphrodites (Kuzmin 2013; Tkach *et al.* 2014). However, the high degree of morphological similarity within the group poses a significant challenge for species identification and delimitation. This limitation may also impact ecological studies by leading to an underestimation of *Rhabdias* diversity (Kuzmin 2013; Tavares-Costa *et al.* 2022). To avoid erroneous identifications within this group, the inclusion of molecular data alongside morphological data may contribute to more robust description of new species (Langford and Janovy 2013; Müller *et al.* 2018; Poulin *et al.* 2019; Tkach *et al.* 2014).

Rhabdias species have a heterogonic life cycle in which parasitic hermaphrodites occur in the host's lungs, while free-living males and females develop in the soil (Kuzmin 2013; Tkach *et al.* 2014). Adult parasites produce eggs or first-stage larvae in the lungs, which are carried by respiratory secretions to the digestive tract and eliminated in the feces (Baker 1979; Langford and Janovy 2009). Once in the environment, these larvae may either develop directly into infective L3 or form a free-living generation whose progeny becomes the infective stage. Infective larvae penetrate the host's skin and migrate through the circulatory system to the lungs, where they mature into hermaphroditic adults (Anderson 2000).

Anurans of the genus *Physalaemus* have a wide distribution in South America, ranging from northern and central Argentina, eastern Bolivia, Paraguay, Uruguay, Brazil, and the Guianas to the plains of southern Venezuela and the llanos of southeastern Colombia (Frost 2025). *Physalaemus albonotatus* (Steindachner, 1864) is a small (18.6–24.1 mm) and nocturnal species

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that inhabits the ground near water bodies or in flooded fields for reproduction. This species has shown resilience to human disturbances as it may be found in grassland pastures and anthropic environments (Uetanabaro *et al.* 2007). *Physalaemus albonotatus* exhibits a generalist and opportunistic feeding behaviour, adjusting its diet according to prey availability. Terrestrial arthropods, especially those belonging to the orders Coleoptera, Collembola, and Hymenoptera, are commonly recorded in its diet (Falico *et al.* 2012). Currently, there are about 20 parasite taxa known for *P. albonotatus*, most from the phylum Nematoda (Campião *et al.* 2014; González and Hamann 2012; González *et al.* 2019, 2025). In this study, we describe a new species of *Rhabdias* in *P. albonotatus* using morphological and molecular data, along with phylogenetic analyses based on DNA sequences of the mitochondrial Cytochrome Oxidase Subunit I (COI) gene.

Material and methods

Host sampling, parasite collection, and identification

We collected 32 individuals of *Physalaemus albonotatus* (Steindachner, 1864) between January 28 and 29, 2018, through visual and auditory searches (Crump and Scott Jr 1994) in the Nascentes do Rio Taquari State Park (17°59′ to 18°15′S; 53°10′ to 53°26′W). The park is located in the Cerrado biome and forms an important ecological corridor, with Dense Arboreal Savanna and Semideciduous Seasonal Forest vegetation (IMASUL 2009). The collected anurans were anesthetized and euthanized using 4% lidocaine applied topically to the ventral body region (CONCEA 2022). After euthanasia, an incision was made in the ventral body region to remove the organs for analysis. All procedures were conducted in compliance with the ethical guidelines of the host institution. The study protocol was approved by the National Council for Animal Experimentation Control (CEUA; Protocol number 1167), and species collection was approved by the Chico Mendes Institute for Biodiversity Conservation (ICMBio; Authorization number 62552-1).

We examined the lungs of the hosts, and the parasites found were washed in saline solution and killed with heated 70% ethanol. After collection, a portion of the parasites was fixed and stored in 70% ethanol for morphological identification, while the other portion was stored in absolute ethanol for molecular analyses. For morphological data collection, the parasites were rehydrated with distilled water, and temporary slides were prepared by clearing the specimens with lactophenol. All parasite collection, fixation, and mounting techniques were performed according to the methods described by Amato *et al.* (1991). The slides were examined using an optical microscope. Apical morphology was observed with manually prepared sections using a razor blade. Illustrations were initially drawn by hand and later created using Inkscape (version 1.3.2). The body measurements of the parasites are presented with the values for the holotype, and the mean and range for the entire type series, with both values enclosed in parentheses (unless otherwise indicated, all measurements are in micrometres). The necropsied frogs were fixed in 10% formaldehyde, preserved in 70% alcohol, and later deposited in the Zoological Reference Collection of the Federal University of Mato Grosso do Sul (ZUFMS-AMP14222-14288).

Molecular and phylogenetic analysis

The specimens stored in absolute ethanol were sectioned at the anterior end, just posterior to esophago-intestinal junction, and in the portion immediately following the female reproductive apparatus. The anterior and posterior extremities were preserved and deposited as voucher specimens (see Pleijel *et al.* 2008) in the Invertebrate Collection of the Federal University of Paraná.

We performed DNA extraction from the median portion of the parasite using the Wizard® Genomic DNA Purification Kit (Promega, Madison, USA). The extracted and purified DNA was subjected to conventional PCR for amplification of the COI gene, using primers from Folmer *et al.* (1994) (LCO1490/Forward – 5′ GGTCAACAATCATAAAGATATTGG 3′ and HC02198/Reverse – 5′ TAAACTTCAGGGTGACCAAAA 3′). The amplified fragments were analysed by agarose gel electrophoresis (0.75%). The amplified fragments were then cut and purified using the PureLink™ Quick Gel Extraction and PCR Purification Kit Combo (Thermo Fisher). Following purification, the samples were subjected to sequencing PCR with the BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Warrington, UK). The samples were subsequently precipitated and sequenced using an ABI PRISM 3500 Genetic Analyzer.

For the phylogenetic analysis, we selected all available sequences of the genus *Rhabdias* from public databases that were unique and showed a similarity above 90%, as determined by the BLASTn tool. To ensure greater robustness of the analysis, only sequences longer than 350 base pairs were included (Savolainen *et al.* 2000). Sequences were edited and aligned in Geneious Prime (https://www.geneious.com) using MUSCLE with default parameters as described by Edgar (2004). The most appropriate substitution model was selected according to the Bayesian Information Criterion (BIC) using jModelTest (Darriba *et al.* 2012), which identified the GTR+I+G model. *Serpentirhabdias viperidicus* was used as the outgroup to root the tree.

In BEAST, 10 independent runs of 20,000,000 generations each were performed, with sampling every 2,000 generations (Bouckaert *et al.* 2018). Parameter convergence and adequacy were assessed in TRACER v.1.7.2 (Rambaut *et al.* 2018), where effective sample size (ESS) values exceeded 200. A burn-in of 10% from each run was applied in LogCombiner, and the final Maximum Clade Credibility (MCC) tree was generated in TreeAnnotator after an additional 10% burn-in. The resulting Bayesian inference tree was visualized in FigTree v.1.4.3 (Rambaut 2018).

Subsequently, a Bayesian implementation of the Poisson Tree Processes (bPTP) lineage delimitation analysis (Zhang *et al.* 2013) was conducted using the bPTP web server (http://species.hits.org/ptp/). The analysis consisted of 500,000 MCMC generations, with a thinning interval of 100 and a burn-in of 10%. Additionally, a Generalized Mixed Yule Coalescent (GMYC) model (Fujisawa and Barraclough, 2013; Pons *et al.* 2006) was implemented in R v. 4.1.1 (R Development Core Team, 2024) using the 'splits' package (Ezard *et al.* 2009). Finally, an Assemble Species by Automatic Partitioning (ASAP) analysis (Puillandre *et al.* 2020) was performed, a distance-based method that uses pairwise genetic distances to infer species partitions. The ASAP delimitation was executed using a simple distance model and default parameters.

Results

Systematics

Family: Rhabdiasidae Railliet, 1915
Genus: *Rhabdias* Stiles and Hassall, 1905
Species: *Rhabdias taquariensis* n. sp. Euclydes, Silva, and Campião

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Taxonomic summary

Host type: *Physalaemus albonotatus* (Steindachner, 1864) (Amphibia: Leptodactylidae).
Type locality: Parque Estadual das Nascentes do Rio Taquari, Costa Rica, Mato Grosso do Sul, Brazil (17°59′ to 18°15′S; 53°10′ to 53° 26′W).
Site of infection: Lungs.
Number of specimens/hosts, prevalence, and mean abundance and mean intensity with their respective standard deviations: A total of 11 nematodes were detected in 7 out of the 32 hosts analysed. P = 21%; 0.34 ± 0.70; 1.57 ± 0.54.
Type material: 1 Holotype (DZUP 541994) and 5 (DZUP 541995) paratypes have been deposited in the Invertebrate Collection of the Federal University of Paraná.
GenBank, accession numbers: PV468349.
ZooBankRegistration:LSDIurn: lsid:zoobank.org:pub:051E9684-D249-472C-9A59-788E3AF025CE.
Etymology: The specific epithet '*taquariensis*' is a noun in apposition to the locality where the new species was discovered in the Parque Estadual das Nascentes do Rio Taquari. This State Park is one of the largest remnants of the Cerrado in Mato Grosso do Sul, and was created to protect the Taquari River springs, one of the main rivers in the Pantanal floodplain.

Description

Body robust, length 4.6 (4.7; 4.5–5.1), and body surface with subtle cuticular inflation along entire length. Lateral pores and ducts present. Body width at reproductive system 378 (355; 316–378), at oesophagus–intestine junction 182 (181; 181–185). Oral opening with six lips: four submedian and two lateral near edges of oral opening. Each lip with papilla; amphids absent. Vestibulum circular in apical view, cylindrical, lumen narrow. Buccal capsule cup-shaped, depth 6 (7; 6–9), width 13 (7; 13–15), depth/width ratio 0.46 (0.52; 0.46–0.6), surface smooth, oesophageal lumen entrance triangular, edges rounded. Oesophagus length 370 (366; 330–398), 8% (8%; 7–9%) of the total body length, claviform, apex rounded, anterior muscular region dilated. Width anterior end of oesophagus is 41 (39; 37–71), width at anterior dilation 54 (50; 49–54), width posterior to dilation 40 (41; 40–44), bulb width 78 (77; 75–78). Nerve ring surrounds oesophagus after anterior dilation 148 (155; 148–164) from anterior. Excretory pore absent. Intestine with thick walls, brown content throughout. Rectum short, funnel-shaped, lined with cuticle. Genital system amphidelphic, vagina transverse, reproductive system pre-equatorially at 2.1 (1.8; 1.6–2.1) mm from anterior extremity, representing 62% (53%; 46–62%) of the total length. Uterus with finely thickened wall, numerous eggs (>100), embryonated eggs near reproductive system. Eggs 92 (90; 86–92) × 46 (48; 45–52) (n = 3 eggs measured in the holotype and paratypes). Tail 224 (199; 186–224), 5% (4%, 4–5%) of body length. Phasmids absent. See Figures 1 and 2 (based on holotype and five paratypes, all gravid hermaphrodites).

Remarks

Rhabdias taquariensis n. sp. belongs to the genus *Rhabdias* due to its diagnostic characteristics, such as the presence of a buccal capsule, externally inflated cuticle, amphidelphic reproductive system, and transversely oriented vagina. We consider *R. taquariensis* n. sp. a new species because it exhibits a distinct combination of morphometric characteristics in the anterior region and well-defined internal structure of the cephalic dilation, which is a unique morphological feature that differentiates it from other species. The internal cephalic dilation observed in *R. taquariensis* n. sp. is characterized by a well-defined, oval-shaped expansion located at the

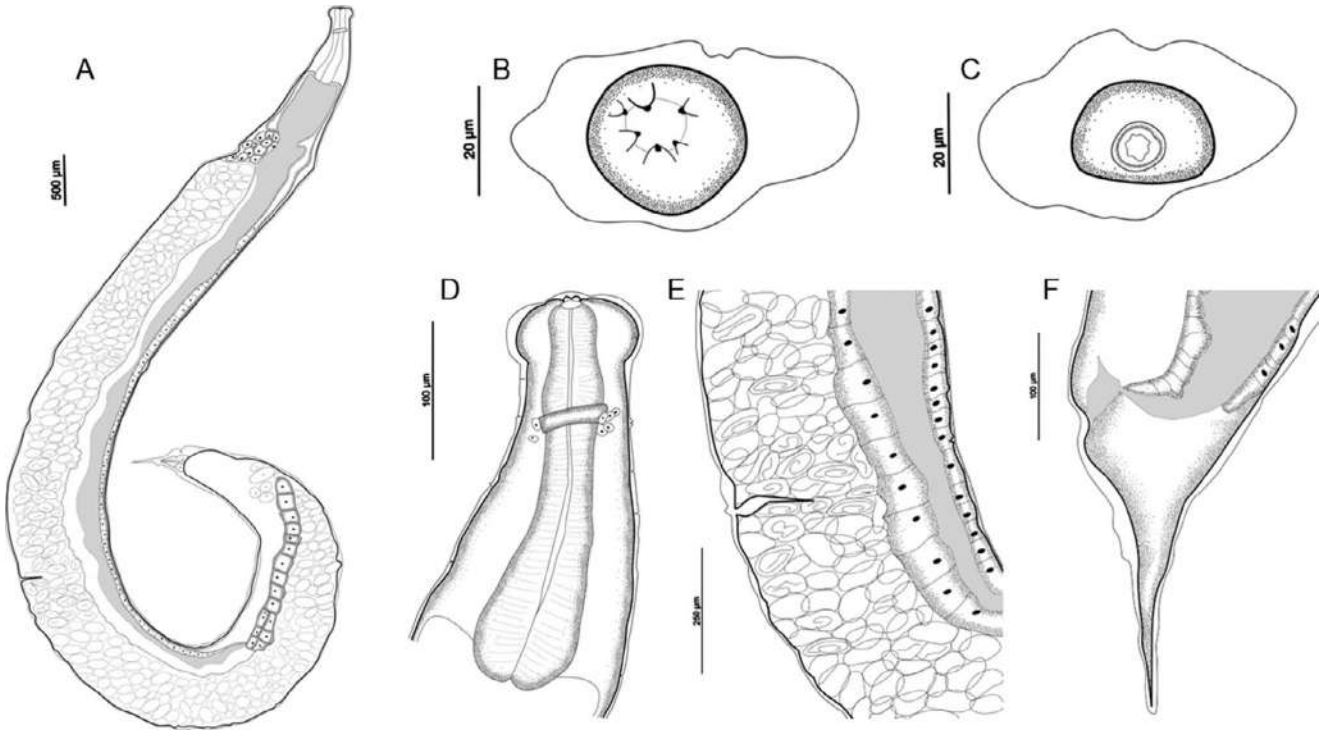

Figure 1. Line drawings of *Rhabdias taquariensis* n. sp. from *Physalaemus albonotatus.* A – Entire body, lateral view. B – En face view of the anterior end of the body. C – Optical section through vestibulum. D – Anterior end of the body, lateral view, showing cephalic dilation. E – Vulva region, lateral view. F – Caudal end, lateral view.

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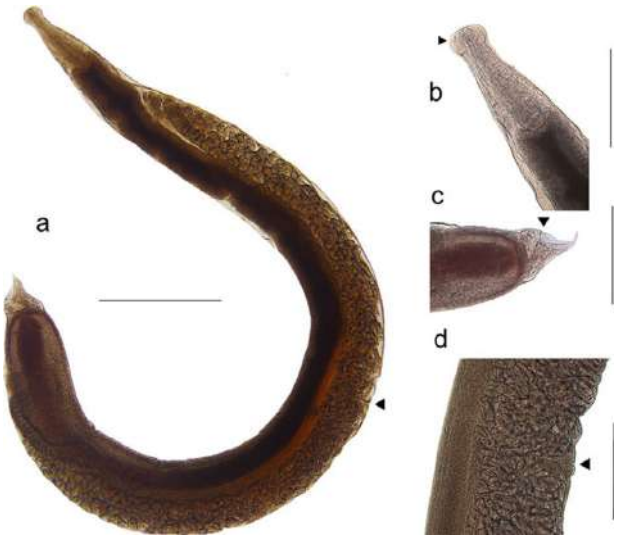

Figure 2. Photomicrographs of *Rhabdias taquariensis* n. sp. A – General view of the body. B – Anterior end; the arrow indicates the internal cephalic dilation. C – Posterior end; the arrow indicates the cloacal opening. D – Larvated eggs; the arrow indicates the vulval opening. Scale bars: A = 1000 μm; B–D = 50 μm.

anterior extremity of the body, near the oral opening (Figure 1B). The apical morphology of *Rhabdias* is highly useful in species description and differentiation. In this context, we propose a species with six lips, four submedial and two more laterally positioned, similar to a group of species that share this characteristic: *R. androgyna*, *R. breviensis*, *R. fuelleborni*, *Rhabdias galactonoti*, *R. glaurungi*, *R. manantlanensis*, *R. pocoto*, *R. tobagoensis*, *R. stenocephala*, and *R. waiapi*.

Rhabdias androgyna Kloss, 1971, described in *Rhinella* cf. *margaritifera* (Laurenti 1768), differs from *R. taquariensis* n. sp. by having a larger body length (*R. androgyna* 9.35–13.39 mm vs. *R. taquariensis* n. sp. 4.521–5.106), as well as a greater width at the oesophagus-intestine junction (*R. androgyna* 269–473 vs. *R. taquariensis* n. sp. 181–185). The oesophagus of *R. androgyna* is also longer (*R. androgyna* 577–618 vs. *R. taquariensis* n. sp. 330–398). Additionally, *Rhabdias androgyna* differs from *R. taquariensis* n. sp. in the buccal capsule diameter (*R. androgyna* 19–27 vs. *R. taquariensis* n. sp. 13–15). The distance from the vulva to the anterior region is greater in *R. androgyna* (3.7–7.2 vs. 2.347–2.851) (Kuzmin *et al.* 2015).

Rhabdias breviensis Nascimento, Gonçalves, Melo, Giese, Furtado, and Santos, 2013, from *Leptodactylus petersii* (Steindachner 1864), has a curved body, length 2.63–3.63 vs. 4.5–5.1; width 370–543 vs. 316–378. Buccal capsule smaller, 7–13 × 4–9 vs. 13–15 × 6–9. The oesophagus of *R. breviensis* is shorter (238–410) than in *R. taquariensis* n. sp. (330–398). Nerve ring to anterior region shorter 41–84 vs. 148–164. The vulva is post-equatorial in *R. breviensis* (65–71% of body length), while it is pre-equatorial in *R. taquariensis* n. sp. (46–62%) (do Nascimento *et al.* 2013).

Rhabdias fuelleborni Travassos, 1926, originally described in *Rhinella diptycha* (= *Bufo marinus*) (Schneider 1799), is larger than *R. taquariensis* n. sp. (10–12 vs. 4.521–5.106) and shows a different pattern of cuticular inflations. Body elongated, anterior end rounded, midbody wider, tapering posteriorly. Cuticle inflated, more prominent at anterior and posterior ends. Oral opening circular, six lips, each with papilla; amphids at base of lateral lips. The buccal capsule is cup-shaped, with a larger diameter (18–19 vs. 13–15). Nerve ring thick-walled. The vulva is pre-equatorial with a transverse aperture, and the tail is conical, gradually tapering (Kuzmin *et al.* 2015; Müller *et al.* 2023).

Rhabdias galactonoti Kuzmin *et al.* (2016), described in *Adelphobates galactonotus* (Steindachner 1864), differs from *R. taquariensis* n. sp. and is larger than *R. taquariensis* n. sp., 5.60–6.04 mm vs. 4.52–5.10 mm. Body elongated, midbody wider, tapering ends. The oesophagus is club-shaped, with an anterior dilation of 39–43 (vs. 50–54) and a bulb measuring 61–73 (vs. 75–78). The nerve ring is positioned 165–184 from the anterior end (vs. 148–164). The vulva is pre-equatorial, located 2.5–2.8 from the anterior end (vs. 46–62% of body length in *R. taquariensis*). The tail is 229–333 long (vs. 186–224), and phasmids are present 91–120 from the tip of the tail, whereas they are absent in *R. taquariensis* (Kuzmin *et al.* 2016).

Rhabdias glaurungi Willkens *et al.* (2020), collected from *Scinax* gr. *ruber* (Laurenti 1768). The oesophagus of *R. glaurungi* is clavate, shorter 314; 310 (292–332) long, representing 6.2; 6.4% (4.9–8.2%) of body length, with a distinct dilation in the middle of the anterior half and a posterior bulb, whereas *R. taquariensis* n. sp. exhibits a longer oesophagus 370 (366; 330–398), 8% (7–9%) of body length with a triangular lumen entrance and rounded edges. Moreover, *R. glaurungi* shows cuticular inflation along the entire body, and slight differences are also present in buccal capsule dimensions (10–16 × 6–9 in *R. glaurungi* vs. 13–15 × 6–9 in *R. taquariensis* n. sp.) (Willkens *et al.* 2020).

Rhabdias manantlanensis Martínez-Salazar (2008), differs from the new species by a consistent set of morphological characters. It has a longer and more slender body (6.48–9.64 mm vs. 4.5–5.1 mm in *R. taquariensis* n. sp.) and slightly inflated cuticle, whereas *R. taquariensis* exhibits subtle cuticular inflation along the entire body. The buccal capsule of *R. manantlanensis* is larger, measuring 11–19 × 19–27, while in *R. taquariensis* n. sp. it is narrower and shallower (6–9 × 13–15), in addition to bearing a triangular entrance to the oesophageal lumen, a feature absent in *R. manantlanensis*. The oesophagus of *R. manantlanensis* is proportionally shorter (5.11–6.57% of body length), contrasting with the longer and more robust oesophagus of *R. taquariensis* n. sp. (7–9% of total body length). The tail is also a diagnostic character, being shorter in *R. manantlanensis* (1.48–3.27% of the body) and relatively longer in *R. taquariensis* (4–5%). Thus, clear differences in body size, oesophageal and tail proportions, and buccal capsule morphology distinguish *R. taquariensis* n. sp. from *R. manantlanensis*.

Rhabdias pocoto Morais *et al.* (2020), found in *Pseudopaludicola pocoto*, differs from the new species by having a generally longer body (3.4–7.4 vs. 4.5–5.1 in *R. taquariensis* n. sp.), a proportionally longer oesophagus (8.9–13.9% vs. 7–9% of body length). The buccal capsule of *R. pocoto* also shows differences in diameter and depth (0.6–1.1 vs. 0.46–0.6). In addition, the vulva of *R. pocoto* is equatorial (39–64% of body length), whereas in *R. taquariensis* n. sp. it is pre-equatorial (46–62%). A remarkable structural difference in *R. pocoto* is the presence of amorphous glands in the anterior body region, which are important for species differentiation and are absent in *R. taquariensis* n. sp. (Morais *et al.* 2020).

Rhabdias tobagoensis Moravec and Kaiser, 1995, described in *Pristimantis incertus* (Lutz, 1927), differs from *R. taquariensis* n. sp. by having a longer body (*R. tobagoensis* 7.34–7.56 mm vs. *R. taquariensis* n. sp. 4.521–5.106 mm). The diameter of the buccal capsule in *R. tobagoensis* is also larger than in *R. taquariensis* n. sp. (*R. tobagoensis* 18–21 vs. *R. taquariensis* n. sp. 15–18). The position of the vulva, which is located near the mid-body and slightly pre- or post-equatorial in *R. tobagoensis* (3.33–3.69 from the anterior end), whereas in *R. taquariensis* n. sp., it is pre-equatorial, about 1.6–2.1

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from the anterior extremity, corresponding to 46–62% of body length. In addition, the tail of *R. tobagoensis* is longer (210–276) compared to *R. taquariensis* n. sp. (186–224) (Moravec and Kaiser 1995).

Rhabdias stenocephala Kuzmin *et al.* (2016), found in *Leptodactylus pentadactylus* (Laurenti 1768) and *Leptodactylus paraensis* (Heyer 2005), differs from *R. taquariensis* n. sp. by its longer body (6.9–8.1 mm vs. 4.5–5.1 mm). The buccal capsule walls are separated into a longer anterior part and a ring-shaped posterior part with circular thickening (depth 8–11, width 15–18, depth/width ratio 0.5–0.7). Whereas in *R. taquariensis* n. sp. the capsule is cup-shaped, with smooth walls, shallower depth (6–9) and greater width (13–15), resulting in a lower depth/width ratio (0.46–0.6). The oesophagus in *R. stenocephala* is longer (385–442, 5–6% of body length) and bears a distinct anterior dilatation and oval posterior bulb, while in *R. taquariensis* n. sp. it is shorter (330–398, 7–9% of body length), claviform, with an anterior muscular dilation. Additionally, *R. stenocephala* has the nerve ring located more posteriorly (192–225 from anterior end vs. 148–164 in *R. taquariensis* n. sp.) (Kuzmin *et al.* 2016).

Rhabdias waiapi Tavares-Costa and Melo (2022), described in *Pristimantis chiastonotus* (Lynch and Hoogmoed 1977), has a more slender body (3.36–4.91 mm long) compared to the more robust body of *R. taquariensis* n. sp. (4.5–5.1 mm). The cuticular inflation in *R. waiapi* is prominent, with a distinct anterior dilation that becomes discrete along the body, whereas in *R. taquariensis* n. sp., it is subtle and uniform throughout. *R. waiapi* exhibits a larger buccal capsule diameter compared to *R. taquariensis* n. sp. (*R. waiapi* 16–19 vs. *R. taquariensis* n. sp. 13–15). The oesophagus of *R. waiapi* is longer (405–533, 7.8–12.6% of body length) compared to *R. taquariensis* (330–398, 7–9%). The position of the vulva also distinguishes the two species: in *R. waiapi*, it is post-equatorial (1.9–2.9 from the anterior end; 51–61% of body length), while in *R. taquariensis* n. sp. it is 1.6–2.1 from the anterior end (46–62%) (Tavares-Costa *et al.* 2022).

Molecular analysis and phylogenety

We obtained a 638-base pair sequence of the COI gene from *Rhabdias taquariensis* n. sp., which did not match any other sequences in the BLASTn search (NCBI). For alignment, we included the sequence of *R. taquariensis* n. sp. along with 49 other *Rhabdias* sequences from GenBank, resulting in an alignment of 588 base pairs. *Rhabdias taquariensis* n. sp. is closest to the species complex consisting of *R. pseudosphaerocephala* (MH548278–MH548289) from *Rhinella marina*, *Rhi. jimi*, *Rhi. schneideri*, and *Boana raniceps* from Brazil (Müller *et al.* 2018), with a genetic distance ranging from 9.7% to 11.5%, and *R. waiapi* (OL689010–OL689012) from *Pristimantis chiastonotus* collected in the Amazon region, Brazil (Tavares-Costa *et al.* 2022), with a 9.9% genetic difference (see Supplementary Table S1).

Phylogenetic inference based on the mitochondrial COI gene showed that *Rhabdias taquariensis* n. sp. formed a monophyletic lineage that is sister to the clade comprising *R.* cf. *stenocephala*, *R. fuelleborni*, *R. guaianensis*, *R. waiapi*, and *Rhabdias* sp.5 (PP = 0.68). The clade containing *R. pseudosphaerocephala* appeared in a more basal position relative to this group. The species delimitation analyses consistently recognized *Rhabdias taquariensis* n. sp. as a distinct lineage among the taxa analysed. Genetic divergence in the COI gene between *R. taquariensis* and other congeners ranged from 9% to 15%, with a mean value of approximately 10.6%. Diagnostic morphological characteristics were congruent with the molecular findings.

Additionally, various clades were supported in accordance with the literature: *Rhabdias matogrossensis* + *R. breviensis* (Alcantara *et al.* 2023), *R. fuelleborni* + *Rhabdias* sp.4 (Müller *et al.* 2018, 2023), *R. waiapi* + *Rhabdias* sp.5 (Müller *et al.* 2023; Tavares-Costa *et al.* 2022), and the species complexes *R. pseudospharocephala* and *Rhabdias* cf. *stenocephala* (Müller *et al.* 2018, 2023) (Figure 3).

Discussion

Rhabdias taquariensis n. sp. represents the 27th species of the genus described from the Neotropical region, the 16th known from Brazil, and the first recorded in the state of Mato Grosso do Sul. *Rhabdias taquariensis* n. sp. can be readily distinguished from its congeners by the presence of a conspicuous internal cephalic dilation, an oval, well-delimited expansion immediately posterior to the oral opening, located at the anterior end of the body. Apical morphology has proven especially informative for species delimitation in *Rhabdias*, and the combination of lip morphology, oesophageal proportions, and body size observed in the new species represents a diagnostic configuration not previously reported for Neotropical members of the genus.

Cuticular inflation patterns also constitute an important taxonomic feature in *Rhabdias* (Müller *et al.* 2023), given their considerable variation among Neotropical species. For instance, considering the sister clade of *R. taquariensis*, congeners such as *R. matogrossensis* and *R. guaianensis*, both reported from leptodactylid hosts in Central Brazil, possess thin external inflations (Alcântara *et al.* 2023). *R. breviensis, R. glaurungi*, and *R. pseudosphaerocephala*, on the other hand, display notably thicker ones. While *R. pseudosphaerocephala* parasitizes *R. marina, R. breviensis* has been recorded in leptodactylid and hylid hosts, and *R. glaurungi* also occurs in a hylid host. Expanding biodiversity knowledge with additional morphological and molecular data is essential to determine whether this trait exhibits phylogenetic conservatism across species and to clarify its functional and evolutionary associations with host use.

Molecular analyses provided additional support for the distinct status of *R. taquariensis* n. sp., revealing a genetic divergence of at least 9% from the other species included in the study (Supplementary Table S1). This level of divergence falls within the range typically observed among distinct *Rhabdias* species, e.g., 9–15% in *R. megacephala*, *R. waiapi*, and *R. guaianensis* (Alcantara *et al.* 2023; Euclydes *et al.* 2024; Müller *et al.* 2018). In the phylogenetic tree, *R. taquariensis* n. sp. occupies a basal position relative to the clade formed by *R.* cf. *stenocephala*, *R. fuelleborni, R. waiapi*, and *R. guaianensis*. Although support for some internal relationships remains moderate, the placement of the new species outside this clade, combined with its unique morphological traits, supports its recognition as an independent lineage.

The topology suggests that the new species may have diverged early from the lineages of *Rhabdias* species that are associated with amphibians that occur in the Atlantic Forest and Amazon rainforests. *Rhabdias* cf. *stenocephala*, for instance, has been described in *Leptodactylus pentadactylus* and *L. paraensis* in the Amazon region (Kuzmin *et al.* 2016), as *R. waiapi*, reported in *Pristimantis chiastonotus* (Tavares-Costa *et al.* 2022); while *R. fuelleborni* was first documented in *Rhinella diptycha* from an Atlantic Forest region (Müller *et al.* 2023). On the other hand, *Rhabdias guaianensis*, described in *Leptodactylus podicipinus*, is associated with

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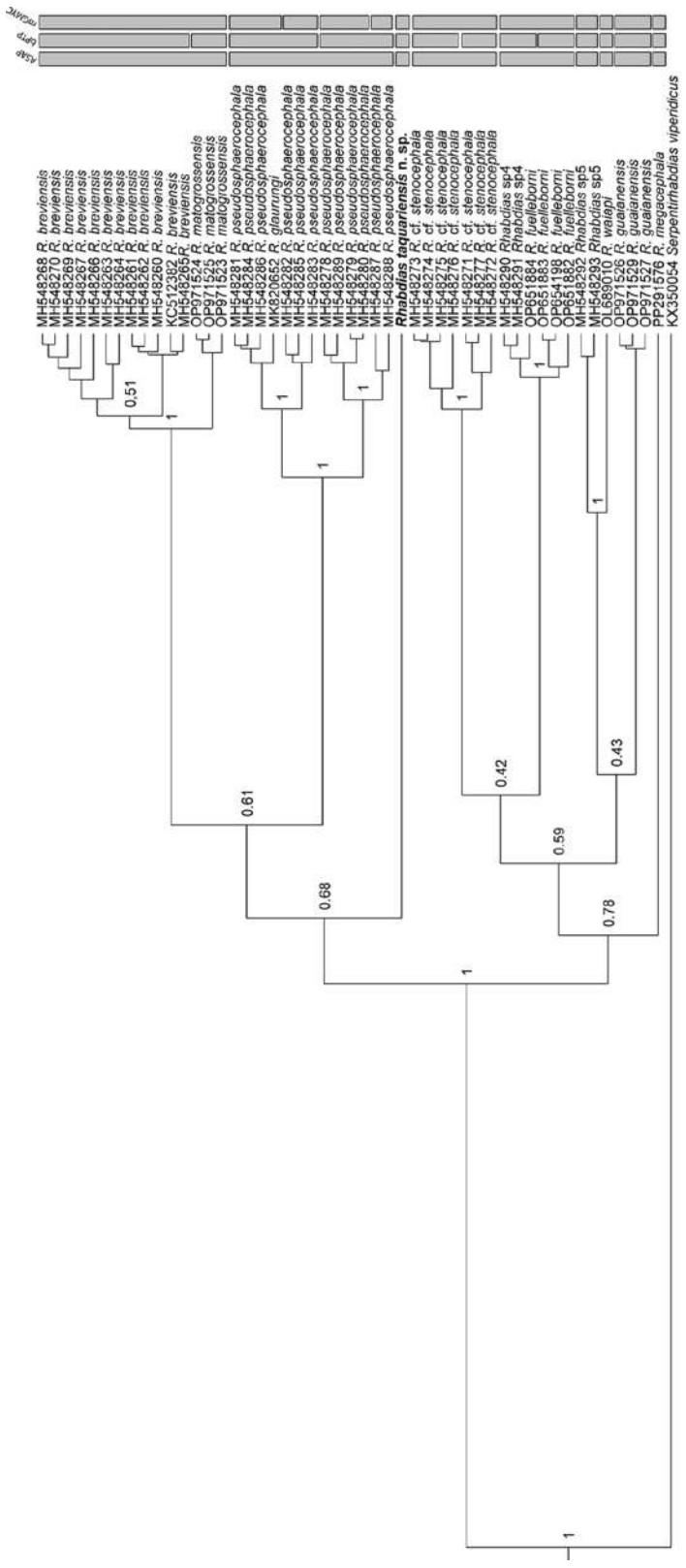

Figure 3. Bayesian phylogenetic tree based on COI (Cytochrome c oxidase subunit I) gene sequences inferred under the GTR+I+G substitution model. The highlighted taxon in bold represents the target species of this study, herein described as *Rhabdias taquariensis* n. sp. Node support values correspond to posterior probabilities. Grey bars on the right indicate species delimitation results obtained from independent methods: ASAP, bPTP, and GMYC. The congruence among these approaches supports the distinctiveness of *R. taquariensis* n. sp. from closely related congeners.

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transitional regions between the Atlantic Forest and Cerrado (Alcântara *et al.* 2023). The phylogenetic position of *R. taquariensis* n. sp. and its occurrence in an ecotonal region connecting the Cerrado and Pantanal reinforces the hypothesis that transitional environments may harbour unrecognized parasite diversity. Its association with *Physalaemus albonotatus*, a host distinct from those associated with the clade comprising species from the Amazon and Atlantic Forest, suggests that *R. taquariensis* divergence may predate the radiation of the species within this clade.

The host *Physalaemus albonotatus* is phylogenetically and ecologically distant from many of the leptodactylid or hylid anurans used by other *Rhabdias* spp. in the present phylogeny. The proposed phylogeny seems consistent with recent models of parasite diversification, which emphasize the influence of host phylogenetic distance on parasite evolution (D'Bastiani *et al.* 2023). Moreover, a combination of ecological opportunity, repeated host switching, and broad host compatibility may contribute to the emergence of novel parasite lineages as proposed for other Neotropical taxa (Nascimento *et al.* 2019). Nevertheless, the limited availability of molecular sequences of *Rhabdias* still constrains broader phylogenetic resolution. Expanding molecular datasets across host taxa and biomes will be essential to fully understand diversification patterns and the role played by host evolutionary history, ecology, and biogeography in shaping the phylogeny of *Rhabdias* species (Tavares-Costa *et al.* 2022; Willkens *et al.* 2020).

A broad range of parasites has been recorded for *Physalaemus* species, including species of Acanthocephala, Monogenoidea, and various families of Nematoda such as Atractidae, Cosmocercidae, Molineidae, Physalopteridae, and Strongyloididae (Campião *et al.* 2014; González et al. 2025; Graça *et al.* 2017; Oliveira *et al.* 2019). *Rhabdias* cf. *breviensis* is one of the few species precisely identified in *Physalaemus petersii*, whereas other studies on parasitic helminths in *Physalaemus* have reported *Rhabdias* only at the generic level (Aguiar *et al.* 2015; do Nascimento *et al.* 2013; Leivas *et al.* 2018; Oliveira *et al.* 2019). Further studies on the diversity of *Rhabdias* in this region are needed, especially in light of the challenges posed by anthropogenic pressures in the Cerrado that threatens all wildlife, including parasitic organisms' diversity.

Supplementary material. The supplementary material for this article can be found at http://doi.org/10.1017/S0022149X25101041.

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Methodology: RE, ICOS, DJS, CEO, LERT, and KMC.
Analysed the data: RE, ICOS, DJS, LFN, LERT, and KMC.
Wrote the paper: RE, ICOS, DJS, LERT, and KMC.
Supervision: KMC.

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