*Parasitology*

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## Research Article

**Cite this article:** Louvard C, Yong RQ-Y, Vermaak A, de Klerk L, Joubert A, Smit NJ (2025) A new species of *Rhipidocotyle* Diesing, 1858 (Trematoda: Bucephalidae) infecting leerfish, *Lichia amia* (L.) (Teleostei: Carangidae), and the first complete life-cycle for a marine trematode from Southern Africa. *Parasitology*, 1–18. https://doi.org/10.1017/S0031182025100681

Received: 4 June 2025
Revised: 17 July 2025
Accepted: 4 August 2025

**Keywords:**
*Bucephalus*; cercaria; Digenea; fish parasites; Life Under Water; mussel; *Perna*; *Prosorhynchoides*; sporocyst

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# A new species of *Rhipidocotyle* Diesing, 1858 (Trematoda: Bucephalidae) infecting leerfish, *Lichia amia* (L.) (Teleostei: Carangidae), and the first complete life-cycle for a marine trematode from Southern Africa

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**Abstract**

We present the first elucidated marine trematode life-cycle for southern Africa, involving intermediate and definitive hosts from the southwestern Indian Ocean, through southern South Africa and to the southeastern Atlantic Ocean in Namibia. Adults of *Rhipidocotyle meridionalis* n. sp. were found infecting leerfish, *Lichia amia* (L.) (Carangiformes: Carangidae), in the Tsitsikamma section of Garden Route National Park, South Africa. Bucephalid sporocysts and cercariae isolated from a brown mussel, *Perna perna* (L.) (Bivalvia: Mytilidae), were subsequently recovered from the same area and are a perfect genetic match with adults of *R. meridionalis* n. sp. based on the partial 28S rDNA region. Metacercariae encysted in multiple organs of the following fishes were found to genetically match this taxon: *Chelon richardsonii* (Smith) (Mugiliformes: Mugilidae), *Dichistius capensis* (Cuvier) (Centrarchiformes: Dichistiidae) and *Diplodus capensis* (Smith) (Eupercaria *i. s.*: Sparidae) from the coast between Swakopmund and Walvis Bay, Namibia; *Chelon dumerili* (Steindachner) (Mugiliformes: Mugilidae), *Chrysoblephus laticeps* (Valenciennes), *Dip. capensis, Sarpa salpa* (L.) and *Sparodon durbanensis* (Castelnau) (Eupercaria *i. s.*: Sparidae) from Tsitsikamma; *Amblyrhynchote honckenii* (Bloch) (Tetraodontiformes: Tetraodontidae) and *Rhabdosargus holubi* (Steindachner) (Eupercaria *i. s.*: Sparidae) from Witsand; *A. honckenii* from Chintsa, De Hoop Nature Reserve and Uvongo; and *Dip. capensis* from Mossel Bay. The southern coast of South Africa, where the type-locality of *R. meridionalis* n. sp. is located, is influenced by both cold Atlantic and warm Indian Ocean currents and is thus subjected to complex hydrological patterns. This life-cycle, linking hosts present in both oceans, informs both the picture of host- and trematode connectivity in southern Africa.

## Introduction

The Bucephalidae Poche, 1907 (Plagiorchiida: Bucephaloidea) comprises 329 currently recognized species in five subfamilies and 26 genera (Atopkin et al., 2022; WoRMS, 2024a). Adults of this family are characterized by the combination of possessing a rhynchus in place of a true oral sucker, the mouth having migrated from the anterior extremity to the mid-ventral body region, the absence of a ventral sucker and the male sexual apparatus having a complex elongated opening into a common genital pore at the body's posterior extremity (Bott and Cribb, 2009).

The bucephalid genus *Rhipidocotyle* Diesing, 1858 comprises 60 currently recognized species (Atopkin et al., 2022; WoRMS, 2024b), many of which were described from carangid definitive hosts. The parasite fauna of carangids has been well studied in the Northern Hemisphere. In the Mediterranean, 10 trematode species from five families, including bucephalids, have been reported infecting the carangid *Lichia amia* (L.) (Stossich, 1887; Looss, 1907; Fischthal, 1980, 1982). For the Bucephalidae, *Bucephalus margaritae* (Ozaki and Ishibashi, 1934) infects *L. amia* from off Israel (Fischthal, 1980, 1982), and *Rhipidocotyle galeata* (Rudolphi, 1819) infects *L. amia* from off Italy [reported by Stossich (1887) as *Monostomum galeatum* (Rudolphi, 1819)] and Tunisia (Derbel et al., 2011). Information on the fauna of *Rhipidocotyle* spp. in/off southern Africa is scarce. The first report of species from this genus was by Reimer (1985), who described three species from off Mozambique. Nunkoo et al. (2017) reported an undetermined *Rhipidocotyle* sp. from oilfish *Ruvettus pretiosus* Cocco (Perciformes: Gempylidae)

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caught in deep water off South Africa. Most recently, Dumbo et al. (2024) described two species of *Rhipidocotyle* infecting the sawtooth barracuda, *Sphyraena putnamae* Jordan & Seale (Sphyraenidae) from off Mozambique (see Table 1 for all records).

Bucephalids infect an exceptional array of bivalve superfamilies as first-intermediate hosts (Cribb et al., 2001). In freshwater, bucephalid first-intermediate stages have been reported from bivalves of the family Unionidae, especially species of *Anodonta* (Lamarck) in northern Europe (Taskinen et al., 1994), and *Eurynia* Rafinesque (Woodhead, 1929) and *Lampsilis* Rafinesque (Kniskern, 1950) in the USA. In the marine environment, first-intermediate stages have been reported from a wide variety of bivalve taxa and families (Giles, 1962; Hutson et al., 2004), albeit seldom reliably identified to species. Species of the family Mytilidae are particularly commonly reported as first-intermediate hosts (Szidat, 1963; Wardle, 1990b; Zeidan et al., 2012; Bagnato et al., 2015; Muñoz et al., 2015). Typical bucephalid sporocysts vegetatively ramify through the host tissue (usually the mantle and gonads), forming a dense network of sporocysts (Wardle, 1988) with narrow tubules interspersed with discrete chambers (Stunkard, 1976). The furcocercous cercariae (Kniskern, 1952) possess two long furcae that, in some species, have been observed tangling cercariae together to form ‘nets’ of multiple individuals (Wardle, 1988). The cercariae actively penetrate the second-intermediate fish hosts and encyst as metacercariae (Muñoz et al., 2015). The metacercariae show little host-specificity, often encysting in small intertidal fishes (Stunkard, 1974) with lower positions in the trophic chain (Kvach and Mierzejewska, 2011). They target a variety of tissues, including fin membranes, gills, under the skin and almost all internal organs (Vidal-Martínez et al., 2012). As adults, bucephalids infect the digestive tract of piscivorous teleost fishes, such as species of the family Carangidae (Carangiformes) (Bray et al., 2019).

Investigations into intermediate stages of marine trematodes in southern Africa have been limited, with all prior records consisting of intermediate stages in molluscan hosts that were unmatched to genus or species (Botes et al., 1999, first published in Botes, 1999; Bower et al., 1994). Lasiak (1993) demonstrated that mytilid bivalves, specifically the indigenous brown mussel *Perna perna* (L.) (Mytilidae) along the South African coast, were extensively infected by bucephalid first-intermediate stages, but these were not matched to genus or species. Calvo-Ugarteburu and McQuaid (1998a, b) noted the presence of bucephalid intermediate stages in *P. perna* (first reported in a conference abstract; Calvo-Ugarteburu and McQuaid, 1994), but these, likewise, were not matched to any adults. The parasite fauna of *L. amia* from this region is poorly known, despite this species being one of the most popular food and gamefishes in southern Africa (Coetzee, 1982). The only trematode record from this fish in the region is that of *Plerurus digitatus* (Looss, 1899) (Hemiuroidea: Hemiuridae) by Bray (1990). As part of an ongoing assessment of South African inshore marine parasite diversity, infections from both *P. perna* and *L. amia*, as well as from various intermediate-host fishes, are characterized both molecularly and morphologically for the first time.

## Materials and methods

As part of broader assessments of the parasitological fauna of marine fishes from coastal southern Africa between 2019 and 2024, a variety of marine fishes were sampled from various localities (Figure 1). In all cases, fishes were transported from the collection sites to the respective field stations in containers of aerated fresh seawater, processed and dissected using standard field protocols. Adult trematodes and metacercariae recovered from the dissections were heat-fixed in near-boiling saline and stored in 70% or 96% ethanol for processing. Brown mussels (*P. perna*) were sampled in the rocky intertidal zone at Tsitsikamma (Garden Route National Park), kept alive in containers of aerated fresh seawater and then dissected for the presence of trematode intermediate stages. No natural cercarial emergence was attempted. Pieces of branching sporocysts were isolated from mussel tissue where possible and preserved in 80% molecular-grade ethanol without heat fixation.

Preserved sporocysts were finely dissected from tissue samples under a dissecting microscope; some were opened to release cercariae for characterisation. Permanent morphological wholemounts of adult trematodes, sporocysts, cercariae and metacercariae were produced using standard procedures for Mayer’s haematoxylin staining and mounting in Canada balsam [see Yong et al. (2016) for full procedure]. Specimens were drawn under a *camera lucida* mounted on a Nikon Eclipse 80i microscope and digitized using Adobe Illustrator version 6.0 (Adobe). Measurements and photomicrographs were made using a Nikon DS-Fi3 digital camera mounted on the same microscope and NIS-Elements BR Cameral Analysis v 5.20 software (Nikon Instruments, Tokyo, Japan). All measurements are in micrometres (μm) and given as a range with the mean in parentheses. Where breadth follows length, the 2 measurements are separated by ‘×’. Specimens for scanning electron microscopy were prepared by chemical dehydration, first in a graded ethanol series followed by a graded hexamethyldisilazane series, then sputter-coated with 60% gold-40% palladium and photographed using a Phenom Pro Desktop scanning electron microscope (Thermo Scientific, Waltham, MA, USA). Type- and voucher specimens are deposited in the Parasite Collection of the National Museum, Bloemfontein, South Africa (NMB).

Molecular sequence data were generated for the large ribosomal subunit gene of the ribosomal DNA region (28S rDNA), the internal transcribed spacer 2 ribosomal DNA region (ITS2 rDNA) and the cytochrome c oxidase subunit 1 mitochondrial region (*cox*1 mtDNA). Genomic DNA was extracted from whole adults, metacercariae and small sporocyst pieces using the PCRBIO Rapid DNA Extraction Kit (PCR Biosystems) following the manufacturer’s protocols with only 10 μL of lysis buffer and 5 μL of proteinase K-containing buffer, with the final extracted product diluted to 300 μL (or to 450 μL for metacercariae). The three target marker regions were conventionally amplified with 25 μL reaction volumes using the primers LSU5 (Littlewood, 1994) or Digl2 (Tkach et al., 2003) and 1500R (Snyder and Tkach, 2001) for the partial 28S rDNA region, 3S (Morgan and Blair, 1995) and ITS2.2 (Cribb et al., 1998) for the ITS2 rDNA region [cf. Yong et al. (2016) for cycle schedules], and DigCox1Fa/DigCox1R (Wee et al., 2017), DICE1F (Moszczynska et al., 2009) and DICE14R (Van Steenkiste et al., 2015) for *cox*1 mtDNA [cf. Wee et al. (2017) and Vermaak et al. (2023) for cycle schedules]. These protocols yielded partial reads for 28S rDNA, including the D1–D3 domains, and partial reads for *cox*1 mtDNA. Sequencing of the ITS2 rDNA region failed, possibly due to the presence of low-complexity repeat regions. Amplicons were visualized via electrophoresis using 1.0% agarose gels stained with SafeView$^{TM}$ Classic (ABM, Canada). Purification and Sanger sequencing were performed by Inqaba Biotechnical Industries (Pretoria, South Africa). Forward and reverse DNA strands were sequenced using the amplification primers, as well

**Table 1.** Species of *Rhipidocotyle* recorded from around Africa

| Species | Host | Location | References |
|---|---|---|---|
| *Rhipidocotyle galeata* (Rudolphi, 1819) Eckmann, 1932 | *Lichia amia* (Carangidae) | Tunisia | Derbel et al. (2011) |
| *Rhipidocotyle angusticollis* Chandler, 1941 | *Epinephelus fasciatus* (Forsskål) (Serranidae) | Egypt | Ozaki & Ishibashi (1934) |
| *Rhipidocotyle eckmanni* Nagaty, 1937 | *Trachinotus bailloni* (Lacépède) (Carangidae) | Egypt | Nagaty (1937) |
| *Rhipidocotyle khalili* Nagaty, 1937 | *Chanos chanos* (Fabricius) (Chanidae) | Egypt | Nagaty (1937) |
| *Rhipidocotyle longicirrus* (Nagaty, 1937) | *Sphyraena barracuda* (Edwards) (Sphyraenidae) | Egypt | Nagaty (1937) |
| *Rhipidocotyle pentagonum* (Ozaki, 1924) | *Caranx compressus* Day | Egypt | Nagaty (1937) |
| | *Caranx* sp. | | |
| *Rhipidocotyle ernsti* Reimer, 1985 | *Gephyroberyx darwinii* (Johnson) (Trachichthyidae) | Mozambique | Reimer (1985) |
| *R. khalili* | *Sphyraena japonica* (Bloch & Schneider) (Sphyraenidae) | Mozambique | Reimer (1985) |
| *Rhipidocotyle lamberti* Reimer, 1985 | *Turrum gymnostethus* (Cuvier) (Carangidae) | Mozambique | Reimer (1985) |
| *Rhipidocotyle laruei* Velasquez, 1959 | *Psettodes erumei* (Bloch & Schneider) (Psettodidae) | Mozambique | Reimer (1985) |
| *Rhipidocotyle longleyi* Manter, 1934 | *Cubiceps whiteleggii* (Waite) (Nomeidae) | Mozambique | Reimer (1985) |
| *Rhipidocotyle nagatyi* Manter, 1940 | *Seriolina nigrofasciata* (Rüppell) (Carangidae) | Mozambique | Reimer (1985) |
| *Rhipidocotyle nolwe Dumbo, Dos Santos & Avenant-Oldewage*, 2024 | *Sphyraena putnamae* Jordan & Seale (Sphyraenidae) | Mozambique | Dumbo et al. (2024) |
| *Rhipidocotyle paruchini* Gavrilyuk-Tkachuk, 1979 | *Otolithes ruber* (Bloch & Schneider) (Sciaenidae) | Off South Africa | Gavrilyuk-Tkachuk (1979) |
| *Rhipidocotyle siphonyaka Dumbo, Dos Santos & Avenant-Oldewage*, 2024 | *S. putnamae* | Mozambique | Dumbo et al. (2024) |
| *Rhipidocotyle sphyraenae* Yamaguti, 1959 | *Verilus cynodon* (Regan) (Acropomatidae) | Mozambique | Reimer (1985) |
| *Rhipidocotyle tonimahnkei* Reimer, 1985 | *S. nigrofasciata* | Mozambique | Reimer (1985) |
| *Rhipidocotyle* sp. | | South Africa | Nunkoo et al. (2017) |
| *Rhipidocotyle capitata* (Linton, 1940) | *Auxis rochei* (Risso) (Scombridae) | Canary Islands | Gijón-Botella et al. (2007) |
| *R. longleyi* | *S. barracuda* | Off Guinea | Szuks (1981) |
| | *Synagrops bellus* (Goode & Bean) (Acropomatidae) | Off Guinea | Szuks (1981) |
| *R. nagatyi* | *Auxis thazard* (Lacépède) (Scombridae) | Off Mauritania | Szuks (1981)[a] |
| | *Katsuwonus pelamis* (L.) (Scombridae) | Canary Islands | Gijón-Botella et al. (2007) |
| *R. pentagonum* | *K. pelamis* | Madeira | Hermida et al. (2019) |
| *Rhipidocotyle viperae* (as *R. galeata*)[b] | *Pomadasys rogerii* (Cuvier) (Haemulidae) | Off Sierra Leone | Szuks (1981) |
| | *Trachinus armatus* Bleeker (Trachinidae) | Off Sierra Leone | Szuks (1981) |

Each region of the African coast is separated by a line: North Africa/Mediterranean, Red Sea, Southern Africa and western Africa. All the records are from the marine environment.

[a] Misspelled as *Rhipidocotyle nagatayi*.

[b] Identified as *R. galeata* by Szuks (1981); recognized as the concept of either *Rhipidocotyle viperae* of van Beneden (1870) or '*Rhipidocotyle viperae*' of Nicoll (1914) by Bartoli et al. (2006).

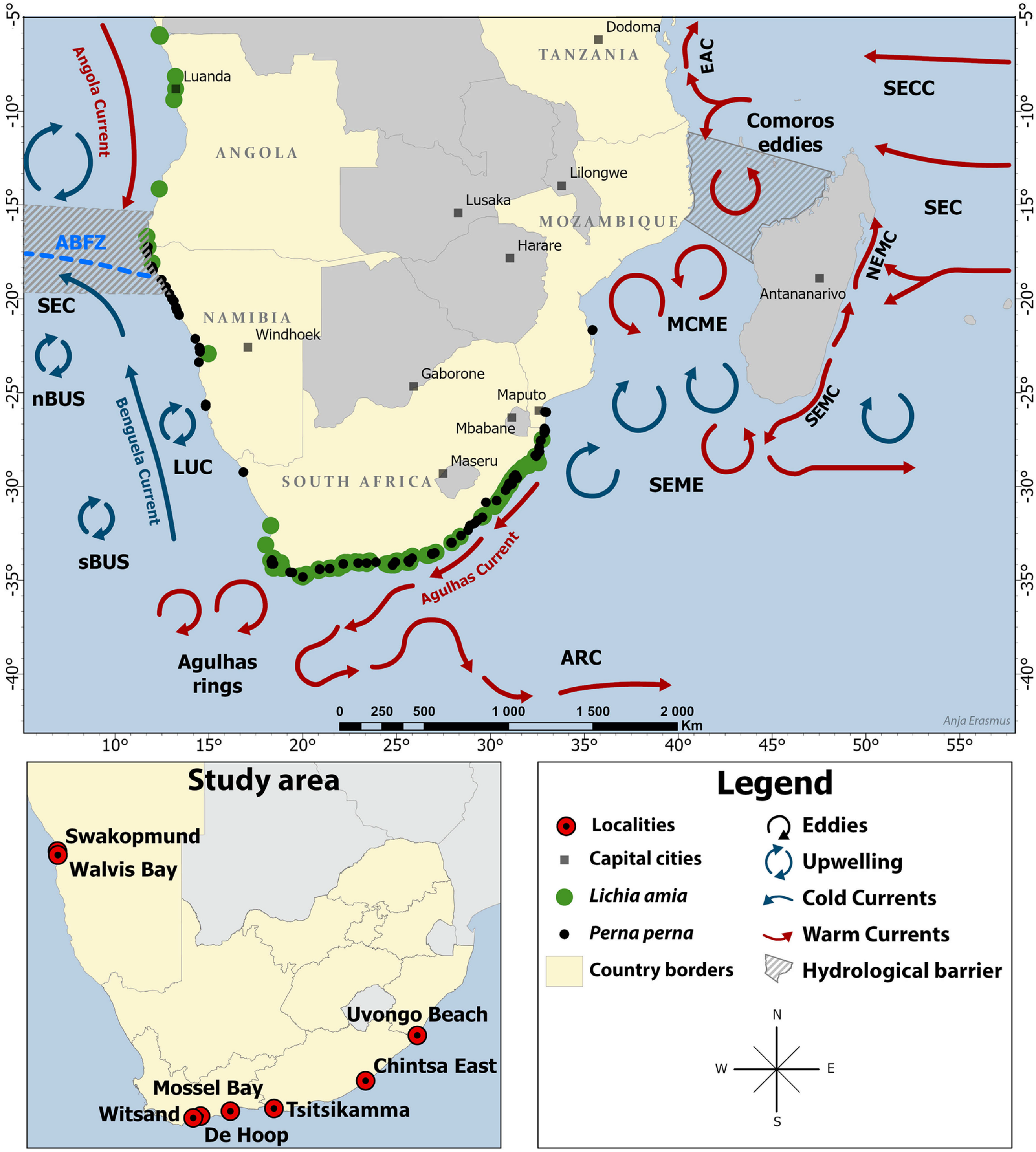

**Figure 1.** Compound map of the distributions of *Lichia amia* and *Perna perna* in Southern Africa in the context of the region's hydrology. The general (large) map shows the distribution of hosts, main marine currents and hydrological barriers. The Study Area map shows sampling sites visited in this study.

as additional internal primers 300F (Littlewood et al., 2000) and ECD2 (Littlewood et al., 1997) for 28S. Contiguous sequences were assembled and edited in Geneious™ version 10.2.2 (Kearse et al., 2012).

Novel 28S sequences produced in this study were used for distance-matrix and phylogenetic analyses. Sequences corresponding to *P. perna* infections were run in NCBI BLASTn for preliminary identification. As sequences of the intermediate stages matched most closely with species of *Rhipidocotyle*, a phylogenetic analysis was conducted on the Bucephalinae Poche, 1907. We used the sequences generated in this study and selected high-quality partial 28S rDNA sequence data for bucephaline species from GenBank (Table 2). Select species of the Heterobucephalopsinae Nolan, Curran, Miller, Cutmore, Cantacessi & Cribb, 2015 and the Prosorhynchinae Odhner, 1905 were used as outgroup taxa in the alignment (Table 2). Sequences were aligned using MUSCLE 3.7

**Table 2.** GenBank accession numbers of sequences of the partial 28S rDNA region used in this study

| Species | GenBank accession | Reference |
|---|---|---|
| *Aenigmatrema grandiovum* Corner, Cribb & Cutmore, 2020 | MT809145 | Corner et al. (2020) |
| *Aenigmatrema inopinatum* Corner, Cribb & Cutmore, 2020 | MT809144 | Corner et al. (2020) |
| *Aenigmatrema undecimtentaculatum* Corner, Cribb & Cutmore, 2020 | MT809143 | Corner et al. (2020) |
| *Bucephalus cynoscion* Hopkins, 1956 | KT273397 | Nolan et al. (2015) |
| *Bucephalus gorgon* (Linton, 1905) Eckmann, 1932 | KT273400 | Nolan et al. (2015) |
| *Bucephalus margaritae* Ozaki & Ishibashi, 1934 | KT273395 | Nolan et al. (2015) |
| *Bucephalus polymorphus* von Baer, 1827 | AY289247 | Stunž et al. (2004) |
| *Bucephalus skrjabini* Akhmerov, 1963 | OM615429 | Atopkin et al. (2022) |
| *Prosorhynchoides aspinosus* Malsawmtluangi & Lalramliana, 2023 | ON557672 | Malsawmtluangi and Lalramliana (2023) |
| *Prosorhynchoides caecorum* (Hopkins, 1956) Bott & Cribb, 2005 | KT273392 | Nolan et al. (2015) |
| *Prosorhynchoides cutmorei* Hammond, Cribb & Bott, 2018 | MG953232 | Hammond et al. (2018) |
| *Prosorhynchoides extenuatus* Curran, Calhoun, Tkach, Warren & Bullard, 2022 | OP348911 | Curran et al. (2022) |
| *Prosorhynchoides galaktionovi* Hammond, Cribb, Nolan & Bott, 2020 | MN310395 | Hammond et al. (2018) |
| *Prosorhynchoides gracilescens* (Rudolphi, 1819) Stunkard, 1976 | AY222224 | Olson et al. (2003) |
| *Prosorhynchoides karvei* (Bhalerao, 1937) Srivastava & Chauhan, 1973 | OM615433 | Atopkin et al. (2022) |
| *Prosorhynchoides kohnae* Hammond, Cribb, Nolan & Bott, 2020 | MN310397 | Hammond et al. (2018) |
| *Prosorhynchoides longoviferus* (Manter, 1940) Curran & Overstreet, 2009 | KT273387 | Nolan et al. (2015) |
| *Prosorhynchoides megacirrus* (Riggin & Sparks, 1962) Curran & Overstreet, 2009 | KT273391 | Nolan et al. (2015) |
| *Prosorhynchoides moretonensis* Hammond, Cribb & Bott, 2018 | MG953230 | Hammond et al. (2018) |
| *Prosorhynchoides ovatus* (Linton, 1900) Dollfus, 1929 | KT273399 | Nolan et al. (2015) |
| *Prosorhynchoides ozakii* (Nagaty, 1937) Margolis & Arthur, 1979 | AB640885 | Baba et al. (2012) |
| *Prosorhynchoides paralichthydis* (Corkum, 1961) Curran & Overstreet, 2009 | KT273398 | Nolan et al. (2015) |
| *Prosorhynchoides scomberomorus* (Corkum, 1968) Curran & Overstreet, 2009 | KT273389 | Nolan et al. (2015) |
| *Prosorhynchoides waeschenbachae* Hammond, Cribb & Bott, 2018 | MG953231 | Hammond et al. (2018) |
| *Rhipidocotyle angusticollis* Chandler, 1941 | KT273383 | Nolan et al. (2015) |
| *Rhipidocotyle campanula* (Dujardin, 1845) Dollfus, 1968 | KF184355 | Petkevičiūtė et al. (2014) |
| *Rhipidocotyle fennica* Gibson, Taskinen & Valtonen, 1992 | KF184363 | Petkevičiūtė et al. (2014) |
| *Rhipidocotyle galeata* (Rudolphi, 1819) Eckmann, 1932 | AY222225 | Olson et al. (2003) |
| *Rhipidocotyle husi* Atopkin, Shedko, Rozhkovan, Nguyen & Besprozvannykh, 2022 | OM615425 | Atopkin et al. (2022) |
| *Rhipidocotyle lepisostei* Hopkins, 1954 | KT273390 | Nolan et al. (2015) |
| *Rhipidocotyle nolwe* Dumbo, Dos Santos & Avenant-Oldewage, 2024 | PQ453500 | Dumbo et al. (2024) |
| *Rhipidocotyle santanaensis* Lunaschi, 2004 | OQ244080 | Montes et al. (2023) |
| *Rhipidocotyle siphonyaka* Dumbo, Dos Santos & Avenant-Oldewage, 2024 | PQ453504 | Dumbo et al. (2024) |
| *Rhipidocotyle meridionalis* n. sp. *ex Chelon richardsoni* | PX124087 | This study |
| *Rhipidocotyle meridionalis* n. sp. *ex Lichia amia* | PX124088 | This study |
| *Rhipidocotyle meridionalis* n. sp. *ex Perna perna* | PX124089 | This study |
| *Rhipidocotyle transversalis* Chandler, 1935 | KT273394 | Nolan et al. (2015) |
| *Rhipidocotyle tridecapapillata* Curran & Overstreet, 2009 | KT273384 | Nolan et al. (2015) |
| **Outgroups** | | |
| *Dollfustrema durum* Nolan, Curran, Miller, Cutmore, Cantacessi & Cribb, 2015 | KT213572 | Nolan et al. (2015) |
| *Heterobucephalopsis yongi* Cutmore, Nolan & Cribb, 2018 | MH754949 | Cutmore et al. (2018) |
| *Prosorhynchus brayi* Cutmore, Nolan & Cribb, 2018 | MH754950 | Cutmore et al. (2018) |

(Edgar, 2004) in MEGA11 (Kumar et al., 2016) with UPGMA clustering for iterations 1 and 2 (gap opening penalty = −400, gap extension penalty = −100). The alignment was trimmed online using Gblocks v.0.9.1 (Castresana, 2000; Dereeper et al., 2008) with parameters of least stringent selection (Kück et al., 2010). The general time reversible model with estimates of invariant sites and gamma-distributed among-site variation (GTR + I + Γ) was used, based on the Akaike information criterion calculated in jModelTest2 v2.1.10 (Darriba et al., 2012). The alignment was converted into the appropriate formats in MESQUITE v.3.81 (Maddison and Maddison, 2018) and subjected to Bayesian inference (BI) and maximum likelihood (ML) phylogenetic analyses using MrBayes v3.2.7a (Ronquist et al., 2012) and RAxML-HPC Blackbox v8.2.12 (Stamatakis, 2014), respectively, on the CIPRES portal (Miller et al., 2010). For the BI analysis, 'nst', gamma shape fixed parameter ('shapepr'), number of discrete categories ('ncat') and fixed proportion of invariable sites ('prinvarpr') parameters were calculated in jModelTest2. The analysis was run over 10 000 000 generations ('ngen = 10 000 000') with 2 runs each containing 4 simultaneous Markov chain Monte Carlo (MCMC) chains ('nchains = 4') and every 1000th tree saved ('samplefreq = 1000'). Samples of substitution model parameters, and tree and branch lengths were summarized using the parameters 'sump burnin = 3000' and 'sumt burnin = 3000'. The ML analysis ran 100 bootstrap pseudo-replicates for both datasets with sequences of the Heterobucephalopsinae and the Prosorhynchinae set as outgroups and branch lengths printed. In parallel, a second 28S rDNA alignment was built in MUSCLE3.7 in MEGA11 using the sequences generated in this study only; the part of the alignment overlapping the ITS2 region was removed, but the alignment was otherwise left untrimmed for the calculation of pairwise distance matrices using *P*-distance and number of differences.

## Results

### General results

The single individual of *L. amia* (L.) caught by seine netting at the Groot River estuary mouth, Tsitsikamma section of Garden Route National Park (Eastern Cape), was infected with an uncharacterized adult bucephalid taxon. Metacercariae of that taxon were recovered from many fishes of the Dichistiidae, Mugilidae, Sparidae and Tetraodontidae from several locations (Table 3). As not all the metacercariae from those fishes could be sequenced and molecularly identified to species, no infection prevalence was calculated. In addition, one of 44 individuals of *P. perna* from Tsitsikamma was found infected with branching sporocysts typical of the Bucephalidae.

### Molecular sequence results

Distance matrices for the 28S rDNA alignment including the newly generated sequences only (1280 bp) show that the metacercarial sequences are all identical save for that of a metacercaria from *Amblyrhynchote honckenii* (Bloch) (Tetraodontiformes: Tetraodontidae) from Witsand. The sequence of that metacercaria differed from that of another metacercaria from the fin of *Chelon richardsonii* (Smith) (Mugiliformes: Mugilidae) (Namibia) by 1 bp (*P*-distance = 0.0008%) (Supplementary Tables 1 and 2). The distance matrices also indicate that all metacercarial sequences are identical to those of adults from *L. amia* and of sporocysts from *P. perna*. Thus, it is considered that all the metacercariae, the adult specimens and the single sporocyst infection belong to the same bucephalid species.

Due to a lack of comparable *cox*1 mtDNA data, the single *cox*1 sequence generated in this study was not aligned and anal-

**Table 3.** Summary of the metacercariae molecularly identified as belonging to the same species as the adult bucephalid from *Lichia amia* and the sporocysts from *Perna perna*

| Sampling location | Host species | Host family |
|---|---|---|
| Chintsa East (Eastern Cape) | *Amblyrhynchote honckenii* (Bloch) | Tetraodontidae |
| De Hoop Nature Reserve (Western Cape) | *A. honckenii* | Tetraodontidae |
| Mossel Bay (Western Cape) | *Diplodus capensis* (Smith) | Sparidae |
| Tsitsikamma (Garden Route National Park) | *Chelon dumerili* (Steindachner) | Mugilidae |
| | *Chrysoblephus laticeps* (Valenciennes) | Sparidae |
| | *Dip. capensis* | Sparidae |
| | *Sarpa salpa* (L.) | Sparidae |
| | *Sparodon durbanensis* (Castelnau) | Sparidae |
| Uvongo Beach (KwaZulu-Natal) | *A. honckenii* | Tetraodontidae |
| Breede River estuary, Witsand (Western Cape) | *A. honckenii* | Tetraodontidae |
| | *Rhabdosargus holubi* (Steindachner) | Sparidae |
| Bird Island, Walvis Bay (Namibia) | *Chelon richardsonii* (Smith) | Mugilidae |
| Vier-kant-klip, Swakopmund (Namibia) | *Dichistius capensis* (Cuvier) | Dichistiidae |
| | *Dip. capensis* | Sparidae |

Sampling locations in South Africa and Namibia are separated by a bold line.

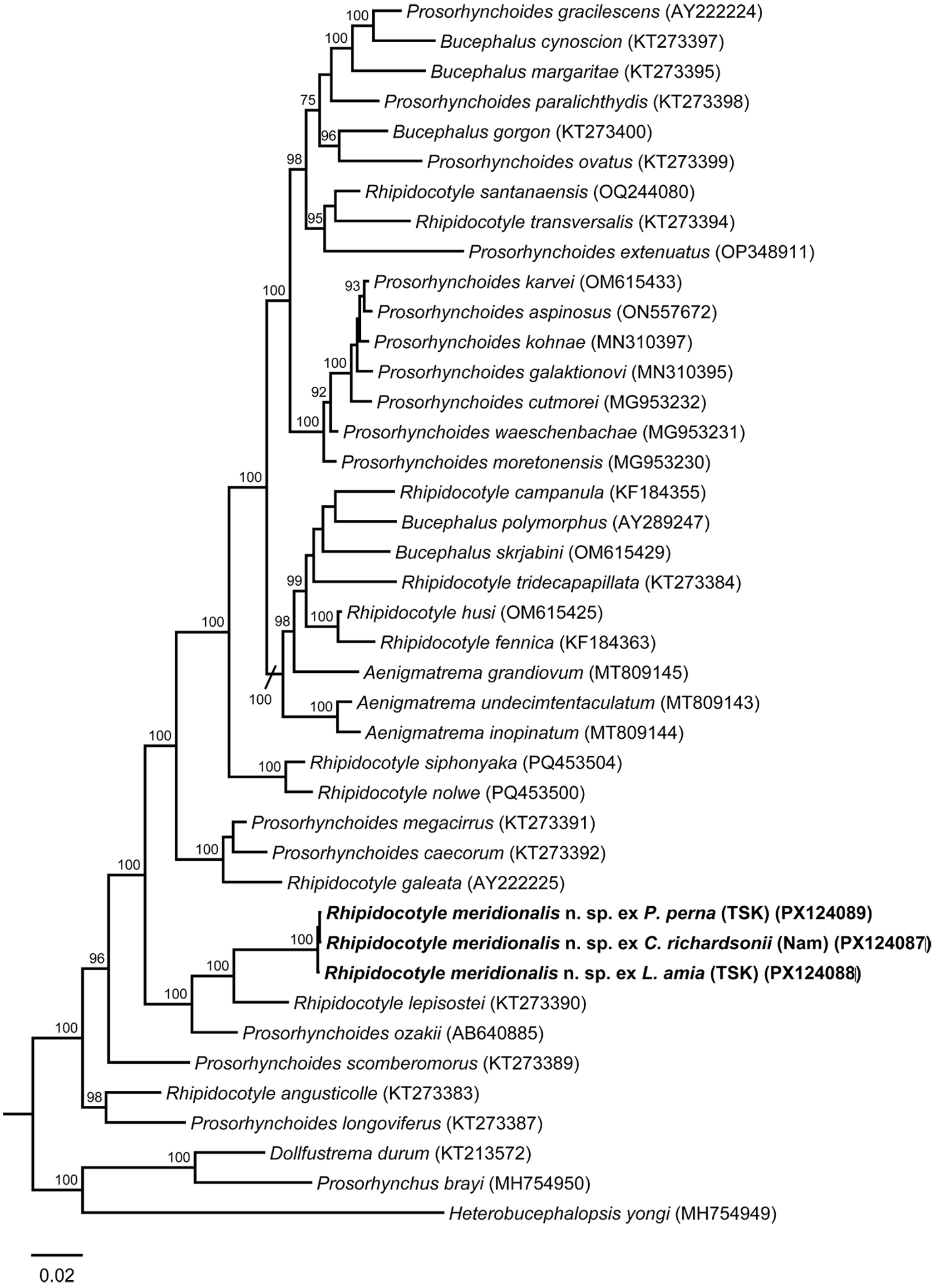

**Figure 2.** Phylogenetic relationships between species of the Bucephalinae inferred with Bayesian Inference analysis of the partial 28S rDNA region from a 1219-bp alignment. Numbers above nodes represent posterior probabilities (%); only values >75% are indicated. In bold: sequences produced in this study. Nam, Namibia; TSK, Tsitsikamma.

ysed, although it has been submitted to GenBank (PX123866). All attempts to generate ITS2 rDNA sequences failed due to extensive repeat codons. Molecular phylogenetic analyses hence focused on the partial 28S rDNA dataset. Alignment of the 28S dataset generated 1219 characters for analyses. Both BI (Figure 2) and ML (Figure 3) analyses of this dataset produced trees with similar topologies: only the placements of *Bucephalus polymorphus* von Baer, 1827, *Bucephalus skrjabini* Akhmerov, 1963, *Prosorhynchoides paralichthydis* (Corkum, 1961) Curran & Overstreet, 2009 and *Rhipidocotyle tridecapapillata* Curran & Overstreet, 2009 differed (Figures 2 and 3). All the bucephaline genera included in the analyses (except those of the outgroup sequences) appear poly- and/or paraphyletic. In both analyses, one of the two identical sequences from the *P. perna* sporocyst infection, the sequence of a metacercaria recovered from the fin of *C. richardsonii* (Namibia), and that of an adult individual from *L. amia* formed a highly supported clade to the exclusion of all other bucephalid species. This clade was sister to *Rhipidocotyle lepisostei*

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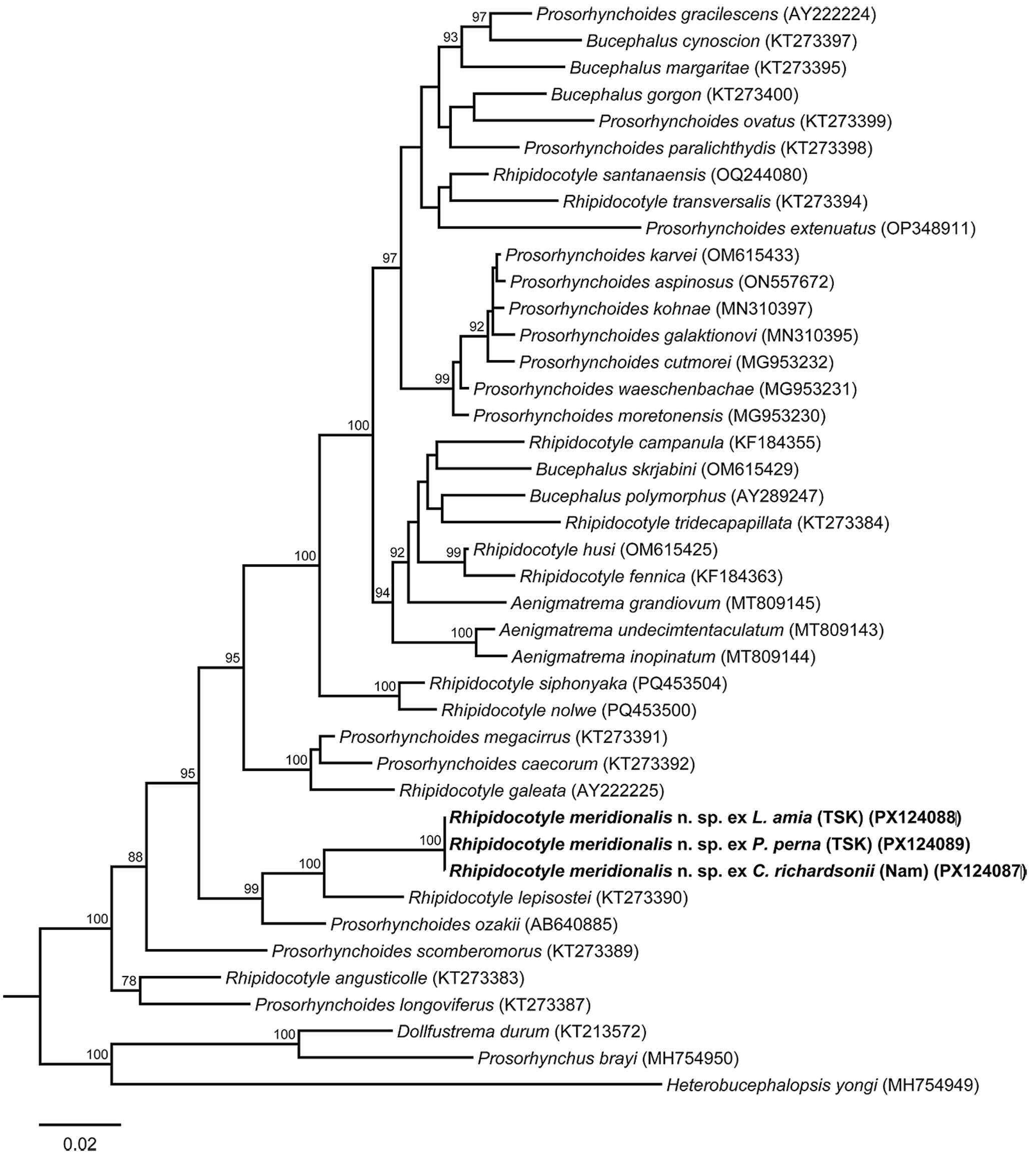

**Figure 3.** Phylogenetic relationships between species of the Bucephalinae inferred with maximum likelihood analysis of the partial 28S rDNA region from a 1219-bp alignment. Numbers above nodes represent bootstrap support values (%); only values > 75% are indicated. In bold: sequences produced in this study. Nam, Namibia; TSK, Tsitsikamma.

Hopkins, 1954 (Figures 2 and 3), a species that infects mullets (Mugilidae) as metacercariae and species of gar (Lepisosteidae) as adults (Wardle, 1990a).

## *Morphological results*

The partial 28S rDNA region (and indeed all other rDNA regions conventionally used for molecular phylogenetics) has been repeatedly shown as uninformative for generic-level phylogenetic inference in the Bucephalidae (e.g. Corner et al., 2020). The use of the key by Overstreet and Curran (2002) on adult specimens, however, indicated that the taxon recovered from *L. amia* is a member of the genus *Rhipidocotyle*, with consistent observable morphological differences between the adult and those of all other bucephalid species.

Morphological diagnosability of the adult specimens, in combination with the lack of a genetic match to any publicly available sequence data in the molecular sequence analyses, supports the proposition of a new species.

## *Taxonomic summary*

Bucephalidae Poche, 1907

***Rhipidocotyle* Diesing, 1858**

**Type species: *Rhipidocotyle galeata* (Rudolphi, 1819) Eckmann, 1932, by subsequent designation**

***Rhipidocotyle meridionalis* n. sp.** (Figures 4 and 5)

**Type and adult host**: *Lichia amia* (L.), leerfish (Carangiformes: Carangidae).

**First-intermediate host**: *Perna perna* (L.), brown mussel (Bivalvia: Mytilidae).

**Second-intermediate hosts**: *Amblyrhynchote honckenii* (Bloch), evileye puffer (Tetraodontiformes: Tetraodontidae); *Chelon dumerili* (Steindachner), grooved mullet (Mugiliformes:

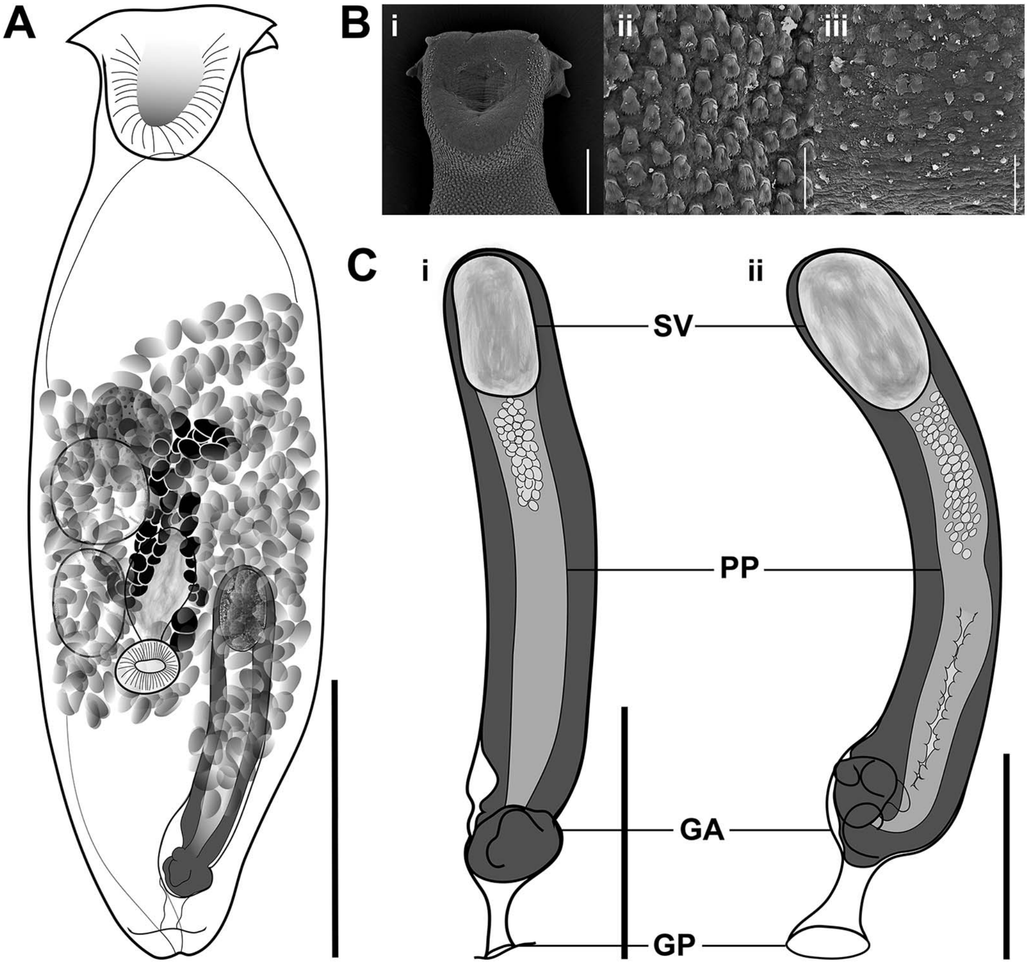

**Figure 4.** (A) Adult of *Rhipidocotyle meridionalis* n. sp. *ex Lichia amia*, holotype (NMB P1179) whole-mount, ventral view. Scale-bar 100 μm. (B) Scanning electron micrographs depicting (i) rhynchus, ventral view; (ii) tegumental spines at mid-body level, anterior to oral opening; and (iii) tegumental spines in area immediately anterior to genital pore, showing increasing sparseness. Scale-bars: (i) 50 μm; (ii) and (iii) 10 μm. (C) Cirrus-sac and male terminal genitalia of (i) holotype (NMB P1179) showing dorso-ventral view and (ii) paratype (NMB P1180) whole-mount showing lateral view. Scale-bars 100 μm. GA, genital atrium containing genital lobe; GP, genital pore; PP, pars prostatica; SV, seminal vesicle.

Mugilidae); *Chelon richardsonii* (Smith), South African mullet (Mugilidae); *Chrysoblephus laticeps* (Valenciennes), red roman (Eupercaria *i. s.*: Sparidae); *Dichistius capensis* (Cuvier), galjoen (Centrarchiformes: Dichistiidae); *Diplodus capensis* (Smith), Cape white seabream (Sparidae); *Rhabdosargus holubi* (Steindachner), Cape stumpnose (Sparidae); *Sarpa salpa* (L.), Salema porgy (Sparidae); *Sparodon durbanensis* (Castelnau), white musselcracker (Sparidae).

**Infection sites**: Adult: intestine. First-intermediate stage (sporocysts): area adjacent to the gonads and the tissue at the base of gill filaments. Second-intermediate stage (metacercariae): most commonly in heart- and fin membrane tissues, but also in fin bases, eyes, intestinal wall, kidney, spleen and muscle tissue.

**Type locality**: Groot River estuary, Tsitsikamma section, Garden Route National Park (33°58′43′′S, 23°33′56′′E).

**Other localities**: Vier-kant-klip fishing area, Swakopmund (22°42′35′′S, 14°31′23′′E) and near Bird Island fishing area, Walvis Bay (22°52′35′′S, 14°32′22′′E), Namibia; Uvongo Beach, KwaZulu Natal, South Africa (30°49′59.8′′S, 30°23′53.3′′E); The Breede River estuary, Witsand, Western Cape, South Africa (34°23′50′′S; 20°50′14′′E); Mossel Bay, Western Cape, South Africa (34°10′46′′S; 22°9′7′′E); De Hoop Nature Reserve (Koppie Alleen), Western Cape, South Africa (34°28′42.1′′S, 20°30′39.9′′E); Chintsa East, Eastern Cape, South Africa (32°50′11′′S; 28°7′1′′ E).

**Prevalence and intensity**: Adult: One of one fish infected with 10 worms. First-intermediate stage: *P. perna* – one of 44 mussels (2.27%) infected. Second-intermediate stage: N/A.

**Material**: Adult: one holotype (NMB P1179) and seven paratypes (NMB P1180–P1186), permanently whole-mounted. First-intermediate stage: one serial, sectioned sporocyst voucher in four slides (NMB P1189) and two cercarial vouchers (NMB P1190–P1191), permanently mounted. Second-intermediate stage: two vouchers (NMB P1187–P1188), permanently whole-mounted.

**Representative DNA sequences**: Adult: One replicate of partial 28S rDNA (PX124088) and *cox*1 mtDNA (PX123866) generated from one whole worm. First-intermediate stage: two identical replicates of partial 28S rDNA generated from one sporocyst infection, one replicate submitted to GenBank (PX124089). Second-intermediate stage (metacercariae): *Ex A. honckenii* – five identical replicates of partial 28S rDNA generated from five individuals *ex* eye, heart and intestine from Chintsa, De Hoop, Uvongo and

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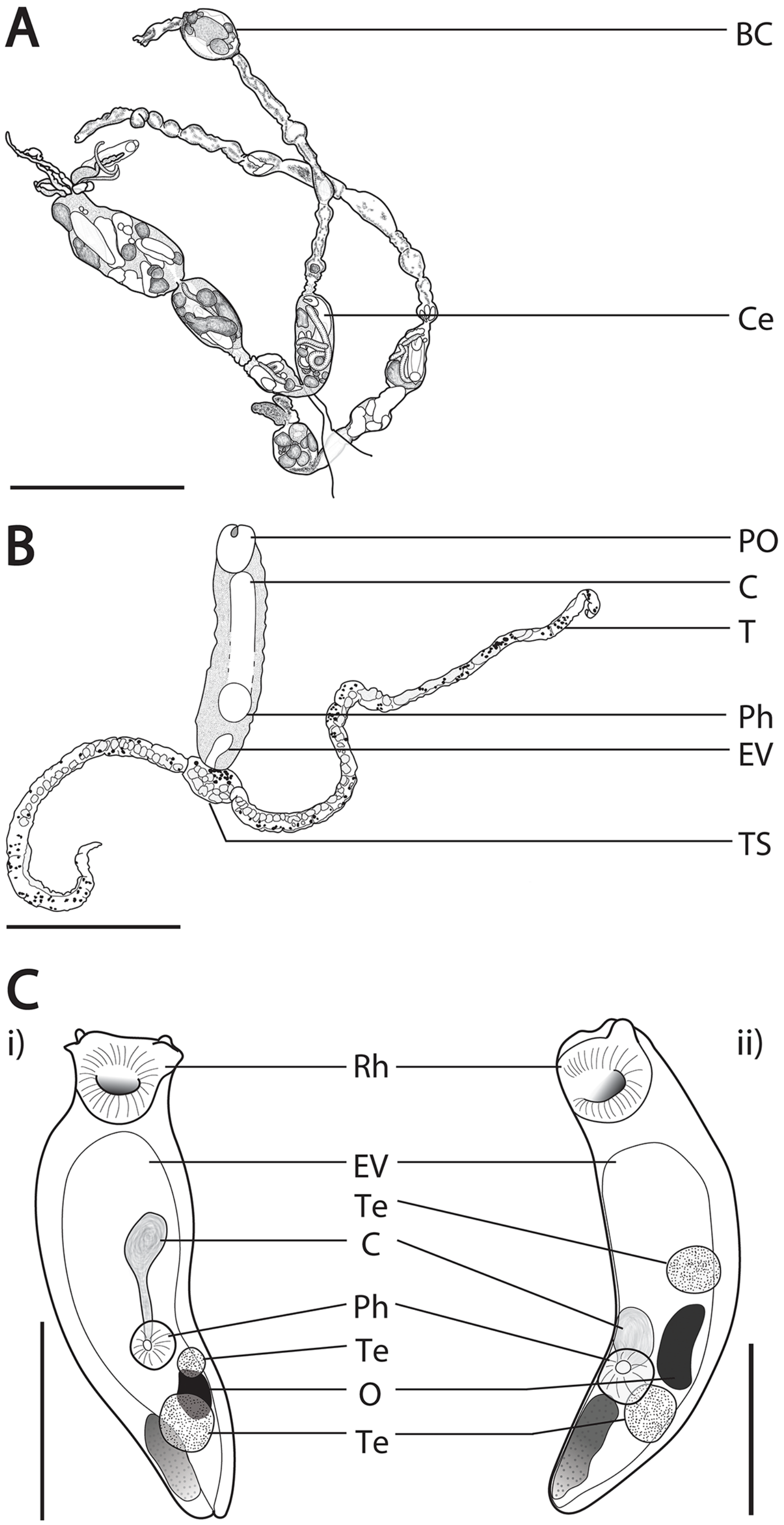

**Figure 5.** Intermediate stages of *Rhipidocotyle meridionalis* n. sp. (A) One section of the sporocyst from *Perna perna* (NMB P1189). (B) Cercaria from *Perna perna* (NMB P1190). (C) Metacercariae ex (i) the heart of a *Dichistius capensis* (NMB P1188), whole mount; (ii) the kidney of *Chelon richardsonii* (NMB P1187) whole-mount. Scales: 150 μm. BC, brood chamber; C, caecum; Ce, cercaria; EV, excretory vesicle; O, ovary; Ph, pharynx; PO, penetrative organ; Rh, rhynchus; T, tail; Te, testis; TS, tail stem.

Witsand, respectively, not submitted to GenBank; *ex C. dumerili* – one replicate of partial 28S rDNA generated from one individual *ex* heart from Tsitsikamma, not submitted to GenBank; *ex C. richardsonii* – four identical replicates of partial 28S rDNA generated from four individuals *ex* fin, heart, kidney and spleen from Namibia, one (from the fin) submitted to GenBank (PX124087); *ex C. laticeps* – one replicate of partial 28S rDNA generated from one individual *ex* heart from Tsitsikamma, not submitted to GenBank; *ex Dic. capensis* – one replicate of partial 28S rDNA generated from one individual *ex* heart from Namibia, not submitted to GenBank;

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*ex Dip. capensis* – three replicates generated from one individual *ex* heart from Namibia, one individual *ex* heart from Mossel Bay and one individual *ex* heart from Tsitsikamma, not submitted to GenBank; *ex R. holubi* – one replicate of partial 28S rDNA generated from one individual *ex* heart from Witsand, not submitted to GenBank; *ex S. salpa* – one replicate of partial 28S rDNA generated from single individual *ex* head muscle from Tsitsikamma, not submitted to GenBank; *ex S. durbanensis* – one replicate of partial 28S rDNA generated from one individual *ex* heart from Tsitsikamma, not submitted to GenBank.

**ZooBank registration**: The species *R. meridionalis* is registered in ZooBank under the code 2FBB65B6-49DA-4082-A041-C2C7A21C86A1.

**Etymology**: The species name 'meridionalis' reflects the fact that *R. meridionalis* is widespread along the southern African coast, having been recorded from seven localities off Namibia and South Africa, and infects *L. amia* south of the equator.

*Description* (Figures 4 and 5).

**Adult** (Figure 4) [based on 8 whole-mounted, unflattened specimens]: Body fusiform, 781–1087 × 214–255 (913 × 239), 3.1–4.3 times longer than broad. Tegumental spines flattened, palmate with serrated edges, arranged in diagonal transverse, tessellate rows, absent from ventral 'face' of rhynchus, dense for most of body length, becoming gradually sparser approaching posterior extremity, absent around genital pore, becoming dense again around posterior terminus/excretory pore, 5 long. Rhynchus with prominent ventral aperture and ventral lip, 115–158 × 103–162 (143 × 140). Rhynchal hood ornamentation comprising paired dorso-lateral lobes bearing 2 papillae each and one ventro-lateral papilla on either corner of rhynchal antero-ventral extremity. Oral opening ventro-medial in hindbody, immediately meeting pharynx. Pharynx globular to subspherical, 38–51 × 43–62 (43 × 53), length:breadth ratio 0.7–0.9 (0.8), 476–729 (594) or 60.0–68.1% (64.9%) of total body length from anterior extremity, 218–311 (270) or 27.9–34.3% (29.7%) of total body length from posterior extremity. Oesophagus 34–90 (60) long, passes straight anteriorly, expanding to form single blind-ended caecum. Caecum simple, saccular, greatly varying in size depending on volume of contents, 68–111 (92) long, 7.3–12.3% (10.3%) of total body length.

Testes 2, of similar size, spherical to oblong, with margins unlobed, arranged in tandem, contiguous or slightly separated, lateral and somewhat anterior to oropharyngeal/caecal complex, 68–132 × 63–83 (96 × 74). Anterior testis 283–503 (391) from anterior body extremity, creating pretesticular space 35.7–54.2% (42.7%) of total body length; posterior testis 242–418 (298) from posterior body extremity, creating post-testicular space 29.1–38.5% (32.5%) of total body length. Cirrus-sac sinistral, elongate, moderately thick-walled, contains seminal vesicle and pars prostatica, 270–356 × 44–53 (314 × 49). Seminal vesicle ovoid to oblong, occupying proximal portion of cirrus-sac, with size varying according to sperm content, 63–94 × 33–53 (78 × 46). Pars prostatica slightly bent, highly glandularized, leading from seminal vesicle to genital lobe, 167–252 × 14–33 (208 × 25). Genital lobe contained in genital atrium; genital atrium leading to common genital pore.

Ovary spherical to subspherical, with margins unlobed, mostly pretesticular, overlapping anterior testis, 63–92 × 50–96 (81 × 75); length:breadth ratio 0.9–1.4 (1.1). Oviduct and egg-forming complex not seen in any specimens. Uterus extensive in midbody; uterine coils overlapping testes, ovary, caecum and most of cirrus-sac, extending anterior to ovary and descending to meet genital atrium. Eggs ovoid, light golden-yellow in colour, 23–27 × 14–16 (25 × 15) ($n = 24$). Vitellarium comprising single looped chain of follicles in poorly-defined, vaguely oblong bundles, medial in midbody, sinistral to testes and ovary, overlapping and anterior to oropharyngeal/caecal complex, 294–494 (405) from anterior extremity, 248–353 (304) from posterior extremity, occupying 13.8–29.1% (22.5%) of total body length. Excretory vesicle large, simple and unbranching, ovoid, extending anteriorly as far as rhynchus, 654–951 (767) long or 74.2–88.1% (83.9%) of total body length.

**First-intermediate stages** (Figure 5A, 5B) [based on 3 serial mounts of one branched sporocyst specimen, 12 whole-mounted cercariae and 17 temporarily mounted cercariae]:

Sporocysts (Figure 5A) arranged in branching orange chains forming dense vegetative network in host tissue; slender branched tubules interspersed with 'bead'-like swollen brood chambers. Brood chambers spherical to subspherical, cylindrical or oblong, increasing in size with volume of content, containing cercariae and germ balls, 133–1668 × 107–200 (793 × 164). Emerged cercariae not obtained. Un-emerged cercariae dissected from preserved sporocysts.

Cercariae (Figure 5B) furcocercous. Cercarial body sausage-shaped, 203–288 × 39–82 (243 × 58). Cephalic organ located on cercarial body opposite tail attachment, short- to elongate-cylindrical with rounded ends, bearing depression at its apex, 27–64 × 19–41 (48 × 28); glandular cells not observed. Mouth and pharynx inconspicuous; pharynx round, 16–22 (19) in diameter, located 28–56 (47) from posterior extremity of cercarial body and 142–181 (165) from anterior extremity. Caecum saccular, empty in all specimens where observed, 61–73 × 26–46 (70 × 36). Reproductive organs and genital pore not observed. Excretory vesicle an empty sac opening into tail stem. Tail stem bluntly oval or bilobed, filled with large, finely granular vesicles concentrated in its posterior part, with small granules concentrated in its anterior part, 22–69 × 21–45 (49 × 32). Furcae 2, starting from opposite sides of tail stem, extensible, blunt-ended, each filled with one row of large granular vesicles and small randomly distributed granules, 165–580 × 13–49 (299 × 21).

**Second-intermediate stages** (Figure 5C) [based on 16 whole-mounted excysted metacercariae]: Metacercarial cysts thin-walled, primarily embedded in fin- and tail ray membranes and in heart tissue, less commonly in kidney, spleen, and in flesh at bases of fins. Cysts contained 1–25 metacercariae, sometimes of varying maturity. Metacercariae elongate cylindrical with pointed posterior extremity, bearing minute spines, 225–526 × 49–153 (348 × 103). Rhynchus large in proportion to body, 37–88 × 30–77 (67 × 57). Pharynx in middle of or in posterior half of body, round to oval, 95–352 (206) from anterior extremity and 50–170 (117) from posterior extremity, 16–36 × 15–35 (28 × 30). Excretory vesicle occupying most of body, reaching to 40–100 (79) from anterior extremity.

## Discussion

### *Differential diagnosis*

Currently, bucephalid genera are primarily differentiated using morphological criteria, mainly on features of the rhynchus. This practice, in light of evidence provided by molecular sequence data, is not particularly useful; however, given the continuing failure of molecular sequences (including those of the 28S rDNA region used here and in recent studies; Corner et al., 2020) to resolve intergeneric relationships among bucephalids in a morphologically sensible manner, the *status quo* is to continue to emphasize morphology (and, particularly, rhynchal structure) in defining bucephalid genera. The bucephalid genus *Rhipidocotyle* is morphologically defined by having a rhynchus that is a simple sucker partially covered by either a simple muscular hood or one with three to five fleshy lobes. In addition, species of *Rhipidocotyle* have pretesticular ovaries and are said to have a curved or slightly bent (i.e. not straight) pars prostatica (Overstreet and Curran, 2002). In all these respects, *R. meridionalis* n. sp. obeys the morphological concept of this genus.

Sixty-seven species (including the new species presented here) are currently recognized in the genus *Rhipidocotyle* (WoRMS, 2024b). Of these, the four species described and known only from immature or progenetic forms (*R. eggletoni* Velasquez, 1959; *R. heptathelata* Stunkard, 1974; *R. johnstonei* Pulsford & Matthews, 1984; and *R. lingualis* Komiya & Tajimi, 1941) are disregarded. A further eight species found exclusively in freshwater are also discounted: *R. gibsoni* Kohn & Fernandes, 1994; *R. husi* Atopkin, Shedko, Rozhkovan, Nguyen & Besprozvannykh, 2022; *R. jeffersoni* (Kohn, 1970), *R. kovalae* Ivanov, 1970; *R. pseudobagri* Wang, 1985; *R. santanaensis* Lunaschi, 2004; *R. tridecapapillata* Curran & Overstreet, 2009; and *R. vachius* Singh & Sinha, 1976. In possessing papillate lobes on either side of its rhynchal hood (as opposed to a simple, unornamented hood), *R. meridionalis* n. sp. can be distinguished from a further 33 species of *Rhipidocotyle*. Of the remaining 21 species, the absence of paired robust, medial lobes (called 'papillae' by Derbel et al. (2011)) on the ventral margin of the rhynchal hood distinguishes the new species from *R. angusticollis* Chandler, 1941; *R. apapillosa* Chauhan, 1943; *R. coiliae* Wang, 1980; *R. galeata* (Rudolphi, 1819); *R. indica* Gupta & Ahmad, 1976 (emend.); *R. khalili* Nagaty, 1937 and *R. theraponi* Gupta & Tandon, 1985. A rhynchal hood configuration of paired dorso-lateral lobes bearing two papillae each and one ventro-lateral papilla on either corner of rhynchal antero-ventral extremity distinguishes *R. meridionalis* n. sp. from *R. anguillae* Wang, 1985; *R. coronata* Tang & Tang, 1976; *R. gazzae* (Shen, 1990); *R. genovi* Dimitrov, Kostadinova & Gibson, 1996; *R. laruei* Velasquez, 1959; *R. microovata* Zhukov, 1977; *R. nicolli* Bartoli, Bray & Gibson, 2006; *R. pentagonum* Eckmann, 1932; *R. pseudorhombi* Nahhas, Sey & Nakahara, 2006; and *R. viperae* (van Beneden, 1870), all of which have two lateral papillae and a pronounced medial, antero-ventral papilla; from *R. longleyi* Manter, 1934 and *R. septpapillata* Krull, 1934, which have seven lobes or papillae spaced evenly across the rhynchal hood; from *R. minima* (Wagener, 1852), which has seven papillae armed with robust spines; from *R. nicolli* Bartoli, Bray & Gibson, 2006, which has three prominent papillae (described as 'dorsal ridges') medial on a fan-shaped hood; from *R. papillosa*, which has 15 papillae arranged apparently evenly along the hood margin; and from *R. sphyraenae* Yamaguti, 1959 which has seven evenly spaced prominences with minute paired papillae around the hood margin. The rhynchi of *R. paruchini* Gavrilyuk-Tkachuk, 1979 and *R. tonimahnkei* Reimer, 1985 are not well described; that of *R. paruchini* is described as 'with a hood, which in contracted specimens has the appearance of a crown', whereas that of *R. tonimahnkei* is not described beyond its size dimensions, though it is compared to those of *R. galeata* (misspelled '*galeara*' by Reimer (1985)) and *R. minima*, and the accompanying illustration indicates the presence of at least 4 papillae or lobes (Gavrilyuk-Tkachuk, 1979; Reimer, 1985). *Rhipidocotyle paruchini* is distinctly larger than *R. meridionalis* n. sp. (1980–2740 μm long vs 781–1087 μm for the latter) and has a longer cirrus-sac, described as 'reaching almost half the body length', whereas that of *R. meridionalis* n. sp. averages just over a third of total body length, maximum 39.6%. The eggs are also larger and longer (32 × 12 μm); those of *R. meridionalis* n. sp. are 23–27 × 14–16 μm (ave. 25 × 15 μm). Finally, the host of *R. paruchini* is a sciaenid, *Otolithes ruber* (Bloch & Schneider, 1801) [as *Otolithes argenteus* (Cuvier, 1830) in Gavrilyuk-Tkachuk (1979)], whereas that of *R. meridionalis* n. sp. is a carangid. *Rhipidocotyle tonimahnkei* is even larger than *R. paruchini* (2200–2580 μm) and is distinctly thin-bodied [317–380 μm maximum breadth, thus at least six times longer than broad, whereas *R. meridionalis* n. sp. is 3.1–4.3 (average 3.9) times longer than broad] and has an oral opening anterior to both the testes and ovary (in *R. meridionalis* n. sp., the oral opening is lateral or posterior to the testes and ovary) and larger eggs (31–38 × 14–18 μm *vs* 23–27 × 14–16 μm in *R. meridionalis* n. sp.).

Derbel et al. (2011) describe an ejaculatory duct in their redescription of the type species of *Rhipidocotyle, R. galeata*. It is not clear what defines the ejaculatory duct in this instance, as it is completely contiguous with the duct of the pars prostatica. Some recent authors, e.g. Curran and Overstreet (2009), define the ejaculatory duct as the portion of the male duct extending beyond the pars prostatica, i.e. in the genital lobe. This distinction, however, does not seem useful: little to no differentiation in the duct before and after it meets the genital lobe and enters the genital atrium has been noted for most descriptions, including in those which draw such a distinction. Such a duct is also not always evident, e.g. in the present specimens. Other recent authors of bucephalid descriptions (Cutmore et al., 2018; Corner et al., 2020; Malsawmtluangi and Lalramliana, 2023) either do not describe an ejaculatory duct or do not distinguish it from the distal section of the prostatic duct where it meets the genital lobe. We prefer to take the latter stance and regard the ejaculatory duct as synonymous with the pars prostatica.

## Life-cycle elucidation within *Rhipidocotyle* and host use by *R. meridionalis n. sp.*

In the marine environment, complete bucephalid life-cycles have been reported by various authors. Most often, these reports were surmised from co-occurrences of first-stage infections and potential fish hosts in the same areas (Chubrik, 1952), morphological comparisons between metacercariae and adults (Matthews, 1974) or incomplete infection experiments (Matthews, 1973a, b; Stunkard, 1976). Most of these reports also pre-date the ability to test identifications with molecular sequencing. The few comprehensive infection experiments available (e.g. Gargouri-Ben Abdallah and Maamouri, 2002) did not rule out the possibility of morphologically similar, sympatric congeners due to the absence of molecular data. Only a handful of bucephalid life-cycles have been fully elucidated using a combination of molecular data on all life-stages and a thorough morphological examination of the adult worm. Pina et al. (2009) used ITS1 rDNA sequencing to identify all stages of the life-cycle of *Bucephalus minimus* (Stossich, 1887) off Portugal, which involved first-intermediate stages in the cockle *Cerastoderma edule* (L.) (Gastropoda: Cardiidae), metacercariae in the sea mullet *Mugil cephalus* L. (Mugilidae) and adults in sea bass *Dicentrarchus labrax* (L.) (Moronidae). Muñoz and Bott (2011) and Muñoz et al. (2015) elucidated the life-cycle of *Prosorhynchoides carvajali* Muñoz & Bott, 2011 from Chile using a combination of experimental infections and identifying natural infections, with first-intermediate stages being found in two mytilid bivalve species, metacercariae in five benthic intertidal fish species and adults in two piscivorous species of *Auchenionchus* Gill (Labrisomidae). These studies present the only uncontroversially elucidated life histories for marine bucephalids, which makes that of *R. meridionalis* the first reliably elucidated life-cycle for a *Rhipidocotyle* species. The use of molecular verification of bucephalid life-stages to fully elucidate life-cycles is scarcely more common in freshwater. Hayashi et al. (2022) and Saito et al. (2025) used ribosomal and mitochondrial DNA sequencing to verify the identity of intermediate and definitive stages of, respectively, *Prosorhynchoides ozakii* (Nagaty, 1937) and a species of *Dollfustrema* Eckmann, 1934 in the Tone River system of Japan; both are invasive species introduced to Japan, possibly from China. The latter species was described as *Dollfustrema invadens* Saito, Iwata, Nitta & Waki, 2025 in Saito et al. (2025), a redescription of an inappropriately proposed taxon from China, '*Dollfustrema hefeiense* Liu in Zhang, Qiu & Ding, 1999'. Both *P. ozakii* and *D. invadens* use the freshwater mytilid *Limnoperna fortunei* (Dunker) as first-intermediate host and a wide range of fish species as second-intermediate hosts (13 recorded for *P. ozakii*, 10 for *D. invadens*). The introduced channel catfish *Ictalurus punctatus* (Rafinesque) is the definitive host for both species. Interestingly, ovigerous adults of *D. invadens* were found encysted in the gills and fins of putative second-intermediate hosts, indicating progenesis is possible for this species. No complete life-cycles have yet been demonstrated using molecular verification for any freshwater species of *Rhipidocotyle*.

*Perna perna* has been reported as a host for bucephalid first-stage infections in Brazil, where it is an aquacultural pest (Pereira Jr et al., 1996; da Silva et al., 2012), and in South Africa (Calvo-Ugarteburu and McQuaid, 1998a, b; Lasiak, 1993). No identification was attempted on South African specimens [Lasiak (1993) first reported his sporocyst and cercarial infection as of a *Bucephalus* species (p. 1) but later justified that the taxon should rather be considered as a bucephalid (p. 2)]. In contrast, infections from Brazil were identified as either *Bucephalus* von Baer, 1827 or *Prosorhynchoides* Dollfus, 1929. However, these identifications relied on sporocyst- and cercarial morphology alone (da Silva et al., 2002), on the assumption of the identity of the infections from previous records without the use of molecular sequence data (Carneiro-Schaefer et al., 2017) or on identifications using phylogenetic placement alone (Gleyce Lima de Oliveira et al., 2022). Notwithstanding that the presence of species of a trematode genus in a defined area does not imply the absence of other confamilials, these approaches are problematic. First, bucephalid sporocysts possess the same morphology in all genera (see Stunkard (1976)) and cercariae do not exhibit enough of the morphological characters necessary for generic-level identification: "So far, there is no way to tell which genus of the Bucephalidae a given cercaria belongs to, until the life-cycle is worked out by means of experimental infections" (Hopkins, 1954) (see also the comment by Lasiak (1993)). Second, most early reports of '*Bucephalus*' referred to first-intermediate stages of any bucephalid and not specifically to a member of the currently accepted genus *Bucephalus*, whereas all adult bucephalids were placed in *Gasterostomum* von Siebold, 1848 or *Prosorhynchus* Odhner, 1905 at the time (see Stunkard (1976) and Lasiak (1993)). *Bucephalus margaritae*, described as a first-stage infection only and never matched to an adult (Ozaki and Ishibashi, 1934), is a prime example of this use. Studies on *P. perna* infections using first-stage morphology alone and/or relying on a confusing generic name are many (Umiji et al., 1976; Magalhães, 1998; Lima et al., 2001; Loureiro et al., 2001; da Silva et al., 2002; Galvão et al., 2006; Garcia and Magalhães, 2008; Carneiro-Schaefer et al., 2017) and should be regarded as questionable. For the same reason, the life-cycle of *B. margaritae* from *P. perna* proposed by da Costa Marchiori et al. (2010) is dubious, even not accounting for it involving a mytilid from Brazil instead of an ostreid from Japan. Third, the extensive polyphyly of *Bucephalus, Prosorhynchoides* and *Rhipidocotyle*, which comprise most known bucephalid species (Corner et al., 2020; this study), makes the identification of first-stage infections impossible *via* molecular phylogenetic placement alone. The report of a *Prosorhynchoides* sp. in *P. perna* by Gleyce Lima de Oliveira et al. (2022) is thus also questionable. For all these reasons, doubt must be cast on the identities of all the *P. perna* bucephalid infections characterised using these three approaches. Those infections should instead be considered as species of the Bucephalidae. To the best of our knowledge, *R. meridionalis* is therefore the first reliably identified bucephalid species infecting mussel species of the genus *Perna* Philipsson.

Three species of carangids are recorded as hosts for bucephalids in southern Africa. Parukhin (1976) reported *Prosorhynchus chorinemi* Yamaguti, 1952 in *Scomberoides lysan* (Forsskål) from coastal Mozambique (location given as 'Sofala Bank'); Bray (1984) reported *B. margaritae* in *Caranx heberi* (Bennett) and *Atropus hedlandensis* (Whitley) (as *Carangoides hedlandensis*) from the east coast of South Africa. *Rhipidocotyle meridionalis* n. sp. is the second species of *Rhipidocotyle* known from *L. amia* after *R. galeata*, reported from the Mediterranean off Italy (Stossich, 1887). It constitutes the first species of *Rhipidocotyle*, and bucephalid, in general, identified to species from the southern coast of South Africa; the report of *Rhipidocotyle* sp. from *R. pretiosus* by Nunkoo et al. (2017) remains unverified beyond generic level.

## Distribution of the hosts of *R. meridionalis* n. sp.

The life-cycle of *R. meridionalis* involves first-intermediate and definitive hosts from southern South Africa and second-intermediate hosts from both that area and the coast of central Namibia. Consequently, the life-cycle of *R. meridionalis* n. sp. completes at least in waters off the Tsitsikamma-Garden Route National Park. *Lichia amia* is distributed across the coastline of southern Africa (Froese and Pauly, 2024) from Sodwana Bay (Indian Ocean coast) to Saldanha Bay (Atlantic coast) (Dunlop et al., 2015) and from northern Namibia to Angola (Henriques et al., 2012). The distribution of *P. perna*, from Cape Town to Mozambique (Bownes and McQuaid, 2006; Zardi et al., 2007) and north of Lüderitz, Namibia (Van Erkom Schurink, 1990; Zardi et al., 2007), partly overlaps that of *L. amia*. As the many known intermediate hosts are also widely distributed, it is thus likely that *R. meridionalis* n. sp. is widely distributed off southern Africa and that the life-cycle of this species can probably complete in other locations. This does not preclude the potential existence of discrete populations of this parasite. The population structure of *R. meridionalis* n. sp. may be inferred from population-genetics data of the hosts.

On South Africa's western coast, the cold Benguela Current flows northwards to the Angola-Benguela Frontal Zone (ABFZ), where it turns into the Equatorial Current (Siegfried et al., 2019). The Benguela Upwelling System (BUS) is divided between northern and southern areas by the Lüderitz Upwelling Cell (LUC) (Bakun, 1996) (Figure 1). On South Africa's eastern coast, the warm Agulhas Current flows south from the Mozambique Channel mesoscale eddies and south-east Madagascar dipole eddies

https://doi.org/10.1017/S0031182025100681

(Vousden, 2016); the westward-flowing North-East Madagascar Current (NEMC) and the resulting northward-flowing East-African Coastal Current (Halo and Raj, 2020) and Comoros eddies (Lett et al., 2024) turn the areas between South Africa-Mozambique and Tanzania-Somalia into separate marine ecosystems (Halo and Raj, 2020) (Figure 1). Consequently, southern Africa is relatively isolated by two hydrological barriers (i.e. the ABFZ-LUC and the NEMC-Comoros eddies) on both its western and eastern coasts. Henriques et al. (2012) showed that gene flow is significantly restricted between Angolan and South African populations of *L. amia* on either side of the BUS. Studies on other coastal fishes have showed similar trends, with sharp population divergences across the LUC and/or the ABFZ (Henriques et al., 2014, 2016a; Reid et al., 2016; Shoopala et al., 2021; Kapula et al., 2022; Forde et al., 2023), although some fishes seem unaffected by these barriers (Henriques et al., 2016b; Schulze et al., 2020; Forde et al., 2023).

Similarly, *P. perna* shows genetic structuring and a division into two sympatric populations, the southern coast + western coast + Namibian population and the eastern coast + Mozambique population, i.e. between Namibian, South African warm-temperate and southeastern tropical-temperate regions (Zardi et al., 2007, 2015; McQuaid et al., 2015). It is therefore possible that the spatial distribution of *R. meridionalis* n. sp. might be conditioned by the population structuring in both its definitive and first-intermediate hosts. Oceanographic barriers, however, do not always have an impact on host mobility. The cases of the copepod *Lepeophtheirus lichiae* Barnard, 1948 (Copepoda: Caligidae), infecting *L. amia* from both the eastern South African coast and the Mediterranean (Sakarya et al., 2019), and of *Rhipidocotyle khalili*, infecting various hosts from off Mozambique and India, the Red Sea and the western Pacific (Nagaty, 1937; Yamaguti, 1953; Madhavi, 1974; Reimer, 1985; Ndiaye et al., 2018), seem like prime examples of trans-barrier movements. That said, neither example has been tested with molecular sequencing methods.

Our ability to infer biogeographic trends for *R. meridionalis* n. sp. is currently limited, as our records of adults and first-intermediate stages are from only one locality (in the case of adults, from a single fish), with records from all other localities comprising those of metacercariae. Even accounting for the fact that not all of the fishes sampled represent likely outlets for transmission (it is unlikely, for example, that *L. amia* feed on *A. honckenii*, a toxic pufferfish), the broadcast nature of second-intermediate stage infections and varying dispersal habits of the fish intermediate hosts mean it is entirely possible that connectivity might be driven, not by the distribution of first-intermediate hosts nor the movements of the definitive hosts, but by those of the second-intermediate hosts. An expanded assessment of all hosts from across southern Africa, factoring in the movement of other highly vagile species, such as mugilids, is therefore desirable.

**Acknowledgements.** The authors are thankful to Prof. Richard Greenfield (University of Johannesburg) for collecting the mussels; to Dr Jessica Schwelm (University of Duisburg-Essen/Institute for Environmental Sciences, RPTU University of Kaiserslautern-Landau, Landau, Germany) for her help in dissecting them; to Dr Anja Erasmus (Water Research Group, Unit for Environmental Science & Management, North-West University (NWU-UESM-WRG)) for producing the maps in Figure 1; to Willem Landman (NWU-UESM) for assistance and guidance with processing and photographing specimens for SEM; and to members of the NWU WRG (UESM) for their assistance with field sample collection. This study is publication no. 979 from the WRG.

**Author contributions.** Conceptualization: C.L., N.J.S. and R.Q.-Y.Y. Investigation: A.V., C.L., L.d.K. and R.Q.-Y.Y. Formal analysis: C.L. and R.Q.-Y.Y. Writing – original draft: C.L. and R.Q.-Y.Y. Writing – review & editing: A.V., C.L., L.d.K., N.J.S. and R.Q.Y.Y. Visualization: C.L., A.J. and R.Q.-Y.Y. Supervision: N.J.S. and R.Q.-Y.Y. Project administration: N.J.S. Funding acquisition: N.J.S.

**Financial support.** This study is funded by the National Research Foundation of South Africa (grants no. MND200420515000 and PMDS23041191140 to A.V. and 132805 to L.d.K.). Opinions, findings and conclusions or recommendations expressed are those of the authors, and the funders accepts no liability whatsoever in this regard. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

**Competing interests.** The authors declare there are no conflicts of interest.

**Ethical standards.** Sampling in South Africa was conducted under permits no. CRC/2020-2021/005-2017/V1 (to Prof. Richard Greenfield, University of Johannesburg), MALH-K2016-005a and SMIT-NJ/2020-004 for Garden Route National Park; South African Department of Forestry, Fisheries and the Environment permit nos. RES2019-103, RES2021-49, RES2022-49, RES2023-26 and RES2024-70 for Uvongo Beach, Chintsa East, Mossel Bay and Witsand; and Cape Nature permit no. CN44-8718289 for De Hoop Nature Reserve. The permit for sample collection in Namibia was provided by the National Commission on Research, Science and Technology of Namibia (permit number RPIV010252022-1). Ethical approval for this study was provided by North-West University's AnimCare Ethics committee (NWU-00440-16-A5, NWU-00565-19-A5 and NWU-00759-22-A5).

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