

Article

Dactylostomum brayi n. sp. (Trematoda: Opecoelidae) from the Fish *Hemibagrus nemurus* (Bagridae) in Khun Thale Swamp, Thailand

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Abstract

During a parasitological survey of freshwater fishes from Khun Thale Swamp, Surat Thani Province, Thailand, a new species of opecoelid trematode, *Dactylostomum brayi* n. sp., was recovered from the intestine of the Asian redbtail catfish *Hemibagrus nemurus* (Bagridae). Of the 1065 fishes examined, representing 33 species, 27 genera, and 16 families, the parasite was found exclusively in *H. nemurus*, with a prevalence of 27.27% and a mean intensity of 2.33 worms per infected host, suggesting a high degree of host specificity. The new species differs from all recognised freshwater congeners in the number of ventral sucker papillae, body proportions relative to the oral sucker, and the morphology of the reproductive organs. This represents the second record of *Dactylostomum* from Thailand, following specimens identified as *Dactylostomum* sp. by R. A. Bray from the same host and deposited in the Natural History Museum, London, in 1983. The present study also provides the first molecular characterisation (18S, ITS2, and 28S rRNA) for the genus *Dactylostomum* and reveals a close phylogenetic relationship with *Dimerosaccus*. These findings contribute to the taxonomy and distribution of *Dactylostomum* and provide a basis for future systematic and phylogenetic studies of this poorly known genus.

Keywords: digenea; integrative taxonomy; freshwater parasite; host specificity; Asian redbtail catfish



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1. Introduction

Studying parasites is important for understanding their ecological and biological impacts on hosts and ecosystems, as parasites can influence various aspects of host biology. For this reason, basic research on parasite taxonomy is an essential first step towards understanding parasite–host relationships. The genus *Dactylostomum* Woolcock, 1935 (Opecoelidae), was originally established by Woolcock [1] with the type species *Dactylostomum gracile* Woolcock, 1935, from the marine fish *Myxus elongatus* Günther, 1861 (Mugilidae) in Australia. Subsequently, eleven additional species were described from marine fishes, the most recent being *Dactylostomum nicolli* Aken'Ova, Cribb & Bray, 2003, from Australia [2].

According to Bray [3], although *Dactylostomum* has traditionally been regarded as a marine genus, it was suggested that the genus may also include representatives from freshwater fishes. Bray [3] recognised the two nomina nuda *Dactylostomum jhansiensis*

Agarwal & Agarwal, 1988, and *Dactylostomum mastacembeli* Agarwal & Agarwal, 1981 (the latter, for which we found no evidence of its existence, appears to be an error in Bray [3]), as belonging to *Dactylostomum*. He also included the genera *Gangatrema* Agarwal & Kumar, 1981, currently regarded as a synonym of *Nicolla* Wisniewski, 1934 [4], and *Opedunculata* Dwivedi, 1975, synonymised with *Dactylostomum* by Cribb [4]. In addition, Bray [3] included *Coitocaecum parvum* Crowcroft, 1945, which is currently retained as a valid species of *Coitocaecum* [4].

Following the review of Bray [3], the following three additional species were described from the spiny eel *Mastacembelus armatus* (Lacepède, 1800) (Mastacembelidae) in India: *Dactylostomum harishii* Agarwal & Agarwal, 1988 [5], *Dactylostomum kashiensis* (Maurya, Agarwal & Singh, 1989) Cribb, 2005 [6], and *Dactylostomum satpali* Agrawal & Sharma, 1989 [7]. However, these descriptions do not conform to current taxonomic standards because of the absence of deposited type material, incomplete morphometric descriptions, and inadequate differential diagnoses separating the proposed taxa from previously described congeners. Consequently, these species may be regarded as *species inquirendae*.

In addition to the nominal species described from *M. armatus*, several species assigned to *Dactylostomum* have been described from other freshwater fishes in India, including *Dactylostomum gayaprashadi* Tripathi & Jauhari, 1991, from *Xenentodon cancila* (Hamilton, 1822) (Belonidae) [8], *Dactylostomum cuchia* Sen, 2011, from *Ophichthys cuchia* (Hamilton, 1822) (Synbranchidae) [9], *D. matatiliansis* Sen, 2014, and *D. govindsagerii* Sen & Siddiqui, 2014, from *Ophichthys cuchia* (Hamilton, 1822) (Synbranchidae) [10,11], and *Dactylostomum ramgangaii* and *Dactylostomum elongatus* Kumar, 2016, from *Sperata seenghala* (Sykes, 1839) (Bagridae) [12]. These taxa are likewise based on inadequate taxonomic descriptions and should also be regarded as *species inquirendae*.

In Australia, *Dactylostomum cribbi* Gibson, Bray & Langdon, 1990, was described from the freshwater fish *Gadopsis marmoratus* Richardson, 1848, an endemic species of southeastern Australia [13]. Consequently, the currently recognised freshwater representatives of *Dactylostomum* comprise *Dactylostomum armatum* (Dwivedi, 1975) Cribb, 2005, *Dactylostomum sapani* (Dwivedi & Dwivedi, 1982) Cribb, 2005 (both originally described in *Opedunculata*), and the above-mentioned *D. cribbi*.

Knowledge of *Dactylostomum* remains limited and is further constrained by the complete absence of molecular characterisation. This is unsurprising, as no recent collections suitable for molecular analyses have been available. Moreover, the most recently described marine species, *D. nicolli*, was preserved in formalin, preventing the generation of DNA sequences [2]. In addition to the three recognised freshwater species, the genus currently comprises the following twelve marine species: *D. gracile*, *D. vitellosum* Manter, 1940, *D. caballeroi* Martin, 1960, *D. winteri* Caballero & Caballero, 1971, *D. epinepheli* Wang, 1982, *D. longivesiculum* Shimazu & Machida, 1985, *D. manteri* Ramadan, 1985, *D. tanegashimense* Shimazu & Machida, 1985, *D. woolcocki* Ramadan, 1985, *D. yamagutii* Ramadan, 1985, *D. griffithsi* Bray, 1987, and *D. nicolli*.

During a parasitological survey of freshwater fishes from Khun Thale Swamp, Surat Thani Province, Thailand, specimens of a trematode attributable to the genus *Dactylostomum* according to the diagnosis of Cribb [4] were recovered. The present study aims to describe this new species and to provide the first molecular characterisation and phylogenetic position in the subfamily Opecoelinae for the genus *Dactylostomum*.

2. Materials and Methods

2.1. Sample Collection

Freshwater fishes were collected from Khun Thale Swamp, Surat Thani Province, Thailand (9°4'3" N, 99°19'52" E), using gill nets and cast nets obtained from a local fisherman

during the dry season (February–April 2020) and the rainy season (September–November 2020). This swamp is the largest freshwater lake-like wetland in Surat Thani Province, covering approximately 203 ha. It is an open freshwater system connected to tributary canals and the Tapi River and is occasionally influenced by tidal seawater intrusion during the dry season, making it an ecologically important wetland [14]. Live fish were transported to the Scientific Laboratory and Equipment Centre (SLEC), Prince of Songkla University, Surat Thani, Thailand (it is a short distance of less than six kilometres, making the logistics of sampling feasible). The whole gastrointestinal tract of each fish was examined for helminths under a stereomicroscope. Fish were identified following [15–17]. The animal study was approved by the Institutional Animal Care and Use Committee, Prince of Songkla University, Thailand, under approval number MHESI 6800.11/965. The study was conducted in accordance with the local legislation and institutional requirements.

2.2. Morphological Study

Digenean trematodes were recovered from the digestive tract under a Zeiss stereomicroscope (Jena, Germany) using 0.85% saline solution. Specimens were rinsed in 0.85% saline solution, fixed in 4% formalin, and gently flattened between a microscope slide and a coverslip under light pressure. They were subsequently stained with acetocarmine, dehydrated through a graded ethanol series, cleared in xylene, and permanently mounted in Permount (Fisher Scientific, NJ, USA) [18]. A total of six gravid, stained specimens were examined morphologically. All examined specimens were deposited in the Museum of Natural Sciences of Barcelona under collection numbers MZB 2026–0497 to MZB 2026–0502. Prevalence and mean intensity were calculated following [19].

2.3. Morphometrical Study

Stained specimens were examined using a Leica DMLB light microscope (Leica Microsystems, Wetzlar, Germany) equipped with a digital camera Euromex (sCMEX) (Duiven, the Netherlands). Identification to the generic level followed the key to Cribb [4]. Measurements are given in micrometres (μm) as the mean followed by the range in parentheses.

2.4. Molecular Study

Molecular analyses of one specimen were performed using rRNA (18S, ITS2 and 28S) markers. DNA was extracted using the DNeasy Blood & Tissue Kit[®] (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The 18S, ITS2 and 28S regions of the rRNA were amplified by PCR. The PCRs were performed in a total volume of 25 μL containing 1x Green GoTaq[®] Reaction Buffer (1.5 mM MgCl_2) (Promega, Madison, WI, USA), 0.2 mM each dNTP, 1 μM of each primer, and 5 U/ μL GoTaq[®] DNA Polymerase (Promega, Madison, WI, USA). The 18S region was amplified using the primers 18S F (5'–ATCCGAAGTAATGGTTAAGAGGG–3') and 18S R (5'–ACCTACGGAAACCTTGTTACG–3'); the ITS2 region using ITS2 F (5'–GCTCGTGTGTCGATGAAGAG–3') and ITS2 R (5'–AGGCTTCGGTGCTGGGCT–3'); and the 28S region using the primers 28S F (5'–GTGAATACCCGCTGAACTTAAGC–3') and 28S R (5'–TCTCCTTGGTCCGTGTTTCAA–3') [20]. The cycling conditions included an initial denaturation at 94 °C for 5 min, followed by 40 cycles of 30 s at 94 °C, 30 s at 52 °C for 28S and 18S, 56 °C for ITS2 (annealing), and 2 min at 72 °C, with a final extension step of 10 min at 72 °C. Amplified PCR products were electrophoretically separated in a 1% (*w/v*) agarose gel stained with Green Gel. Negative controls were always included in the PCRs to check for contamination. Relevant bands were sent for purification and sequencing, and all sequences have been deposited in GenBank (Table 1). Sequencing was performed by the Genomics Unit of the Scientific and Technological Centres (CCiTUB), University of Barcelona.

Table 1. GenBank accession numbers and ITS2 genetic distances for Opecoelidae species included in the phylogenetic analyses. Sequences generated in the present study are indicated in bold. Outgroups are indicated as OG. N.C: New Caledonia. Numbers in brackets link GenBank accession numbers to their corresponding host and locality.

Species	Host	Locality	18S rRNA	28S rRNA	ITS2 rRNA	ITS2 Pairwise Distances (%)
<i>Dactylostomum brayi</i> n. sp.	<i>Hemibagrus nemurus</i>	Thailand	PZ787884	PZ787886	PZ787885	-
<i>Opegaster mezcail</i>	<i>Tomicodon zebra</i> <i>Labrisomus xanti</i>	Mexico Mexico		PV577190 PV577186	-	-
<i>Opecoelus adsphaericus</i>	<i>Girella nigricans</i>	Mexico		PV577185 PV577184	-	-
<i>Anomalotrema koiae</i>	<i>Sebastes viviparus</i> <i>Microankhatus fedorovi</i>	UK North Pacific	KU320582	KU320595 MH161429	-	-
<i>Dimerosaccus oncorhynchi</i>	<i>Liobagrus reini</i> ⁽¹⁾ <i>Salvelinus curilus</i> ⁽²⁾	Japan ⁽¹⁾ Russia ⁽²⁾	LC856516 ⁽¹⁾	FR870262 ⁽²⁾	LC856509 ⁽¹⁾	9.6
<i>Discoverytrema markowskii</i>	<i>Muranolepis marmorata</i>	North Pacific	-	MH161431	-	-
<i>Discoverytrema gibsoni</i>	<i>Muranolepis marmorata</i>	North Pacific	-	MH161430	-	-
<i>Pseudopecoelus vulgaris</i>	<i>Sebastes</i> sp.	North Pacific	-	MH161436	-	-
<i>Pseudopecoeloides tenuis</i>	<i>Priacanthus hamrur</i>	N.C.	KU320592	KU320605	-	-
<i>Opecoeloides pedicathedrae</i>	<i>Scomberomorus cavalla</i>	Brazil	OQ101906	-	-	-
<i>Opecoeloides fimbriatus</i>	<i>Micropogonias undulatus</i>	USA	-	KJ001211	-	-
<i>Opecoeloides furcatus</i>	<i>Mullus surmuletus</i>	France	-	AF151937	AJ241790	13.7
<i>Buticulotrema thermichthysi</i>	<i>Thermichthys hollisi</i>	Pacific Ocean	KF733987	KF733984	-	-
<i>Urochis acheilognathi</i>	<i>Tanakia lanceolata</i> ⁽³⁾ <i>Tanakia limbata</i> ⁽⁴⁾	Japan ⁽³⁾ Japan ⁽⁴⁾	LC856515 ⁽³⁾	KX553945 ⁽⁴⁾	KX553945 ⁽⁴⁾	17.5
<i>Neoplagioporus kajika</i>	<i>Cottus pollux</i> ⁽⁵⁾ <i>Triplophysa fuxianensis</i> ⁽⁶⁾	Japan ⁽⁵⁾ China ⁽⁶⁾	LC856512 ⁽⁵⁾	-	MH257741 ⁽⁶⁾	21.5
<i>Neoplagioporus zacconis</i>	<i>Opsariichthys platypus</i>	Japan	-	KX553949	KX553949	17.2
<i>Gaevskajatrema perezi</i>	? fish ⁽⁷⁾ <i>Symphodus roissali</i> ⁽⁸⁾	Corsica ⁽⁷⁾ Corsica ⁽⁸⁾	-	AF184255 ⁽⁷⁾	AJ241800 ⁽⁸⁾	21.5
<i>Bathypodocotyle margolisi</i>	<i>Coryphaenoides mediterraneus</i>	UK	KU320583	KU320596	-	-
<i>Scorpidotrema longistipes</i>	<i>Scorpiis georgiana</i>	Australia	MK052939	MK052936	MK052933	17.6
<i>Holsworthotrema chaoderma</i>	<i>Kyphosus gladius</i>	Australia	MK052941	MK052938	MK052935	18.8

Table 1. Cont.

Species	Host	Locality	18S rRNA	28S rRNA	ITS2 rRNA	ITS2 Pairwise Distances (%)
<i>Helicometra fasciata</i>	<i>Epinephelus fasciatus</i> ⁽⁹⁾ <i>Symphodus rostratus</i> ⁽¹⁰⁾	N.C. ⁽⁹⁾ Corsica ⁽¹⁰⁾	KU320584 ⁽⁹⁾	KU320597 ⁽⁹⁾	AJ241793 ⁽¹⁰⁾	24.0
<i>Macvicaria dubia</i>	<i>Oblada melanura</i>	Tunisia	-	KR149470	KR149489	19.6
<i>Maculifer diodontis</i>	<i>Diodon hystrix</i>	Australia	-	MH933879	MH933873	22.9
<i>Trilobovarium parvatis</i>	<i>Lethrinus nebulosus</i>	Australia	KY551561	KY551562	KY551560	23.3
<i>Choerodonicola arothokoros</i>	<i>Scarus ghobban</i>	Australia	MG844417	MG844418	MG844416	23.8
<i>Pseudoplagioporus mediocris</i>	<i>Lethrinus atkinsoni</i>	Australia	MN081739	-	MN078306	22.7
<i>Pseudopycnadena fischthali</i>	<i>Diplodus vulgaris</i>	Algeria	-	MF166851	MF166841	19.0
<i>Shimazuia lethrini</i>	<i>Lethrinus atkinsoni</i>	Australia	MN081745	-	MN078315	24.3
<i>Fairfaxia lethrini</i>	<i>Lethrinus nebulosus</i>	Australia	MN081742	-	MN078311	24.4
<i>Podocotyloides gracilis</i>	<i>Diagramma picta</i>	Australia	MF805692	MF805693	MF805691	21.4
<i>Hamacreadium mutabile</i>	<i>Lutjanus fulviflamma</i>	N.C.	KU320588	KU320601	-	-
<i>Allopodocotyle epinepheli</i>	<i>Epinephelus cyanopodus</i> ⁽¹¹⁾ <i>Salarius fasciatus</i> ⁽¹²⁾	N.C. ⁽¹¹⁾ Australia ⁽¹²⁾	KU320585 ⁽¹¹⁾	KU320598 ⁽¹¹⁾	MN067859 ⁽¹²⁾	25.8
<i>Pacificreadium serrani</i>	<i>Plectropomus leopardus</i>	N.C.	KU320589	KU320602	DQ083433	26.6
<i>Pedunculacetabulum inopinipugnus</i>	<i>Plectorhinchus chrysotaenia</i>	Australia	MF805699	MF805700	MF805698	20.7
<i>Polypipapiliotrema stenometra</i>	<i>Chaetodon quadrimaculatus</i>	Hawaii	MH823959	MH823954	MH823948	22.3
<i>Pseudoheterolebes stellaglobulus</i>	<i>Dicotylichthys punctulatus</i>	Australia	MH933882	MH933877	MH933870	22.9
<i>Opistholebes amplicoeus</i>	<i>Lagocephalus lunaris</i>	Australia	MH933881	MH933875	MH933868	22.1
<i>Heterolebes maculosus</i>	<i>Diodon hystrix</i> ⁽¹³⁾ <i>Diodon hystrix</i> ⁽¹⁴⁾ <i>Diodon liturosus</i> ⁽¹⁵⁾	Australia ⁽¹³⁾ Australia ⁽¹⁴⁾ Australia ⁽¹⁵⁾	AY222109 ⁽¹³⁾	MH933878 ⁽¹⁴⁾	MH933872 ⁽¹⁵⁾	21.7
<i>Plagioporus shawi</i>	<i>Oncorhynchus tshawytscha</i>	USA	-	KX553951	KX553951	17.2
<i>Preptetos caballeroi</i> (OG)	<i>Naso vlamingi</i>	Australia	AJ287563	AY222236	-	-

2.5. Phylogenetic Analysis

To infer the position and determine the sister-group relationships of *Dactylostomum* within an Opecoelidae phylogenetic framework, a concatenated phylogenetic tree was constructed using the partial 18S rRNA region, the ITS2 and the partial 28S rRNA region (see species and outgroup data in Table 1). Alignments of the individual regions were performed using MAFFT (available at <http://www.ebi.ac.uk/Tools/msa/mafft/>) (accessed on

20 July 2026) and the concatenation was performed using MEGA 12.1.2 [21]. Phylogenetic and molecular evolutionary analyses were conducted using the aligned and concatenated nucleotide sequences and inferred using both the maximum-likelihood (ML) method in MEGA 12.1.2 and Bayesian inference (BI) using BEAST [22]. To determine the evolutionary model that best fitted our dataset, jModeltest 2.1.1 [23] was employed, with model selection based on the Akaike information criterion (AIC). The results indicated that the general time-reversible model with an estimate of gamma-distributed among-site rate variation and invariant sites (GTR + G + I) was the most appropriate for the analyses. The number of substitution types was fixed to 6. The 4×4 substitution model was used, while rate variation across sites was modelled with a gamma distribution. Node support was evaluated using the ultrafast bootstrap with 1000 replicates [24]. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Four Markov Chain Monte Carlo (MCMC) chains were run for 10,000 generations, with sampling performed every 10 generations, with the first 250 sampled trees discarded as burn-in. Finally, a 50% majority-rule consensus tree was constructed. The concatenated 18S–ITS2–28S tree involved 44 sequences with a total of 6700 positions in the final dataset. For the ITS2 sequences, evolutionary divergence was calculated using p-distance with 1000 bootstrap replicates in MEGA 12.1.2.

3. Results

A total of 1065 freshwater fish belonging to 16 families, 27 genera, and 33 species were collected and examined for trematodes. Of the species examined, six of 22 *Hemibagrus nemurus* (Valenciennes, 1840) (Bagridae) were infected with a trematode belonging to the genus *Dactylostomum*. The 22 fish examined ranged from 24 to 46 cm (35 cm) in length and from 118 to 800 g (436 g) in weight. The prevalence, mean intensity, and intensity range were 27.27%, 2.33, and 1–4 worms per infected host, respectively.

The only other bagrid examined was *Mystus nigriceps* (Valenciennes, 1840), of which both specimens examined were uninfected. All remaining fish species were negative for *Dactylostomum*.

3.1. Systematic Account

Phylum: Platyhelminthes Minot, 1876;

Class: Trematoda Rudolphi, 1808;

Order: Plagiorchiida La Rue, 1957;

Family: Opecoelidae Ozaki, 1925;

Genus: *Dactylostomum* Woolcock, 1935.

***Dactylostomum brayi* n. sp.**

Type host: *Hemibagrus nemurus* (Valenciennes, 1840) (Siluriformes: Bagridae), Asian redbtail catfish;

Type locality: Khun Thale Swamp, Surat Thani Province, Thailand (9°4′3″ N, 99°19′52″ E);

Site of infection: Intestine;

Type specimens: Holotype MZB 2026–0497; paratypes MZB 2026–0498 to MZB 2026–0502, deposited in the Museum of Natural Sciences of Barcelona, Barcelona, Spain;

Date of collection: November 2020;

Prevalence, mean intensity, and intensity range: Six of twenty-two (27.27%, 2.33, and 1–4 worms per infected host, respectively);

GenBank accession numbers: 18S rDNA (PZ787884), 28S rDNA (PZ787886), and ITS2 (PZ787885);

Etymology: The specific epithet *brayi* honours Dr Rodney A. Bray (Natural History Museum, London) in recognition of his outstanding contributions to the taxonomy and systematics of digenean trematodes, including the family Opecoelidae. The name also acknowledges his examination and deposition of specimens of the same species collected in Thailand more than four decades before the present study;

Description. Based on six whole-mounted specimens, including two mounted in lateral view and four in ventral view (Figure 1a–d). Measurements are in micrometres and are given as the range followed by the mean in parentheses.

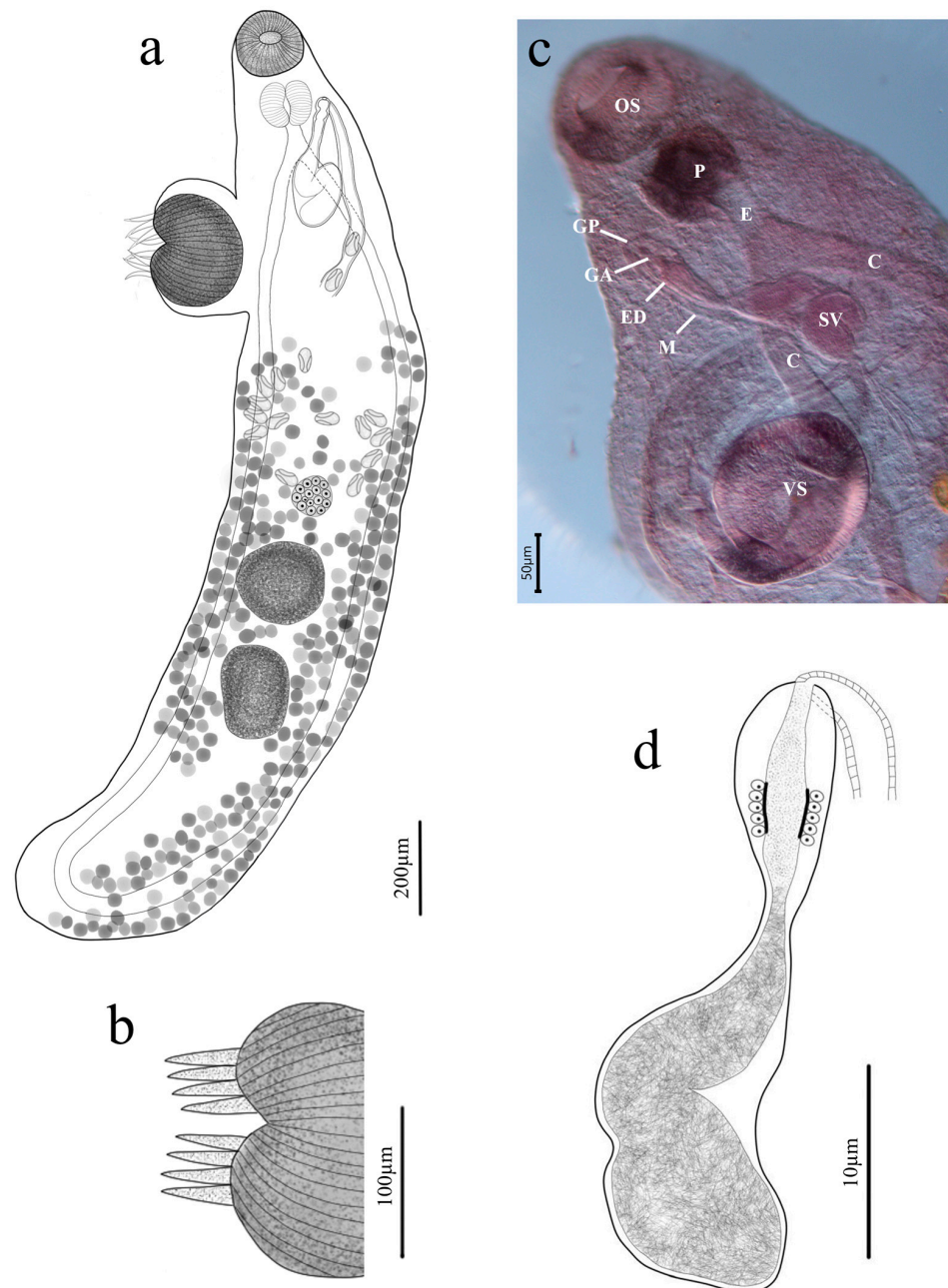


Figure 1. *Dactylostomum brayi* n. sp. from the intestine of *Hemibagrus nemurus* (Valenciennes, 1840). (a) Line drawing of the whole worm in lateral view showing the general arrangement of the internal organs; inset showing the ventral sucker bearing eight prominent horn-like papillae. Scale bar = 200 μm. (b) Photomicrograph of the anterior region in lateral view. Abbreviations: C, caeca; E, oesophagus; ED, ejaculatory duct; GA, genital atrium; GP, genital pore; M, metraterm; OS, oral sucker; P, pharynx; SV, seminal vesicle; VS, ventral sucker. Scale bar = 50 μm. (c) Detail of the ventral sucker. Scale bar = 100 μm. (d) Terminal genitalia. Scale bar = 10 μm.

Body elongate, slender, with body surface unarmed, 1629–2299 (1946)-long and 306–463 (390)-wide (Figure 1a). Oral sucker oval, subterminal, 107–137 (118) long and 99–146 (127) wide. Prepharynx 3–21 (10)-long. Pharynx oval, well-developed, 84–124 (106)-long and 94–113 (104)-wide. Oesophagus 28–73 (50)-long. Intestinal bifurcation in posterior forebody (Figure 1b). Intestinal caeca extending posteriorly and uniting to form a cyclocoel near posterior extremity of body. Distance from posterior margin of cyclocoel to posterior extremity of body 62–103 (86) (this measurement could not be obtained in one specimen). Ventral sucker larger than oral sucker, oval, pedunculate, 147–274 (191)-long and 140–208 (168)-wide, protuberant, bearing eight horn-like papillae on luminal margin (Figure 1b). Oral sucker: ventral sucker width ratio 1:1.2–1.4 (1.3). Forebody representing 15.7–27.5% of body length.

Seminal vesicle short, sigmoid to sinuous, situated anteriorly at mid-level of ventral sucker and continuing as tubular ejaculatory duct opening into conspicuous genital atrium. Genital pore situated approximately at mid-pharyngeal level, apparently sinistrally sub-lateral. External seminal vesicle entirely enclosed within thin, delicate membranous sac; cirrus-sac wall thin around seminal vesicle, becoming thicker at level of ejaculatory duct and genital atrium. Pars prostatica and prostatic gland cells observed (Figure 1d).

Anterior testis 76–197 (157)-long and 107–210 (166)-wide. Posterior testis 142–215 (191)-long and 122–201 (166)-wide. Inter-testicular distance 9–67 (35). Post-testicular region 502–673 (571)-long.

Ovary transversely oval, irregular, 58–90 (77)-long and 67–98 (84)-wide; separated from ventral sucker by 176–418 (274) and from anterior testis by 14–54 (30). Eggs operculate, 7–83 (28) in number, 34–54 (45)-long and 17–33 (26)-wide ($n = 54$). Metraterm muscular, running alongside cirrus sac, with its proximal end at approximately level of pars prostatica. Uterus extending between ovary and genital pore. Vitellarium follicular; vitelline fields extending from posterior extremity to approximately midway between ovary and ventral sucker, –157–102 (–26) anterior to ventral sucker; follicles mainly lateral to caeca, confluent ventrally posterior to posterior testis and ovary, confluent dorsally posterior to testes, almost confluent dorsally between testes and between anterior testis and ovary, with distinct dorsal gap at level of caeca. Excretory pore terminal. Excretory vesicle I-shaped, tubular, extending anteriorly dorsal to cyclocoel and reaching level of ovary.

Remarks. The present specimens are assigned to *Dactylostomum* Woolcock, 1935, based on the presence of prominent horn-like papillae on the ventral sucker, the principal diagnostic character of the genus within Opecoelinae [4]. *Dactylostomum brayi* n. sp. differs from all previously described freshwater species of *Dactylostomum* in the number of ventral sucker papillae, body proportions versus oral sucker, and the morphology of the reproductive organs.

Related to the marine species, *Dactylostomum brayi* n. sp. can be assigned to Group B of Bray [3], characterised by the vitellarium being confined to the hindbody. More specifically, it belongs to Group B1 of Aken'Ova et al. [2], comprising species with simple ventral sucker papillae and an entire to irregular ovary. This group includes *D. griffithsi*, *D. woolcocki*, *D. winteri*, and *D. nicolli*. Although *D. brayi* n. sp. is a freshwater species, it can be readily distinguished from the first three marine species. *Dactylostomum griffithsi* possesses six or seven horn-like papillae rather than eight [3], *D. woolcocki* bears six horn-like papillae [25], and *D. winteri* possesses only three horn-like papillae [26,27].

Among the currently recognised freshwater species, *D. cribbi* shares the presence of four pairs of ventral sucker papillae with *D. brayi* n. sp. but differs in possessing a markedly larger and more muscular ventral sucker (more than one-third of body length) and a maximum of seven eggs, compared with up to 83 eggs in *D. brayi* n. sp. [13]. *Dactylostomum armatum* differs from the new species in having six ventral sucker papillae instead of eight

and in having vitelline fields extending anteriorly to the caecal bifurcation [4,28], whereas in *D. brayi* n. sp. the posterior extent of the vitelline fields remains well posterior to the caecal bifurcation. *D. govindsagerii* and *D. matatiliansis* differ from *D. brayi* n. sp. in having a vitellarium that extends into the forebody. In addition, *D. govindsagerii* has three dactylae on the ventral sucker, whereas *D. matatiliansis* is described as lacking dactylae [10,11]. *Dactylostomum sapani* can be readily distinguished by the presence of four ventral sucker papillae rather than the eight present in *D. brayi* n. sp., and by having transversely elongated testes not separated compared with testes tandem, oval to nearly spherical, well separated in *D. brayi* n. sp. In addition, *D. brayi* n. sp. has smaller eggs than all other freshwater species of the genus.

3.2. Molecular Phylogenetic Analyses

In the ML and BI analyses of the concatenated 18S–ITS2–28S rRNA sequences, *D. brayi* n. sp. belongs to a monophyletic and well-supported clade including all species of the subfamily Opecoelinae (node supports 100/1). There are no other *Dactylostomum* molecular sequences available; however, *D. brayi* n. sp. appears as the sister taxon, with strong support, to *Dimerosaccus oncorhynchi* (Eguchi, 1931) Shimazu, 1980, in all three phylogenetic trees (node supports 100/1.0) (Figure 2).

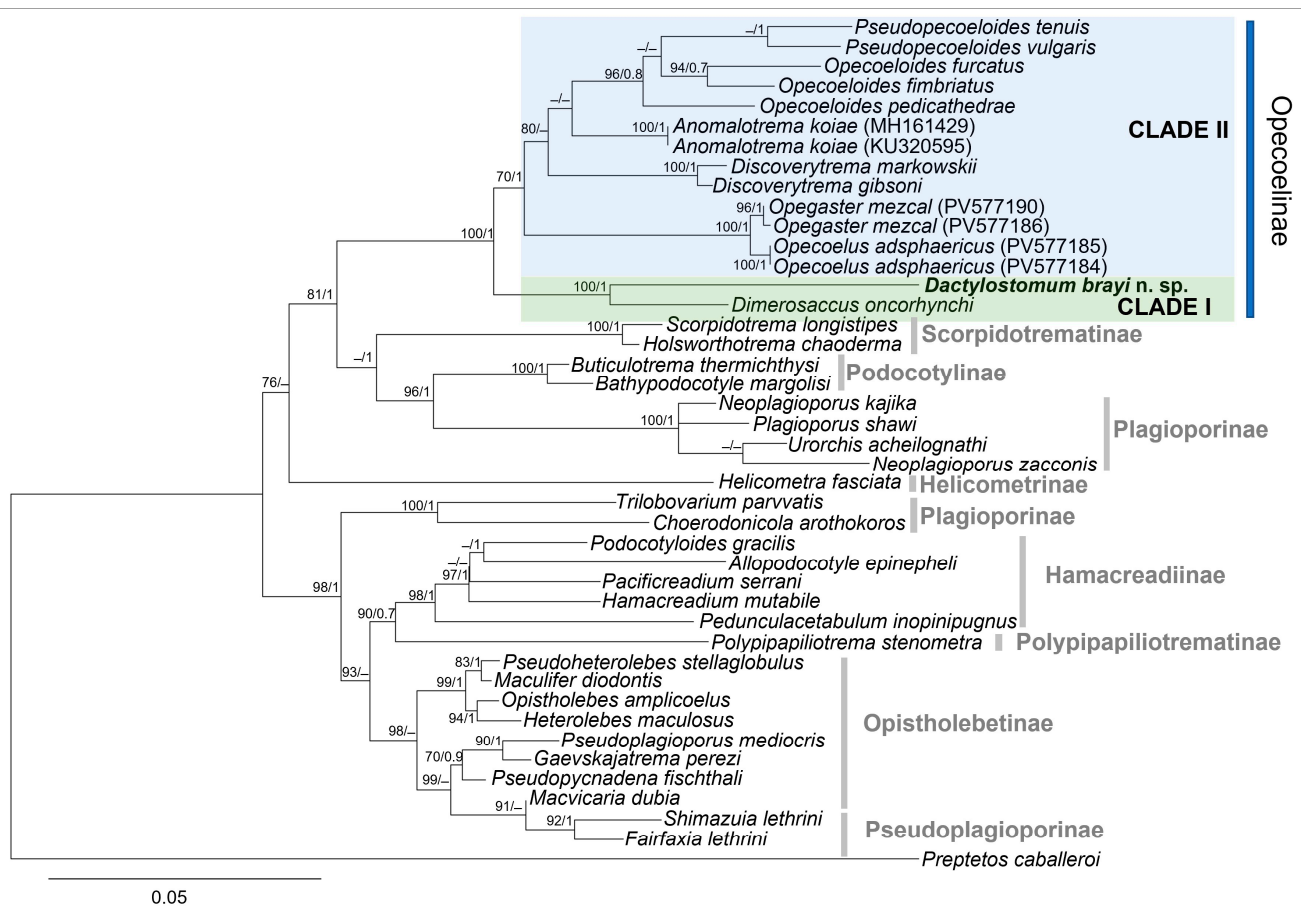


Figure 2. Phylogenetic relationships of Opecoelidae species inferred from maximum-likelihood analysis and Bayesian inference of concatenated 18S–ITS2–28S ribosomal RNA sequences. Newly generated sequences are shown in bold. Bootstrap support values lower than 70% and posterior probabilities lower than 0.8 are indicated by a dash. The scale bar represents the expected number of substitutions per site.

The Opecoelinae clade included the following two distinct subclades: Clade I (node supports 100/1), encompassing *D. brayi* n. sp. and *D. oncorhynchi*, and Clade II (node supports 70/1), containing the other available sequences (species of the genera *Pseudopecoelus*, *Pseudopecoeloides*, *Opecoeloides*, *Anomalotrema*, *Discoverytrema*, *Opegaster*, *Opecoelus*, *Paropecoelus*, *Poracanthium*, and *Coitocaecum*).

The genetic distance between the ITS2 sequences of *D. brayi* n. sp. and *D. oncorhynchi* was 9.6% (Table 1), while genetic distances between *D. brayi* n. sp. and the other species within Clade II varied between 11.3 and 20.8%. The genetic distances among species within Clade II varied between 5.9 and 18.5%. Considering the genetic variability of other subfamilies within Opecoelidae, such as Plagioporinae (4.5–11.2%) and Hamacreadinae (9.5–13.9%), the placement of *Dactylostomum* and *Dimerosaccus* within the Opecoelinae subfamily is supported.

4. Discussion

Despite several parasitological studies reporting digenean trematodes from freshwater fishes in Thailand, including the Asian redtail catfish *Hemibagrus nemurus*, there have been no published records of *Dactylostomum*. Nevertheless, the helminth collection of the Natural History Museum, London, contains four specimens identified by R. A. Bray as *Dactylostomum* sp. (catalogue numbers 1983.8.8.101–104). These specimens were collected in Thailand in 1982 by Supranee Chinabut, former Director of the Aquatic Animal Health Research and Development Division, Bangkok, Thailand, from the bagrid catfishes *Hemibagrus wyckii* (Bleeker, 1858) and *H. nemurus*. The occurrence of *Dactylostomum* in *H. nemurus* more than four decades before the present study further supports this fish as the natural host of the newly described species. Furthermore, considering that the present survey examined 1065 freshwater fishes representing 33 species, 27 genera, and 16 families, and that the parasite was recovered exclusively from *H. nemurus*, the available evidence indicates a high degree of host specificity. However, the host specificity of other *Dactylostomum* species is difficult to assess reliably because sampling designs have not been equivalent among studies, with many surveys not involving intensive sampling within a defined area covering the full local fish community, as was done in the present study.

The present study also provides the first molecular characterisation for the genus *Dactylostomum*, establishing a foundation for future phylogenetic studies. Phylogenetic analysis revealed that this genus, together with *Dimerosaccus*, constitutes a well-supported clade within the Opecoelinae, separated from the other genera. The subfamily Opecoelinae is characterised by possessing both a naked seminal vesicle and a uterine seminal receptacle but lacking both a distinct cirrus sac containing an internal seminal vesicle and a canalicular seminal receptacle [29].

The morphological similarities observed in the male reproductive system of *Dactylostomum* and *Dimerosaccus* may provide useful evidence for assessing their possible evolutionary relationship. Shedko et al. [30] redescribed the terminal genitalia of *Dimerosaccus oncorhynchi*, the type species and only representative of the opecoelid genus *Dimerosaccus* [4], which possesses an elongated, sinuous external seminal vesicle surrounded by compact prostatic gland cells and entirely enclosed within a thin, delicate membranous sac that connects to a highly reduced distal cirrus sac. The presence of a similarly specialised and modified cirrus sac in both taxa may represent a shared morphological feature and could be consistent with common ancestry. However, given that molecular sequences are currently available for only one species of each genus, the available data are insufficient to determine whether this similarity represents a synapomorphy uniting *Dactylostomum* and *Dimerosaccus*. Moreover, other molecularly uncharacterised or unsampled taxa within Opecoelinae may prove to be more closely related to *Dactylostomum*. Therefore, the ap-

parent close relationship between these genera should be considered tentative, pending broader taxon sampling and additional molecular and morphological data. On the other hand, obtaining molecular characterisation from the marine species of *Dactylostomum* will be essential to determine whether the freshwater and marine representatives constitute a single evolutionary lineage or represent distinct evolutionary units that may warrant taxonomic re-evaluation.

5. Conclusions

The present study describes *Dactylostomum brayi* n. sp. from the intestine of the Asian redbtail catfish *Hemibagrus nemurus* in Khun Thale Swamp, southern Thailand. The new species is distinguished from all recognised congeners by a combination of morphological characters, particularly the presence of eight prominent papillae on the ventral sucker, together with features of the reproductive organs. This represents the second record of *Dactylostomum* from Thailand and the first molecular characterisation of the genus. The newly generated DNA sequences provide an essential reference for future phylogenetic and taxonomic studies and will facilitate comparisons with marine representatives to clarify the evolutionary relationships within *Dactylostomum*.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/taxonomy6040059/s1>, Table S1: List of fishes (excluding family Bagridae that data is included in main text) that were examined in Khun Thale Swamp and resulted negative for the presence of *Dactylostomum brayi* n. sp.

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