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A new species of *Creptotrema* (Allocreadiidae) in *Cambeva davisi* (Siluriformes) from river streams in fragments of the Atlantic Rainforest, southern Brazil

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Research Paper

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Abstract

We described *Creptotrema cambeve* n. sp. from the intestine of the Brazilian catfish *Cambeva davisi* (Haseman, 1911) (= *Trichomycterus davisi*) based on integrative analyses using morphological and molecular approaches. *Creptotrema cambeve* n. sp. closely resembles morphologically *Creptotrema paraense* Vicente, Santos & Souza, 1978, which was described from a siluriform fish in Northern Brazil; however, the two species differ mainly in the oral and ventral sucker sizes and the distribution of the vitellaria. The phylogenetic analyses of the 28S rDNA placed the sequences of the new species in a monophyletic clade together with all other *Creptotrema* spp. and as sister taxon of *Creptotrema cruste* Alcantara, Ebert, Ferreira-Silva, Forti, Morais, Pérez-Ponce de León & Silva, 2024, a parasite from a Brazilian anuran. Genetic divergences between the new species and other *Creptotrema* spp. varied from 0.2% to 4.3%, further corroborating the distinction of the new taxon. *Creptotrema cambeve* n. sp. is the 18th nominal *Creptotrema* species known from South America and the 22nd erected in the genus. This is the first study reporting a trematode in *Cambeva* spp. hosts and the second parasitological survey carried out for *Ca. davisi*, a poorly known small endemic fish.

Introduction

The Neotropical region comprises the greatest richness of freshwater fishes globally (Lowe-McConnell, 1999), with Brazil standing out for a large portion of this diversity because of its broad territory and the several hydrographic basins therein (Rosa and Menezes, 1996). Associated with this great fish diversity, there must be a high parasite diversity which remains poorly studied. Knowledge about helminth diversity is primary, as is their association with their hosts and the environment in which they are inserted since this parasite-host-environment relationship is linked to the population diversity of an entire ecosystem (Brooks and Hoberg, 2000).

Trichomycteridae Bleeker, 1858 is one of the most species-rich families of Siluriformes (de Pinna, 1998), distributed exclusively in the Neotropical region. Trichomycterids are characterized, among other particularities, by possessing a highly modified opercular apparatus, adapted to locomote in rocky environments (de Pinna, 1998). Among genera in Trichomycteridae, the recently erected *Cambeva* Katz, Barbosa, Mattos & Costa, 2018, which was supported exclusively by molecular data (Katz *et al.*, 2018), comprises species previously allocated to *Trichomycterus* Valenciennes, 1832, including *Cambeva davisi* (Haseman, 1911) (= *Trichomycterus davisi*), the type species of the genus (Katz *et al.*, 2018). Because of this recent taxonomic rearrangement and that only some of the species previously allocated in *Trichomycterus* were transferred to *Cambeva* (Katz *et al.*, 2018), and, despite new species of the genus have been described recently, specific data on the biology and parasitological aspects of *Cambeva* spp. are scarcely available in the literature. For *Ca. davisi*, a small freshwater fish endemic to upper Paraná, Iguaçu, and Ribeira de Iguapé River basins in Brazil and Argentina (Fricke *et al.*, 2024), the only parasitological records are restricted to *Sebekia oxycephala* (Sambon, 1922) (Pentastomida) found in the gonads, liver, and gastrointestinal tract, and *Minilernaea floricapitella* Thatcher & Huergo, 2005 (Crustacea) found in the gills (Parra *et al.*, 2021). For other

Cambeva spp. and *Trichomycterus* spp. that have been relocated in *Cambeva*, there have been no other parasite taxa previously reported so far.

Among the digeneans of Allocreadiidae Looss, 1902, the genus *Creptotrema* (Travassos, Artigas & Pereira, 1928) includes parasites of anurans and freshwater teleosts belonging to the orders Characiformes, Gymnotiformes, Perciformes, and Siluriformes, distributed across the Neotropical region (Franceschini *et al.*, 2021; Liquin *et al.*, 2022; Alcantara *et al.*, 2024).

Franceschini *et al.* (2021) recently proposed a revised diagnosis of *Creptotrema*, which is mainly characterized by possessing ovary and testes with entire or slightly lobed margins, the presence of small domelike papillae over the margin of the oral sucker and ventral sucker, with variable arrangements (more visible by scanning electron microscopy [SEM] analyses), an oral sucker subterminal, rounded or funnel-shaped, with highly variable morphology of the lobes (may form a discrete lobe on either side of the oral sucker, without prominent dorsolateral lobes ("auricles") and free ends on dorsal view; or assume the same morphology described by Razo-Mendivil *et al.* (2014)); and a uterus pretesticular, intertesticular or post-testicular (depending on the number of eggs and the maturity of the specimens). The genus currently includes 21 valid species (Alcantara *et al.*, 2024), with 17 of those occurring in South America, *i.e.*, the type-species *Creptotrema creptotrema* Travassos, Artigas & Pereira, 1928, *Creptotrema conconae* Franceschini, Aguiar, Zago, Yamada, Ebert & Silva, 2021, *Creptotrema diagonale* (Curran, Tkach & Overstreet, 2011), *Creptotrema foliaceum* (Curran, Tkach & Overstreet, 2011), *Creptotrema guacurarii* Montes, Barneche, Croci, Balcazar, Almirón, Martorelli & Pérez-Ponce de León, 2021, *Creptotrema lamothei* Curran, 2008, *Creptotrema lynchi* Brooks, 1976, *Creptotrema macrorchis* (Szidat, 1954), *Creptotrema megacetabulare* Franceschini, Aguiar, Zago, Yamada, Ebert & Silva, 2021, *Creptotrema ocloye* (Liquin, Gilardoni, Cremonte, Saravia, Cristóbal & Davies, 2022) Alcantara, Ebert, Ferreira-Silva, Forti, Morais, Pérez-Ponce de León and Silva, 2024, *Creptotrema pati* Lunaschi, 1985, *Creptotrema paraense* Vicente, Santos & Souza, 1978, *Creptotrema platense* (Szidat, 1954), *Creptotrema schubarti* Franceschini, Aguiar, Zago, Yamada, Ebert & Silva, 2021, *Creptotrema stenopteri* (Mañé-Garzón & Gascón, 1973), *Creptotrema sucumbiosa* Curran, 2008, and *Creptotrema cruste* Alcantara, Ebert, Ferreira-Silva, Forti, Morais, Pérez-Ponce de León & Silva, 2024.

During ecological surveys in river streams located in fragments of the Atlantic Rainforest in Paraná State, southern Brazil, we were able to discover and describe a new species of *Creptotrema* from the poorly studied Neotropical freshwater fish *Ca. davisi*. By using integrative approaches, we provide a detailed morphological assessment of the specimens (light and scanning electron microscopy) and molecular analyses of the nuclear 28S rDNA gene to confirm its phylogenetic position and assignation within the genus *Creptotrema*.

Material and methods

Host sampling and parasitological procedures

A total of 100 fishes identified as *Ca. davisi* were sampled during three field expeditions (October 2015, December 2017, and October 2018) in river streams from the Tibagi River Basin, located in fragments of Atlantic Rainforest in Mauá da Serra ("Rio do Couro") (23°57'15" S; 51°06'00" W) and Londrina ("Riacho do Bule") (23° 27'20" S; 51°16'32" W) municipalities, Paraná State, southern Brazil (Figure 1). The fish hosts were captured using sieves and transferred to the laboratory, where they were weighed, measured, euthanized, and necropsied. All internal organs and body cavities were separately analyzed under a stereomicroscope to search for helminth parasites. Voucher specimens of fishes were fixed in 10% formalin,

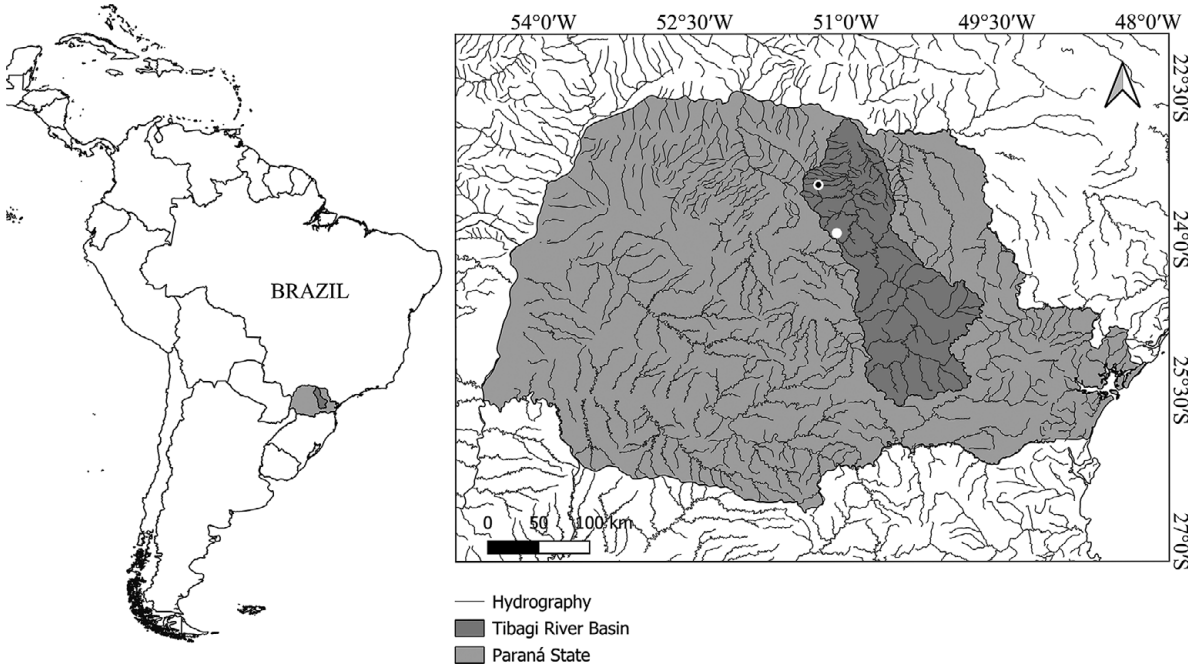

Figure 1. Map with hydrography basins of Paraná State, Brazil, showing the Tibagi Basin and the localities where specimens of *Creptotrema cambeve* n. sp. were collected associated with the siluriform fish *Cambeva davisi*. Black dot indicates the type locality of the new species (23°27'20" S; 51°16'32" W) and the white dot the second locality (23°57'15" S; 51°06'00" W) where the new species was also found.

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preserved in 70% ethanol, and deposited in the Zoology Museum of the State University of Londrina (UEL) (MZUEL 20438)

Trematodes were collected from the intestines of the fishes. Some parasites were fixed in 70% ethanol under slight pressure of a coverslip for 10 minutes and then transferred to 70% ethanol for further morphological analyses. Other specimens were fixed in 96% ethanol for molecular analyses. Posteriorly, some of the worms were stained with chloridric carmine and then cleared with eugenol or creosote in temporary mounts, according to Amato *et al.* (1991). Four specimens were fixed in hot ethanol (70%) for SEM analyses. After fixation, these specimens were dehydrated in a graded alcohol series and critically point-dried. The worms were mounted on an aluminum stub using conductive double-sided tape, coated with gold-palladium, and examined with a Quanta 200 SEM (FEI Company; from Laboratório de Microscopia Eletrônica e Microanálise, Londrina State University, UEL, Brazil) (adapted from Allison *et al.*, 1972).

Morphological description and measurements were obtained using the computerized image system Zeiss Axio Shat AX10 and followed Travassos *et al.* (1928), Scholz *et al.* (2004), Kohn *et al.* (2007), Razo-Mendivil *et al.* (2014), Franceschini *et al.* (2021), and Alcantara *et al.* (2024). Illustrations of the structures were produced with the aid of a camera lucida mounted on a Leica DMLS microscope with phase-contrast optics. Measurements are given in micrometers and averages are in the brackets following the minimum and maximum measurements.

Holotype and paratypes of the new species of *Creptotrema* were deposited in the Helminthological Collection of the Oswaldo Cruz Institute (CHIOC – Holotype: number 39676a; Paratypes: numbers 39676b-c), Rio de Janeiro State, Brazil. Voucher specimens were deposited in the Helminthological Collection of the Department of Biodiversity and Biostatistics of the Institute of Biosciences, São Paulo State University, UNESP (CHIBB – number 10629), Botucatu, São Paulo State, Brazil.

Sequence generation

Total genomic DNA was extracted from two whole ethanol-fixed worms, with the DNeasy Blood & Tissue Kit (Qiagen) following the manufacturer's recommended protocol, in a final volume of 50 µL. Fragments of the nuclear 28S rDNA gene were obtained through polymerase chain reaction (PCR) amplifications using Ready-to-Go PCR beads (GE Healthcare), 3.0 µL of extracted DNA, and 1.0 µL of each PCR primer in a total volume of 25 µL. The following primers and thermocycling profile were used; LSU (5′-TAG GTC GAC CCG CTG AAY TTA AGC A-3′) and 1500R (5′-GCT ATC CTG AGG GAA ACT TCG-3′) (Olson *et al.*, 2003), with initial DNA denaturation for 7 min at 95 °C followed by 40 cycles with 40 s for DNA denaturation at 95 °C, 45 s for primer annealing at 57 °C, 1 min and 30 s at 72 °C for primer extension, and a final extension step of 10 min at 72 °C, followed by storage at 4 °C. PCR products (2.0 µL volume) were run on an agarose gel (1%) using gel red and loading buffer to confirm amplicon size and yield. PCR amplicons were purified using the QIAquick PCR Purification Kit (Qiagen) following the manufacturer's instructions. Automated sequencing was performed directly on purified PCR products using BigDye v.3.1 Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems). The internal primer 1200R (5′-GCA TAG TTC ACC ATC TTT CGG-3′) (Olson *et al.*, 2003) was used for sequencing. Sequences were run onto an Applied Biosystems ABI 3500 DNA genetic sequencer. The sequences were assembled and edited with Sequencher v.5.2.4 (Gene Codes, Ann Arbor, MI, USA), and then subjected to BLAST analysis to confirm identity. We also attempted to generate sequences of the COI mtDNA gene of the new *Creptotrema* species using the primers JB3/JB4 (Morgan and Blair, 1998) and the barcode region primers MplatCOX1dF/BARCOXR (Moszczynska *et al.*, 2009; Hernández-Mena *et al.*, 2017); however, it was unsuccessful.

Alignments and data analyses

A dataset was built including the newly generated 28S rDNA sequences combined with published sequences of Allocreadiidae as the ingroup, and sequences of *Prosthenhystera* Travassos, 1922 (Callodistomidae), *Dicrocoelium* Dujardin, 1845 (Dicrocoeliidae), *Degeneria* Campbell, 1977 (Gorgoderidae), and *Phyllodistomum* Braun, 1899 (Gorgoderidae) as the outgroup. The sequences were aligned using the MUSCLE algorithm implemented on Geneious 7.1.3 (Kearse *et al.*, 2012), with default settings. The parasite species, their hosts, localities of collection, the GenBank accession numbers, and references of all sequences used in the alignment are provided in Supplementary Table 1. Before the phylogenetic analyses, the best-fitting model for nucleotide substitution for the dataset was determined using the Akaike information criterion in the JModelTest software (Posada, 2008) as GTR+I+G. Phylogenies were reconstructed under Bayesian Inference (BI) and maximum-likelihood (ML) criteria. The BI was run on the MrBayes v.3.2 program implemented on the CIPRES web portal (Miller *et al.*, 2015). For the Markov chain Monte Carlo search, chains were run with 10 million generations, saving one tree every 100 generations with the first 25% discarded as burn-in; only nodes with posterior probabilities greater than 0.90 were considered well-supported. The ML analyses were estimated on the RAxML v.8 (Stamatakis, 2014) implemented on the CIPRES web portal (Miller *et al.*, 2015) using random starting trees for each dataset and 1000 bootstrap replicates; only nodes with bootstrap values greater than 80% were considered well supported. The final trees were visualized using FigTree v.1.3.1 (Rambaut, 2009) and edited in CorelDraw V8.

The pairwise genetic distances within and among sequences for the alignment was calculated using the Kimura-2-parameter (K2P) model and a bootstrap procedure with 1000 replicates in MEGA7 software (Kimura, 1980; Kumar *et al.*, 2016).

Description

Family Allocreadiidae Looss 1902

Genus *Creptotrema* (Travassos, Artigas & Pereira, 1928)

Creptotrema cambeve n. sp. (Figs. 2-3)

Description is based on 18 specimens (two from "Riacho do Bule," municipality of Londrina, and 16 from "Rio do Couro," municipality of Mauá da Serra). Body elongated, slightly narrowing at pharyngeal level, tegument unspined, 729–2,525 (1,637) long, 141–476 (322) wide (Figures 2 and 3a). Oral sucker subterminal, well-developed, funnel-shaped, with a single muscular lobe on either side; muscular lobes with a short base located ventrolaterally which do not stretch to dorsolateral view (Figure 3b); oral sucker surrounded with 19 main domelike papillae symmetrically arranged: four apical, 10 arranged along the muscular lobes, and five displayed in one row immediately below the muscular lobes (Figure 3b); oral sucker 103–227 (172) long, 97–236 (161) wide (including lobes). Mouth subterminal with oral opening triangular shaped. Prepharynx absent. Pharynx muscular and ovoid, 35–110 (57) long, 37–127 (67) wide (n = 7). Esophagus straight or slightly

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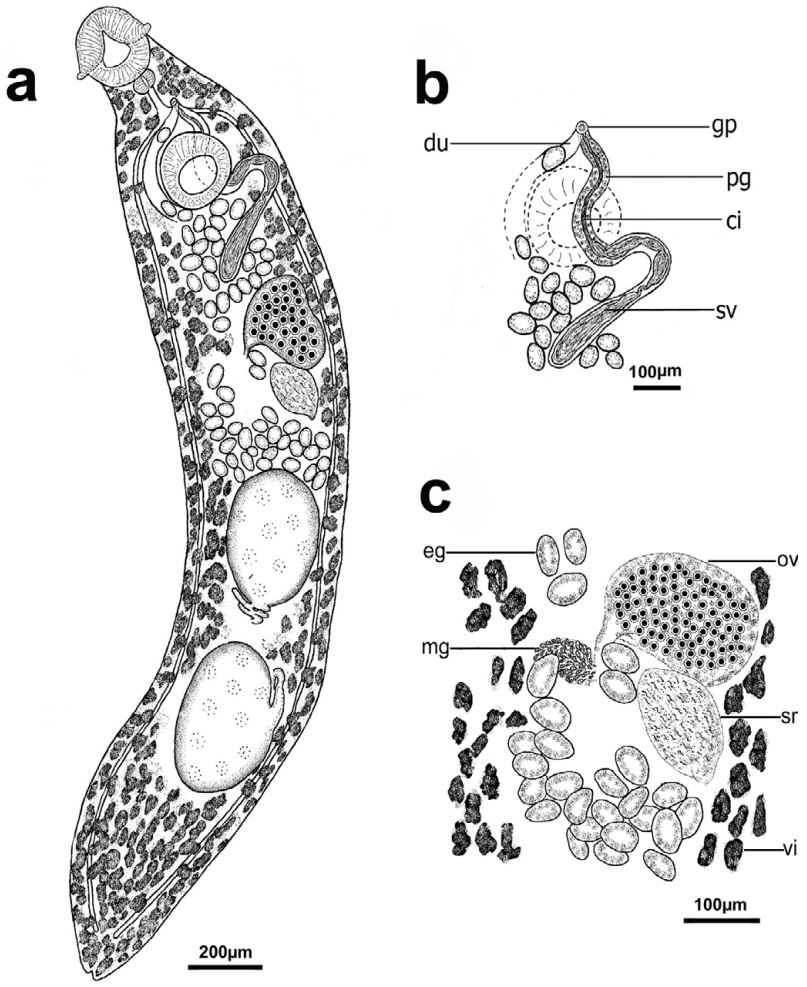

Figure 2. Holotype of *Creptotrema cambeve* n. sp. from *Cambeva davisi* from river streams in Atlantic Rainforest fragments, Southern Brazil. a) Ventral view of whole mount specimen. b) Ventral view of the region of cirrus-sac with cirrus (ci), duct uterine (du), genital pore (gp), prostatic glands (pg), and seminal vesicle (sv). c) Female reproductive complex with eggs (eg), Mehlis' gland (mg), ovary (ov), seminal receptacle (sr), and vitelline follicles (vi).

curved in some specimens, 103 long and 27 wide (n = 1). Intestinal caecal bifurcation preacetabular, at the level of genital pore or posterior. Intestinal caecum thin and slightly apparent reaching posterior extremity of the body. Ventral sucker spherical (Figures 2a and 3c), 97–219 (162) long, 109–195 (144) wide, and slightly smaller than oral sucker in most of the specimens. Ratio of oral and ventral suckers 1:1.0–1.4 (1.1) long, 1:1.1–1.6 (1.3) wide (considering ventrolateral lobes). Ventral sucker localized at one third of the body and 53–368 (175) from oral sucker. Testes two, oval, entire, in tandem in most of the specimens, in posterior half of the body. Anterior testis, 110–281 (193) long, 66–282 (172) wide. Posterior testis 129–345 (235) long, 68–235 (168) wide. Distance between testes 0–92 (29), posterior testis almost contiguous with anterior testis. Distance between anterior testis and anterior end of body 411–1157 (866). Distance between posterior testis and posterior end of body 97–446 (265). Initial portions of spermatic ducts are thick and very apparent in some specimens. Cirrus-sac (Figures 2a and 2b) long and curved 223–639 (428) long (n = 8), 22–54 (39) wide (n = 8), located dorsally to ventral sucker with distal portion posteriorly to ventral sucker. Cirrus-sac containing elongate internal seminal vesicle, pars prostatic, and wide muscular ejaculatory duct. Seminal vesicle very large and conspicuous occupying two thirds of the cirrus-sac. Genital pore median and anterior to intestinal bifurcation (in some specimens, it tends to be at the level of intestinal bifurcation), opening on ventral surface. Ovary pre-testicular, pre-equatorial, irregular shaped, 79–212 (142) long, 68–191 (119) wide, situated on left side of the body between ventral sucker and anterior testis. Distance between ovary and anterior testis 30–275 (169). Seminal receptacle large and subspherical, localized posterior to ovary, 150 long, 95 wide (n = 2). Laurer's canal not observed. Mehlis' gland immediately posterior and laterally to ovary and anterior to seminal receptacle (Figure 2c). Small vitelline follicles numerous and widely distributed from pharyngeal level to posterior end of the body, occupying inter and extracaecal spaces, and confluent in post-testicular region. Uterus large localized between anterior testis and genital pore. Uterine loops predominantly intracaecal. Terminal portion of uterus forming a metraterm opening into the genital pore. Eggs non-embryonated 52–71 (63) long, 29–46 (39) wide. Excretory pore terminal (Figure 3d). Excretory vesicle I shaped, terminating at posterior margin of posterior testis (observed in a few specimens).

Taxonomic summary

Type-host: *Cambeva davisi* (Haseman, 1911) (Siluriformes: Trichomycteridae)

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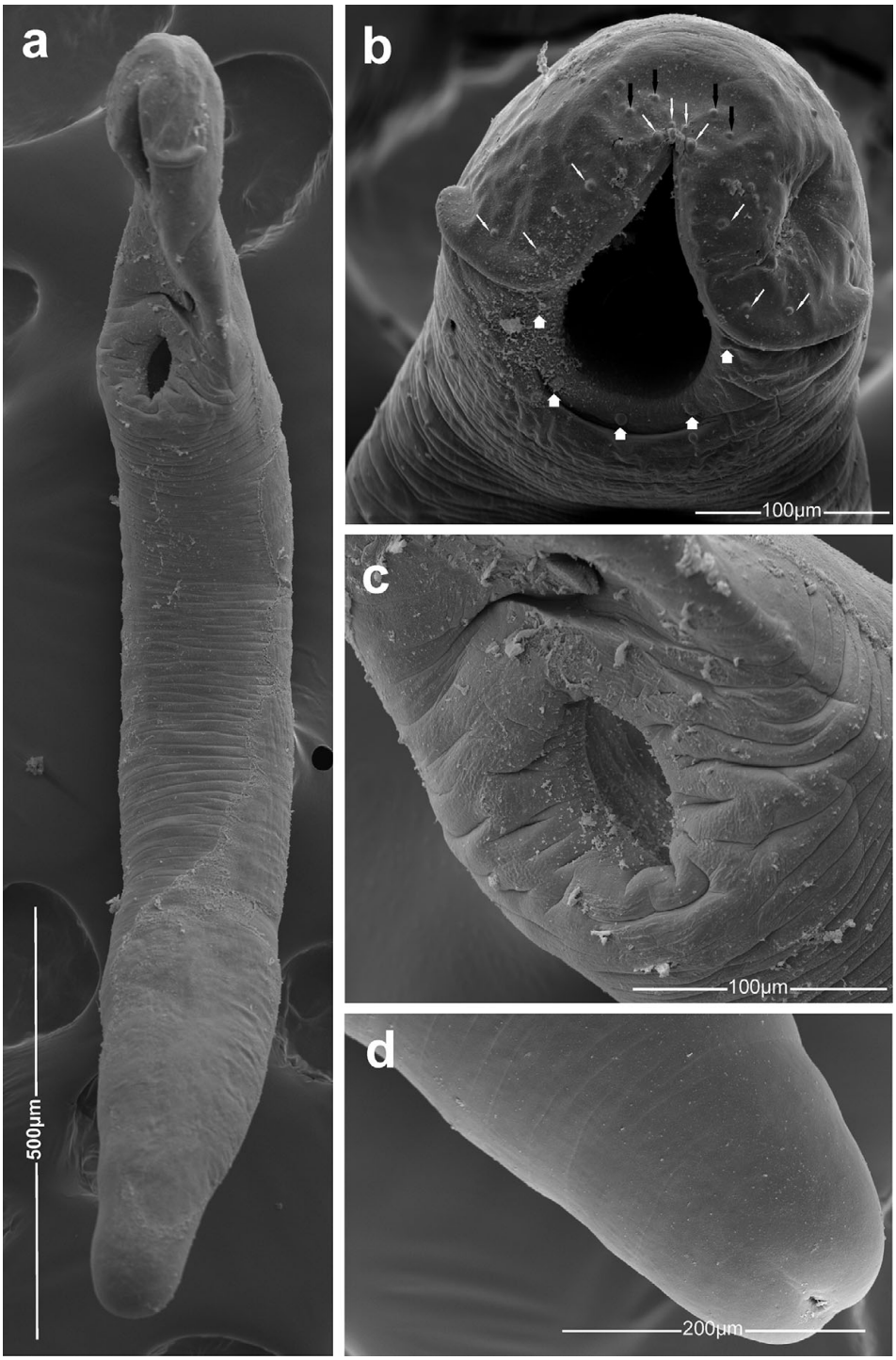

Figure 3. Scanning electron micrographs of *Creptotrema cambeve* n. sp. from *Cambeva davisi* from streams in Atlantic Rainforest fragments, Southern Brazil. a) Total view of body; b) oral sucker bearing muscular lobes and the distribution of the main 19 papillae: four apical (black arrows), 10 arranged along the muscular lobes (white arrows), and five displayed in one row immediately below the muscular lobes (white thick arrows); c) genital atrium and ventral sucker; d) Posterior end showing the terminal excretory pore.

Type locality: a small stream of the Tibagi River basin ("Riacho do Bule") in a forest fragment adjacent to the Mata dos Godoy State Park, municipality of Londrina, Paraná State, Southern Brazil (23° 27'20" S; 51°16'32" W).

Other localities: a small stream of Tibagi River basin ("Rio do Couro"), Mauá da Serra municipality, Paraná State, Southern Brazil (23°57'15" S; 51°06'00" W).

Site of infection: intestine.

Prevalence: 8%.

Specimens deposited: Holotype (CHIOC 39676a) from Londrina; paratypes: 1 specimen from Londrina (CHIOC 39676b) and 16 specimens from Mauá da Serra (CHIOC 39676b and CHIBB 10629).

Representative DNA sequences: 28S rDNA gene – PQ159269 and PQ159270

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Etymology: Specific epithet refers to the genus of the fish host *Cambeva davisi* in which the new species was first described.

Remarks

Creptotrema cambeve n. sp. follows the diagnosis of *Creptotrema* as recently amended by Franceschini *et al.* (2021). The new species closely resembles *C. paraense* mainly in body shape, triangular oral opening, long cirrus-sac located posteriorly to the ventral sucker, and subspherical testes positioned in tandem; however, *Creptotrema cambeve* n. sp. presents oral and ventral suckers with nearly equal sizes whereas in *C. paraense* the oral sucker is larger than the ventral sucker; also, the vitelline follicles are distributed from the pharyngeal region to the end of the body in the new species, whereas in *C. paraense* the vitelline follicles do not reach the oral sucker margin. The genital pore in *Creptotrema paraense* is lateral and the ovary is more rounded with regular-shaped than that of the new species (irregular in shape). Moreover, *C. paraense* was described from a siluriform fish, *Pimelodus* spp. (Pimelodidae), in the state of Pará, Northern Brazil, (Vicente *et al.*, 1978), Amazon rainforest, whereas the new species was found in *Ca. davisi*, a small siluriform from the family Trichomycteridae in the South of Brazil. Among other *Creptotrema* spp., the new species can be distinguished from *C. astyanace, C. platense, C. macrorchis, C. stenopteri, C. foliaceum, C. diagonale, C. totonacapanense*, *C. lobatum*, *C. tica*, *C. ocloye*, and *C. conconae* by lacking dorsolateral muscular lobes on each side of oral sucker which stretching from ventrolateral to dorsolateral side. *Creptotrema cambeve* n. sp. presents two lateral vitelline fields extending along the body which are confluent in post-testicular region, differing from *C. creptotrema, C. foliaceum, C. guacurarii, C. lamothei, C. lynchi, C. macrorchis, C. megacetabulare, C. pati, C. platense, C. schubarti, C. stenopteri*, and *C. totonacapanense*, that possess lateral vitelline fields extending to the posterior end of the body, but not confluent in post-testicular region. *Creptotrema cambeve* n. sp. have a similar distribution of the vitelline follicles and similar testes position as *C. cruste*, but the two species clearly differ from each other as *C. cruste* presents a bivalve shell-shaped musculature at the opening of the ventral sucker as a specific differential character among all *Creptotrema* spp.; besides, the ventral sucker in *C. cruste* is much larger than the oral sucker, whereas in the new species, the suckers are nearly equal sizes. *Creptotrema sucumbiosa* present oblique testes with coincident zones and bilobed ovary, differently from the new species which has separated testes in tandem and entire ovary. The new species can be differentiated from *Creptotrema ocloye* by the intestinal caeca length (reaching posterior extremity of the body *vs* ending between posterior testis and posterior end of body), the ovary position (between acetabulum and anterior testis *vs* immediately posterior to or overlapping ventral sucker) and testes position (in tandem *vs* oblique). *Creptotrema conconae* differs from the new species in the shape of the testes (round *vs* oval), the size and anterior extension of the vitelline follicles (large, from the level of the esophagus to the posterior end of body *vs* small, from pharyngeal level to the posterior end of the body), the genital pore aperture (slightly posterior to intestinal bifurcation *vs* slightly anterior to intestinal bifurcation), the cirrus-sac extension (reaching the ovary posteriorly *vs* reaching the ovary anteriorly), and the size and quantity of eggs (larger and less numerous in *C. conconae*). In *C. megacetabulare* and *C. schubarti*, the testes are symmetrical and elongated, whereas in the new species the testes are oval and in tandem.

The SEM analyses of *Creptotrema cambeve* n. sp. were compared with those of *C. totonacapanense* (Razo-Mendivil *et al.*, 2014), *C. lobatum* (Hernández-Mena *et al.*, 2016), *C. tica* (Hernández-Mena *et al.*, 2019), *C. creptotrema* (Franceschini *et al.*, 2021), and *C. ocloye* (Liquin *et al.*, 2022). *Creptotrema totonacapanense*, *C. lobatum*, and *C. tica* possess a single pair of muscular lobes stretching from the ventrolateral to the dorsolateral side of the oral sucker (easily observed in dorsolateral view of the oral sucker). *Creptotrema totonacapanense* and *C. lobatum* present 19 domelike papillae on the oral sucker surface arranged in four rows (four apical, six anterior, four on the inner surface, and five on the outer surface); *C. tica* possesses 21 domelike papillae arranged in four rows (four apical, eight anterior, four on the inner surface, and five on the outer surface). *Creptotrema creptotrema* possesses a discrete single ventrolateral muscular lobe on either side of the oral sucker, stretching from the ventral side to the lateral area, but not extending to the dorsal region, without free dorsal ends and, although Kohn (1984) has mentioned the presence of papillae in *C. creptotrema*, papillose or rugose teguments on the suckers were not observed in SEM analyses provided by Franceschini *et al.* (2021). *Creptotrema ocloye* possesses an oral sucker with a pair of ventral muscular lobes with a broad base that stretches and fuses on the dorsal side, with 19 domelike papillae arranged in four rows (four apical, four pre-anterior, six anterior, and five on the outer surface). Comparatively, the single ventrolateral lobes on the oral sucker of *Creptotrema cambeve* n. sp. do not extend to the dorsolateral view, and the 19 main papillae are symmetrically organized as follows: four apical, ten arranged along the muscular lobes, and five organized in one row immediately below of the muscular lobes (Figure 3b).

Molecular phylogenetic analyses

The alignment of the 28S rDNA gene consisted of 60 sequences, including two new sequences of *Creptotrema cambeve* n. sp. (PQ159269, 1,064 bp; PQ159270, 1,015 bp), and after trimming to the shortest sequence, the final alignment remained with 1,031 positions. Both the BI and ML analyses yielded identical phylogenetic results. The representative phylogenetic reconstruction (Figure 4) placed the sequences of the new species within a highly supported monophyletic clade formed by all *Creptotrema* spp. used in the analyses, except for *Creptotrema funduli* (which was proposed as *taxon inquirendum* by Franceschini *et al.* (2021) in their revised diagnosis of the genus). *Creptotrema cambeve* n. sp. was recovered in a supported clade together with *C. cruste.* This clade, for its turn, was placed as the early divergent to all other *Creptotrema* spp. Within allocreadiids, a highly supported monophyletic group was formed including the sequences of *Creptotrematina* Yamaguti, 1954 as sister to *Creptotrema* and *Wallinia.* This last clade, for its turn, was positioned as a sister to a monophyletic clade which included species of *Paracreptotrema* Choudhury, Pérez Ponce de León, Brooks & Daverdin, 2006.

The 28S rDNA pairwise intraspecific genetic divergence among the two sequences of *Creptotrema cambeve* n. sp. was null. Interspecific genetic divergence between the new species and its sister species, *C. cruste*, was 0.20%, and between the new species and other *Creptotrema* spp., it ranged from 2.3% (*C. astyanace*) to 4.3% (*C. conconae*). Among closely related genera, the interspecific divergence varied from 4.1 to 5.4% between *Wallinia* spp., and 4.8 to 5.5% between *Creptotrematina* spp.

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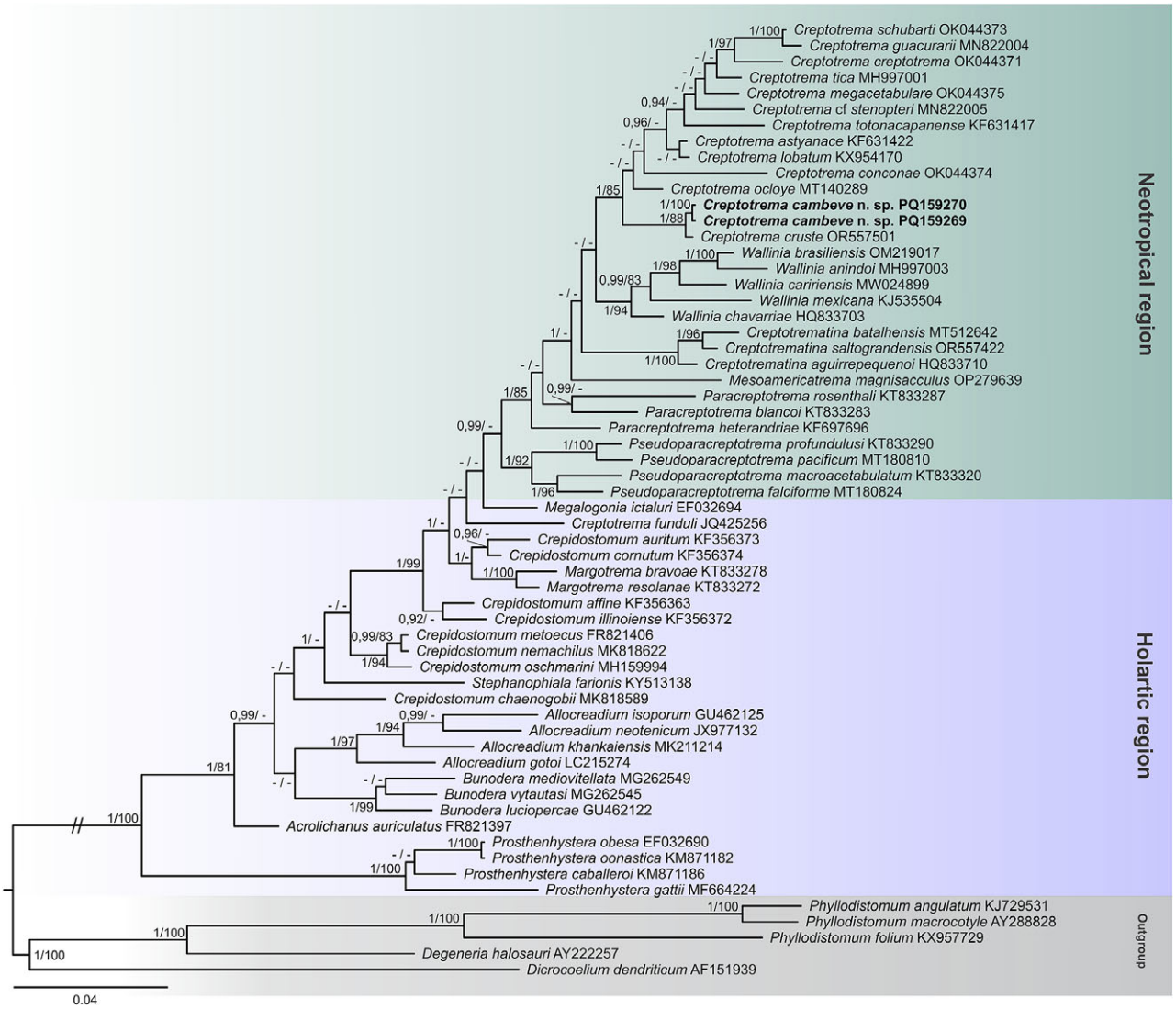

Figure 4. Bayesian phylogenetic reconstruction of Allocreadiidae using 28S rDNA data. The support values at the branching points are shown as the Bayesian posterior probabilities followed by maximum likelihood bootstrap values. Dashes represent values considered not supported (<0.90 posterior probability; <80 bootstrap values). The newly generated sequences are presented in bold. The branch length scale bar indicates the mean number of substitutions per site. The trematodes species names are followed by their correspondent GenBank accession numbers.

Discussion

The use of integrative taxonomy with molecular and morphological analyses including light and scanning electron microscopy along with host and geographical association enabled us to distinguish a new species of *Creptotrema*, namely *Creptotrema cambeve* n. sp., from the siluriform fish *Ca. davisi* from Brazil.

Although *Creptotrema cambeve* n. sp. and *C. paraense* have some similar morphological characters, they can be differentiated by the sucker proportions, ovary shape, position of the genital pore and distribution of vitelline follicles. Moreover, the new species and *C. paraense* were found in fishes of different families (Trichomycteridae and Pimelodidae) and they are located in extreme regions of Brazil (South and North) with different biomes (Atlantic Rain Forest and Amazon Forest). Unfortunately, the description of *C. paraense* was not analyzed using SEM to be compared because the ultrastructure of the body surface may reveal additional differences between the two species. Some morphological analyses based solely on light microscopy are insufficient to state the exact number of muscular papillae (Razo-Mendivil *et al.*, 2014; Hernández-Mena *et al.*, 2016). Moreover, to date, there is no molecular information regarding *C. paraense*, which impedes a genetic comparison between the two species. Therefore, we definitely encourage inventory studies to collect new information, especially molecular and morphological data including SEM analyses, to overcome this troublesome in the relationship among these two species and *Creptotrema* spp. as a whole.

The phylogenetic results based on the partial sequences of the 28S rDNA gene from Allocreadiidae are consistent with previously published topologies for the family, which resolved the genus *Creptotrema* as monophyletic (Franceschini *et al.*, 2021; Liquin *et al.*, 2022; Mendoza-Garfias *et al.*, 2022; Alcantara *et al.*, 2024), with the new species forming a clade together with *C. cruste*, being this clade placed as the early divergent of all other *Creptotrema* spp., in agreement with Alcantara *et al.* (2024).

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The 28S rDNA interspecific genetic divergence found between *Creptotrema cambeve* n. sp., and its sister species *C. cruste* was 0.20%. Morphologically, the two species are completely different: *C. cruste* possess a unique differential character among *Creptotrema* spp. that is a bivalve shell-shaped musculature at the opening of the ventral sucker, which is absent in the new species. Despite the two species being found in a close geographical region (Tibagi river *vs* Iguaçu National Park, both in Paraná state, Brazil), *C. cruste* is a parasite of the anuran *Crossodactylus schmidti* Gallardo, 1961, whereas *Creptotrema cambeve* n. sp. is found in a siluriform fish. In the study of Hernández-Mena *et al.* (2016), the 28S rDNA genetic divergence between the sister species *C. lobatum* and *C. astyanace* was 0.29%. Even though those authors still considered them as two distinct species because they were morphologically different, they were found in different fish hosts (bryconids *vs* characids) and presented different geographical distributions (Mexico *vs* Costa Rica). Likewise, Franceschini *et al.* (2021) found 28S rDNA interspecific divergence of 0.4% between sequences of *C. schubarti* and *C. guacurarii*. Despite these two species being found in characid hosts and the same river basin (Paraná River basin), albeit from relatively distant locations (Middle Paranapanema River, Upper Paraná River basin in Brazil *vs* Iguazu River, Lower Paraná River basin in Argentina), Franceschini *et al.* (2021) observed enough morphological differences between the two species to consider them as separate.

Although the 28S rRNA gene remains the basis of the species-based molecular classification scheme of trematodes thus far (Pérez-Ponce de Léon *et al.*, 2019), as a conserved gene, it is not an ideal molecular marker for species delimitation. Atopkin *et al.* (2020), however, stated that the use of 28S rDNA low values of divergence to establish interspecific differences among allocreadiids should be used in combination with other characters such as host association and distribution to accomplish a more accurate species delimitation. In agreement with Atopkin *et al.* (2020), therefore, we state that our phylogenetic analyses and interspecific divergence values along with morphological, host, and geographical associations yielded in this study strongly validate *Creptotrema cambeve* n. sp. as a new taxon. Unfortunately, we failed in amplifying COI mtDNA fragments of *Creptotrema cambeve* n. sp. COI mtDNA phylogenetic analyses are known to provide a finer resolution for species boundaries than the 28S rRNA gene, and therefore, could help to interpret the limits in intra and interspecific values of divergence within *Creptotrema* spp. (Vilas *et al.*, 2005; Razo-Mendivil *et al.*, 2013; Pérez-Ponce de León *et al.*, 2015). This issue should be overcome in future studies.

The occurrence of a trematode species has never been previously recorded for any species belonging to *Cambeva*. Besides, only trematodes of genera *Clinostomum* Leidy, 1856, *Pygidiopsis* Looss, 1907, and *Genarchella* Travassos, Artigas & Pereira, 1928 have been previously recorded to species of *Trichomycterus* (*sensu stricto*) and none to *Trichomycterus* spp. which were relocated in *Cambeva* (Parra *et al.*, 2021). Thus, this is the first study reporting a trematode in *Cambeva* spp. and the second parasitological survey carried out for *Ca. davisi*, a poorly known small endemic fish.

Creptotrema cambeve n. sp. represents the 18th nominal *Creptotrema* species known from South America and the 22nd erected in the genus. Although *Creptotrema* spp. occur in several species of Siluriformes, Characiformes, and Gymnotiformes from the Neotropical region, *Creptotrema cambeve* n. sp. is the first reported infecting a siluriform of the family Trichomycteridae. Therefore, the present study provides information on host-parasite relationships and distribution, contributing to the knowledge of the parasitic fauna of Brazilian freshwater fish and allocreadids from the Neotropical Region.

Supplementary material. The supplementary material for this article can be found at http://doi.org/10.1017/S0022149X2400052X.

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Competing interest. The authors declare that they have no conflicts of interest.

Ethical standard. All applicable international, national, and/or institutional guidelines for the use and care of animals were followed. Specimens were collected under license number ICMBio/SISBIO 12120-1). According to Brazilian laws, species registration for scientific research purposes was carried out at SisGen (AC60F17).

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