

A new classification for deep-sea opecoelid trematodes based on the phylogenetic position of some unusual taxa from shallow-water, herbivorous fishes off south-west Australia

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We report on *Scorpidotrema longistipes* and two new species belonging to a new genus: ***Holsworthotrema enboubalichthys* gen. et sp. nov.** and ***Holsworthotrema chaoderma* sp. nov.** These taxa are the first representatives of the subfamily Stenakrinae included in molecular phylogenetic analyses of the Opecoelidae. They resolve among the deep-sea + freshwater Plagioporinae (*s.l.*) clade, but exploit shallow-water marine fishes. Based on phylogeny, ecology and morphology, we reorganize this clade into four subfamilies: the Plagioporinae (*s.s.*) for freshwater taxa, the Stenakrinae as currently defined with the addition of *Holsworthotrema*, the **Bathycreadiinae subf. nov.** for *Bathycreadium* and the Podocotylineae, a resurrected concept, for *Podocotyle*, *Bathypodocotyle* gen. nov., *Buticulotrema*, *Halosaurotrema* gen. nov., *Macrourimegatrema*, *Neolebouria* and *Tellervotrema*. *Bathypodocotyle* is proposed for species from deep-sea fishes previously assigned to *Allopodocotyle*, and *Halosaurotrema* for *Gaevskajatrema halosauropsi*. Two deep-sea opecoelids represented by sequence data, *Neolebouria lanceolata* and *Podocotyloides brevis*, remain inadequately accommodated. These taxa are sister to the Stenakrinae and may require another new subfamily, but it is not yet clear which morphological and/or ecological characters would distinguish such a concept. Evidently, neither belongs to its nominal genus; we propose to accommodate these taxa in ***Mesobathylebouria* gen. nov.** and ***Abyssopedunculus* gen. nov.**, respectively.

ADDITIONAL KEYWORDS: Digenea – endemic – Kyphosidae – *Kyphosus* – new species – Opecoelidae – parasites – phylogeny – Plagioporinae – Platyhelminthes – *Scorpiis* – Stenakrinae.

INTRODUCTION

The Opecoelidae Ozaki, 1925 is the largest family of trematodes and among the best represented in fishes of the deep-sea (Bray, 2004). Most opecoelids from deep-sea fishes nominally belong to the Plagioporinae Manter, 1947. Phylogenetically informative rDNA sequence data are available from specimens representative of eight such species: *Allopodocotyle margolisi* Gibson, 1995, *Bathycreadium brayi* Pérez-del-Olmo *et al.*, 2014, *Buticulotrema thermichthysi* Bray *et al.*, 2014, *Gaevskajatrema halosauropsi* Bray & Campbell, 1996, *Neolebouria georgiensis* Gibson, 1976, *Neolebouria lanceolata* Price, 1934, *Podocotyle atomon* (Rudolphi,

1802) Odhner, 1905 and *Podocotyloides brevis* Andres & Overstreet, 2013. On the basis of phylogenetic analyses, four of these eight, *A. margolisi*, *G. halosauropsi*, *N. lanceolata* and *P. brevis*, are not congeneric with the type-species of their respective nominal genera and thus are inadequately classified (Bray *et al.*, 2016; Faltýnková *et al.*, 2017; Martin *et al.*, 2018c). In previous, family-wide phylogenetic analyses of the Opecoelidae, the deep-sea taxa have formed a well-supported, relatively derived clade, together with taxa from freshwater Holarctic fishes, including representatives of *Plagioporus* Stafford, 1904, the type-genus of the Plagioporinae (Fayton & Andres, 2016; Fayton *et al.*, 2017, 2018; Sokolov *et al.*, 2018).

The concept of the Plagioporinae (*s.l.*) is based on the presence of two morphological characters, a well-defined cirrus-sac entirely enclosing the seminal vesicle and a canalicular seminal receptacle (Gibson &

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Bray, 1982; Cribb, 2005). However, this diagnosis is insufficient, because it fails to distinguish between two phylogenetically distinct lineages, the freshwater + deep-sea taxa and a major clade comprising taxa from marine fishes not of the deep-sea, which also includes the Opistholebetinae Fukui, 1929 (Bray *et al.*, 2016; Martin *et al.*, 2018b). Furthermore, *B. thermichthysi*, one of the species belonging to the deep-sea clade for which sequence data have been generated, lacks a cirrus-sac entirely (Bray *et al.*, 2014). The phylogenetic grouping of freshwater and deep-sea plagioporine (*s.l.*) taxa remains unexplained. *Podocotyle atomon* is known from both deep-sea and shallow-water marine fishes from the north Atlantic, but, based on sequence data generated from cercariae collected in the White Sea, it does not resolve as intermediate between deep-sea and freshwater taxa (Sokolov *et al.*, 2018). Thus, it is currently unclear as to whether the concept of the Plagioporinae should be restricted to the seemingly ecologically distinct freshwater clade, or should be expanded to include all, or some, of the deep-sea taxa. In this study, we introduce the first sequence data generated from specimens relating to opecoelids that are known only from shallow-water, marine fishes, but which resolve among the taxa from deep-sea or freshwater fishes. We discuss the implications these data have for working towards a functional subfamilial classification of the Opecoelidae.

MATERIAL AND METHODS

SPECIMEN COLLECTION AND HOST IDENTIFICATION

This is the first in a series of reports on the trematode fauna of *Kyphosus* (Perciformes: Kyphosidae) fishes from the Indo-West Pacific. We have examined a total of 116 individual *Kyphosus* fishes of seven species between 1994 and 2018 (Table 1), the majority of which were collected in Australian waters, on the Great Barrier Reef and in Moreton Bay, Queensland; from off the Yorke Peninsula, South Australia; and on Ningaloo Reef and off Perth, Western Australia. Fewer individuals have been examined from other Indo-West Pacific localities, specifically Japan, Palau, French Polynesia and South Africa. Fishes were collected mostly by spear, with some caught by line or purchased from local fishermen. Trematodes were collected as per Cribb & Bray (2010). Most specimens, including all of those considered in this study, were fixed, without pressure, in near-boiling saline and preserved in 80% ethanol.

Identification of hosts is as per Knudsen & Clements (2013a, b, 2016). However, species of *Kyphosus* fishes often form mixed schools and have overlapping meristic characteristics, making identification difficult (Knudsen & Clements, 2013a, b, 2016). Therefore, between 2015 and 2018, when there was any doubt as to

the identity of the host, a tissue sample was taken and identity was confirmed with two molecular markers: a fragment of the cytochrome *b* gene (*Cytb*) amplified with the primers L15416_CYB_F03 (5'-AAT YTC CTT CCA YCC DTA CTT C-3') and H15995_CYB_R06 (5'-AGA ATC CTA GCT TTG GGA GTT G-3') (Knudsen & Clements, 2016), and a fragment of the 16S rRNA gene amplified with the universal primers 16SarL (5'-CGC CTG TTT ATC AAA AAC AT-3') and 16SbrH (5'-CCG GTC TGA ACT CAG ATC ACG T-3') (Palumbi *et al.*, 1991). Amplification was carried out on a MJ Research PTC-150 thermocycler with the following profiles: (*Cytb*) an initial denaturation at 94 °C for 3 min, followed by 10 cycles of 94 °C for 15 s, 60 °C annealing for 30 s (dropping 1 °C per cycle) and 72 °C extension for 90 s; then an additional 30 cycles of denaturation at 94 °C for 15 s, 50 °C annealing for 30 s, and 72 °C extension for 90 s; with a final extension of 72 °C for 5 min; (16S) an initial 94 °C denaturation for 3 min, followed by 40 cycles of 94 °C denaturation for 30 s, 50 °C annealing for 30 s and 72 °C extension for 30 s, with a final extension at 72 °C for 10 min. Amplicons were purified with Bioline ISOLATE II PCR and Gel Kit, following the manufacturer's instructions. Sequence data were generated using the amplification primers via cycle sequencing with an AB3730xl capillary sequencer and ABI Big Dye v.3.1 chemistry, performed by the Australian Genome Research Facility (AGRF), Brisbane. Sequences generated from *Kyphosus* fishes were uploaded to GenBank with the accession numbers MH933897–920 (*Cytb*); MH933921–44 (16S).

MORPHOLOGICAL STUDY

Preserved specimens were stained with haematoxylin, dehydrated with a graded ethanol series, cleared with methyl-salicylate and mounted in Canada balsam. Some dehydrated specimens were selected for scanning electron microscopy (SEM); these specimens were transferred to hexamethyldisilazane, air-dried overnight and mounted on 12.5 mm pin-stubs using an adhesive carbon tab. Before SEM, specimens were coated with 15 nm of iridium with a Quorumtech Q150TS sputter coater. SEM images were obtained on a Hitachi SU3500 SEM in secondary electron mode. Morphometric data were taken from both ventral and lateral mounts, using the software package cellSens Standard v.1.13 via live feed from an Olympus SC50 camera mounted onto an Olympus BX53 compound microscope. Illustrations were made with the assistance of a drawing tube and digitised in Adobe Illustrator CS6. Type and voucher material were deposited into the Western Australia Museum (WAM). To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code*

Table 1. *Kyphosus* (Perciformes: Kyphosidae) fishes examined for intestinal digeneans between 1994 and 2018. Identification is as per [Knudsen & Clements \(2013a, b, 2016\)](#). Host-locality combinations marked with an asterisk have been confirmed with molecular sequence data, available at GenBank (MH933897–44)

	<i>K. bigibbus</i>	<i>K. cinerascens</i>	<i>K. cornelii</i>	<i>K. elegans</i>	<i>K. gladius</i>	<i>K. sydneyanus</i>	<i>K. vaigiensis</i>
Australia, Queensland, Great Barrier Reef, off Lizard Island	-	13*	-	-	-	-	16*
Australia, Queensland, Great Barrier Reef, off Heron Island	1*	2	-	-	-	-	12
Australia, Queensland, Moreton Bay	22*	7*	-	-	-	1	1
Australia, South Australia, Yorke Peninsula, Point Riley	-	-	-	-	-	5*	-
Australia, West Australia, Ningaloo Reef	1	1	-	-	-	2	2
Australia, West Australia, off Perth	-	-	7*	-	1*	9*	-
French Polynesia, Tuamotus, Rangiroa lagoon	-	4*	-	1*	-	-	2*
French Polynesia, off Moorea	-	-	-	-	-	-	1
Japan, Okinawa, Zampa Lagoon	-	-	-	-	-	-	1*
Palau	1	-	-	-	-	-	-
South Africa, KwaZulu-Natal, Sodwana Bay	-	2*	-	-	-	-	1*
Total	25	29	7	1	1	17	37

of *Zoological Nomenclature* ([ICZN, 2012](#)), details of all new genera and species have been submitted to ZooBank and, for each, the Life Science Identifier (LSID) is reported in the taxonomic summary below.

MOLECULAR AND PHYLOGENETIC STUDY

Genomic DNA were extracted from a total of five hologenophores, and genetic sequence data were generated for the second internal transcribed spacer region (ITS2 rDNA) and the small (18S) and large (28S) ribosomal subunit RNA coding regions. Extraction, amplification and sequencing protocols, including primers, are detailed in [Martin et al. \(2017\)](#). ITS2 data were annotated using the ITS2 database ([Keller et al., 2009](#)) and were used to barcode species. The phylogenetic positions of species were determined with two maximum likelihood and Bayesian inference analyses. The first set of analyses considered 28S data available at GenBank for taxa resolving to the deep-sea + freshwater opecoelid clade, with available data for taxa belonging to the subfamily Opecoelinae Ozaki, 1925 as the outgroup. The second set of analyses considered a

concatenated 28S + 18S dataset, comprising all opecoelid taxa available from GenBank with *Zalophotrema hepaticum* Stunkard & Alvey, 1929 (Brachycladioidea: Brachycladiidae) as the outgroup ([Table 2](#)), based on broader analyses of the Digenea ([Littlewood et al., 2015](#)). Data for *Biospeedotrema biospeedoi* Bray et al., 2014 and *Zdzitowieckitrema incognitum* Sokolov et al., 2017, nominal opecoelids from deep-sea fishes, were omitted, because previous analyses suggest they do not belong in the family ([Shedko et al., 2015](#); [Sokolov et al., 2017](#)). Alignments were constructed using MUSCLE v.3.7 ([Edgar, 2004](#)) implemented in MEGA v.6 ([Tamura et al., 2013](#)) with ClustalW sequence weighting and UPGMB clustering for iterations 1 and 2. The alignments were trimmed and, in the larger, family-wide analysis, indels greater than a single base-position and affecting >5% of sequences were removed, amounting to about 3% and 2% of the 28S and 18S alignments, respectively. The resultant concatenated alignment comprised 1323 and 1816 base-positions of 28S and 18S rDNA, respectively. The shorter, 28S-only alignment included 1301 base-positions. Maximum likelihood and Bayesian inference analyses used the implementations

Table 2. GenBank accession numbers for the sequence data used in phylogenetic analyses

Taxon	GenBank ID (18S)	GenBank ID (28S)	Reference
<i>Abyssopodocotyle brevis</i> (Andres & Overstreet, 2013) comb. nov. ^a		KJ001212	Andres <i>et al.</i> (2014a)
<i>Allopodocotyle epinepheli</i> (Yamaguti, 1942)	KU320585	KU320598	Bray <i>et al.</i> (2016)
<i>Allopodocotyle</i> sp. A	KU320586	KU320599	Bray <i>et al.</i> (2016)
<i>Allopodocotyle</i> sp. B	KU320594	KU320607	Bray <i>et al.</i> (2016)
<i>Anomalotrema koiae</i> Gibson & Bray, 1984	KU320582	KU320595	Bray <i>et al.</i> (2016)
<i>Bathycreadium brayi</i> Pérez-del-Olmo, Dallarés, Carrassón & Kostadinova, 2014 ^b		JN085948	Constenla <i>et al.</i> (2011)
<i>Bathypodocotyle margolisi</i> Gibson, 1955 ^c	KU320583	KU320596	Bray <i>et al.</i> (2016)
<i>Bentholebouria blatta</i> Bray & Justine, 2009	KU320593	KU320606	Bray <i>et al.</i> (2016)
<i>Bentholebouria colubrosa</i> Andres <i>et al.</i> , 2014		KJ001207	Andres <i>et al.</i> (2014a)
<i>Buticulotrema thermichthysi</i> Bray <i>et al.</i> , 2014	KF733987	KF733984	Bray <i>et al.</i> (2012)
<i>Cainocreadium labracis</i> (Dujardin, 1845)		JQ694144	Born-Torrijos <i>et al.</i> (2012)
<i>Cainocreadium lintoni</i> (Siddiqi & Cable, 1960)		KJ001208	Andres <i>et al.</i> (2014a)
<i>Choerodonicola arothokoros</i> Martin <i>et al.</i> , 2018	MG844417	MG844418	Martin <i>et al.</i> (2018a)
<i>Choerodonicola renko</i> Machida, 2014	MG844420	MG844421	Martin <i>et al.</i> (2018a)
<i>Dimerosaccus oncorhynchi</i> (Eguchi, 1931)		FR870262	Shedko <i>et al.</i> (2015)
<i>Discoverytrema gibsoni</i> Zdzitowiecki, 1990		MH161430	Sokolov <i>et al.</i> (2018)
<i>Discoverytrema markowskii</i> Gibson, 1976		MH161431	Sokolov <i>et al.</i> (2018)
<i>Gaevskajatrema perezii</i> (Mathias, 1926)		AF184255	Tkach <i>et al.</i> (2001)
<i>Halosaurotrema halosauropsi</i> (Bray & Campbell, 1996) ^d	AJ287514	AY222207	Cribb <i>et al.</i> (2001); Olson <i>et al.</i> (2003)
<i>Hamacreadium cribbi</i> Bray & Justine, 2016 ^e	KU320590	KU320603	Bray <i>et al.</i> (2016)
<i>Hamacreadium mutabile</i> Linton, 1910		KJ001209	Andres <i>et al.</i> (2014a)
<i>Hamacreadium</i> sp. ^f	KU320588	KU320601	Bray <i>et al.</i> (2016)
<i>Helicometra epinepheli</i> Yamaguti, 1934 ^g	KU320584	KU320597	Bray <i>et al.</i> (2016)
<i>Helicometra equilata</i> (Manter, 1933) ^h	KU320587	KU320600	Bray <i>et al.</i> (2016)
<i>Helicometra manteri</i> Andres <i>et al.</i> , 2014		KJ701238	Andres <i>et al.</i> (2014b)
<i>Holsworthotrema choaderma</i> gen. et sp. nov.	MK052941	MK052938	present study
<i>Holsworthotrema enboubalichthys</i> gen. et sp. nov.	MK052940	MK052937	present study
<i>Maculifer</i> sp.	AY222109	AY222211	Olson <i>et al.</i> (2003)
<i>Macvicaria bartolii</i> Antar <i>et al.</i> , 2014		KR149471	Antar <i>et al.</i> (2015)
<i>Macvicaria crassigula</i> (Linton, 1910)		KJ701237	Andres <i>et al.</i> (2014b)
<i>Macvicaria dubia</i> (Stossich, 1905)		KR149470	Antar <i>et al.</i> (2015)
<i>Macvicaria gibsoni</i> Rima, Marzoug, Pérez-del-Olmo, Kostadinova, Bouderbala & Georgieva, 2017		MF166845	Rima <i>et al.</i> (2017)
<i>Macvicaria maamouriae</i> Antar <i>et al.</i> , 2014		KR149468	Antar <i>et al.</i> (2015)
<i>Macvicaria macassarensis</i> (Yamaguti, 1952)	AJ287533	AY222208	Cribb <i>et al.</i> (2001); Olson <i>et al.</i> (2003)
<i>Macvicaria magellanica</i> Laskowski <i>et al.</i> , 2013		KU212191	Hildebrand <i>et al.</i> (2016)
<i>Macvicaria mormyri</i> (Stossich, 1885)		AF184256	Tkach <i>et al.</i> (2001)
<i>Macvicaria muraenolepidis</i> Zdzitowiecki, 1990		MH161432	Sokolov <i>et al.</i> (2018)
<i>Macvicaria obovata</i> (Mollin, 1859)		JQ694146	Born-Torrijos <i>et al.</i> (2012)
<i>Macvicaria pennelli</i> (Leiper & Atkinson, 1914)		MH892477	Faltýnková <i>et al.</i> , 2017
<i>Macvicaria</i> sp.		MH892476	Faltýnková <i>et al.</i> , 2017
<i>Magnaosimum brooksae</i> Martin <i>et al.</i> , 2018	MG813906	MG813907	Martin <i>et al.</i> (2018b)
<i>Mesobathylebouria lanceolata</i> (Price, 1934) ⁱ		KJ001210	Andres <i>et al.</i> (2014a)
<i>Neolebouria georgiensis</i> Gibson, 1976		MH892478	Faltýnková <i>et al.</i> , 2017
<i>Neoplagioporus ayu</i> (Takahashi, 1928)		KX553947	Fayton and Andres (2016)
<i>Neoplagioporus elongatus</i> (Goto & Ozaki, 1930)		KX553948	Fayton and Andres (2016)
<i>Neoplagioporus zacconis</i> (Yamaguti, 1934)		KX553949	Fayton and Andres (2016)

Table 2. Continued

Taxon	GenBank ID (18S)	GenBank ID (28S)	Reference
<i>Nicolla skrjabini</i> (Iwanitzky, 1928) Dollfus, 1960		MH161438	Sokolov <i>et al.</i> (2018)
<i>Opistholebes amplicoeus</i> Nicoll, 1915	AJ287550	AY222210	Cribb <i>et al.</i> (2001); Olson <i>et al.</i> (2003)
<i>Opecoeloides furcatus</i> (Bremser in Rudolphi, 1819)		AF151937	Tkach <i>et al.</i> (2001)
<i>Opecoeloides fimbriatus</i> (Linton, 1934)		KJ001211	Andres <i>et al.</i> (2014a)
<i>Pacificreadium serrani</i> (Nagaty & Abdel-Aal, 1962)	KU320589	KU320602	Bray <i>et al.</i> (2016)
<i>Pedunculacetabulum inopinipugnus</i>	MF805699	MF805700	Martin <i>et al.</i> (2018c)
<i>Peracreadium idoneum</i> (Nicoll, 1909)	AJ287558	AY222209	Cribb <i>et al.</i> (2001); Olson <i>et al.</i> (2003)
<i>Plagioporus aliffi</i> Fayton <i>et al.</i> , 2017		KX905056	Fayton <i>et al.</i> (2017)
<i>Plagioporus boleosomi</i> (Pearse, 1924)		KX553953	Fayton and Andres (2016)
<i>Plagioporus carolini</i> Fayton <i>et al.</i> , 2018		MG214680	Fayton <i>et al.</i> (2018)
<i>Plagioporus chiliticorum</i> (Barger & Esch, 1999)		KX553943	Fayton and Andres (2016)
<i>Plagioporus fonti</i> Fayton <i>et al.</i> , 2017		KX905054	Fayton <i>et al.</i> (2017)
<i>Plagioporus hageli</i> Fayton & Andres, 2016		KX553950	Fayton and Andres (2016)
<i>Plagioporus ictaluri</i> Fayton <i>et al.</i> , 2018		MG214679	Fayton <i>et al.</i> (2018)
<i>Plagioporus kolipinskii</i> Tracey, Choudhury <i>et al.</i> , 2009		KX553952	Fayton and Andres (2016)
<i>Plagioporus limus</i> Fayton <i>et al.</i> , 2017		KX905055	Fayton <i>et al.</i> (2017)
<i>Plagioporus loboides</i> (Curran <i>et al.</i> , 2007) ⁱ		EF523477	Curran <i>et al.</i> (2007)
<i>Plagioporus shawi</i> (McIntosh, 1939)		KX553951	Fayton and Andres (2016)
<i>Plagioporus sinitsini</i> Mueller, 1934		KX553944	Fayton and Andres (2016)
<i>Podocotyle atomon</i> (Rudolphi, 1802)		MH161437	Sokolov <i>et al.</i> (2018)
<i>Podocotyloides australis</i>	MF805695	MF805696	Martin <i>et al.</i> (2018c)
<i>Podocotyloides gracilis</i> (Yamaguti, 1952) Pritchard, 1966	MF805692	MF805693	Martin <i>et al.</i> (2018c)
<i>Podocotyloides parupenei</i> (Manter, 1962) Pritchard, 1966	MF926408	MF926409	Martin <i>et al.</i> (2018c)
<i>Podocotyloides stenometra</i> Pritchard, 1966	MF926405	MF926406	Martin <i>et al.</i> (2018c)
<i>Propycnadenoides philippinensis</i> Fischthal & Kuntz, 1964	KU320591	KU320604	Bray <i>et al.</i> (2016)
<i>Pseudopecoeloides tenuis</i> Yamaguti, 1940	KU320592	KU320605	Bray <i>et al.</i> (2016)
<i>Pseudopecoelus</i> cf. <i>vulgaris</i>		MH161436	Sokolov <i>et al.</i> (2018)
<i>Pseudopycnadena fischthali</i> Saad-Fares & Maillard, 1986		MF166851	Rima <i>et al.</i> (2017)
<i>Pseudopycnadena tendu</i> Bray & Justine, 2007		FJ788506	Bray and Justine (2009)
<i>Scorpidotrema longistipes</i> Aken'Ova & Cribb, 2003	MK052939	MK052936	present study
<i>Sphaerostoma bramae</i> (Müller, 1776)		MH161435	Sokolov <i>et al.</i> (2018)
<i>Trilobovarium parvatis</i> Martin, Cutmore & Cribb, 2017	KY551561	KY551562	Martin <i>et al.</i> (2017)
<i>Urorchis acheilognathi</i> Yamaguti, 1934		KX553945	Fayton and Andres (2016)
<i>Urorchis goro</i> Ozaki, 1927		KX553946	Fayton and Andres (2016)
<i>Zalophotrema hepaticum</i> Stunkard & Alvey, 1929 (Brachycladiidae)	AJ224884	AY222255	Cribb <i>et al.</i> (2001); Olson <i>et al.</i> (2003)

The following taxa are registered at GenBank under names different to that provided in the table: ^a*Podocotyloides brevis* Andres & Overstreet, 2013 syn. nov.

^b*Bathycreadium elongata* (Maillard, 1970) Bray, 1973, see Pérez-del-Olmo *et al.* (2014).

^c*Allopodocotyle margolisi* Gibson, 1995 syn. nov.

^d*Gaevskajatrema halosauropsi* Bray & Campbell, 1996 syn. nov.

^e*Hamacreadium* sp., see Bray & Justine (2016).

^f*Hamacreadium mutabile* Linton, 1910, see Bray & Justine (2016).

^g*Helicometra fasciata* (Rudolphi, 1819) Odhner, 1902.

^h*Helicometra boseli* Nagaty, 1956, see Blend and Dronen (2015a).

ⁱ*Neolebouria lanceolata* (Price, 1934) Reimer, 1987 syn. nov.

^j*Plagiocirrus loboides* Curran *et al.*, 2007, see Fayton & Andres (2016).

of RAxML v.8 (Stamatakis, 2014) and MrBayes v.3.2.6 (Ronquist *et al.*, 2012) in the CIPRES portal (Miller *et al.*, 2010), with the closest estimation of the GTR+I+ Γ model of evolution, based on the implementation of the Akaike information criterion in PartitionFinder v.1.1.1 (Lanfear *et al.*, 2012). The maximum likelihood analyses ran 1000 bootstrap pseudoreplicates. The Bayesian inference analyses ran four chains and 10 000 000 iterations, at which point the average standard deviation of split frequencies were <0.005. Chains were sampled every 1000 iterations and the first 2500 samples were discarded as burn-in.

RESULTS

IDENTIFICATION OF SPECIES

We discovered infections of opecoelids in three species of *Kyphosus* and also *Scorpid georgiana* Valenciennes (Perciformes: Scorpididae), a closely related and ecologically similar fish, from off Perth in south-west Australia. No other infections of opecoelids were detected among any species of *Kyphosus* examined elsewhere in the Indo-West Pacific. The opecoelid from *S. georgiana* is immediately recognizable as *Scorpidotrema longistipes* Aken'Ova & Cribb, 2003, a distinctive species in a monotypic genus belonging to the Stenakrinae Yamaguti, 1970. It is most obviously distinguished by a small ventral sucker affixed to an exceptionally long peduncle and is known only from *S. georgiana* in south-western Australian waters. The opecoelids recovered from *Kyphosus* fishes clearly represent two congeneric species, with apparently distinct definitive host ranges: one was found only in the western buffalo bream *K. cornelii* (Whitley) and the other in the gladius sea chub *K. gladius* Knudsen & Clements and the silver drummer *K. sydneyanus* (Günther), but not in *K. cornelii*. Molecularly, these two species differ at 2, 7 and 2 base-positions in ITS2, 28S and 18S rDNA, respectively. These two species appear to be new to science. They are consistent with the concept of the Stenakrinae and bear some resemblance to *S. longistipes*, but cannot be adequately accommodated into that or any other existing opecoelid genus. Thus, a new genus, *Holsworthotrema* gen. nov., and two new species are proposed below.

PHYLOGENETIC ASSESSMENT

Scorpidotrema longistipes and the two new species of *Holsworthotrema* formed a strongly supported clade nested within the deep-sea + freshwater Plagioporinae (*s.l.*) clade. The two alignments produced similar topologies for both maximum likelihood and Bayesian inference analyses; a phylogram based on maximum

likelihood analysis of the broader, concatenated alignment is shown in Figure 1. In analyses of both datasets, the deep-sea + freshwater clade was well supported and comprised five distinct, strongly supported subclades, one of which included all of, and only, the represented taxa from freshwater fishes. In analyses based on both datasets, *S. longistipes* and the two new species resolved as sister to *Neolebouria laceolata* + *Podocotyloides brevis* with strong support, and *Bathycreadium brayi* resolved as sister to the freshwater clade with strong support. However, the relative position of the *Allopodocotyle margolisi* + *Buticulotrema thermichthysi* + *Gaeuskajatrema halosauropsi* + *Neolebouria georgiensis* + *Podocotyle atomon* clade differed in the two sets of analyses. In the broader, family-wide analyses, this clade resolved as sister to *B. brayi* + the freshwater taxa, whereas in the analyses restricted to the deep-sea + freshwater clade, it resolved as sister to *N. laceolata* + *P. brevis* and *S. longistipes* + *Holsworthotrema* species. The arrangement in the concatenated, family-wide analyses had better support than that in the narrower analyses considering only 28S data (maximum likelihood probability = 87% vs. 54%).

TAXONOMY

The phylogenetic analyses demonstrate that the deep-sea + freshwater clade now includes representatives presently assigned to three subfamily concepts, the Plagioporinae, Opecoelininae Gibson & Bray, 1984 (*B. thermichthysi*) and Stenakrinae (*S. longistipes*), with the Plagioporinae (*s.l.*) clearly paraphyletic relative to the other two. Thus, either the Plagioporinae must be redefined to accommodate these taxa yet remain distinguishable from nominal plagioporine taxa resolving to the major marine clade, or it must be restricted. If it is to be restricted, nominal plagioporine taxa from deep-sea fishes must be accommodated into either new subfamilies or an expanded concept of the Stenakrinae and/or Opecoelininae. Several species of the type-genus of the Plagioporinae, *Plagioporus*, are represented in the analyses and resolve to the freshwater clade. A strict concept for the subfamily must, therefore, include that clade, but might be expanded to include *B. brayi*, and possibly also the *A. margolisi* + *B. thermichthysi* + *G. halosauropsi* + *N. georgiensis* + *P. atomon* clade, if the broader of the two phylogenetic analyses is considered correct. No solution is immediately appealing, and the problem is exacerbated by the unconvincing classification of several species represented in the analyses; specifically, *A. margolisi*, *G. halosauropsi*, *N. lanceolata* and *P. brevis* are known to not belong in their nominal genera on the basis of sequence data (Bray *et al.*, 2016; Faltýnková *et al.*, 2017; Martin *et al.*, 2018c). Our solution is to propose, in addition to *Holsworthotrema*, four new generic diagnoses

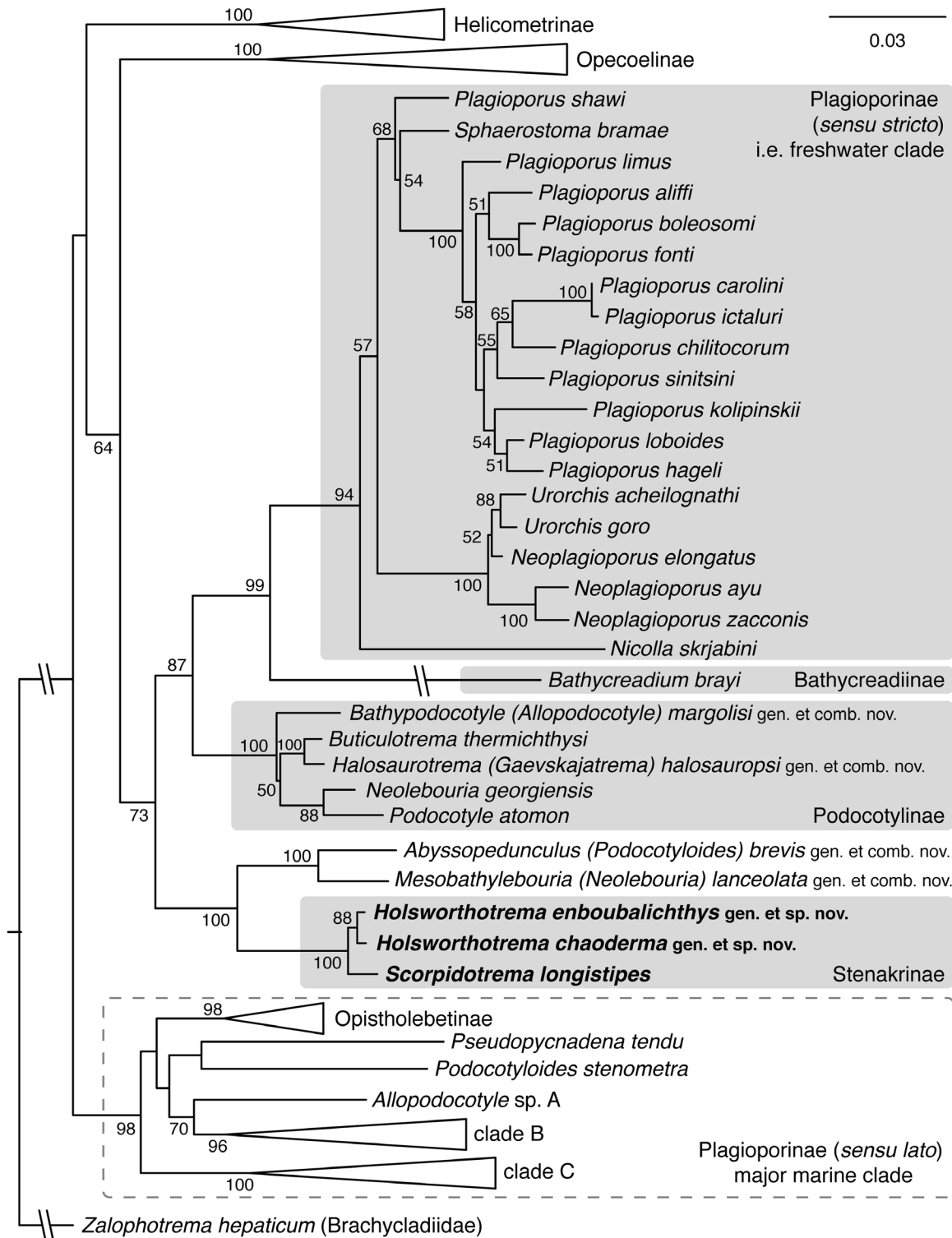


Figure 1. Phylogenetic tree resulting from maximum likelihood analysis of a concatenated 28S + 18S rDNA alignment comprising all data for the Opecoelidae available at GenBank. *Zalophotrema hepaticum* Stunkard & Alvey, 1929 (Brachycladiidae) designated as outgroup. New data in bold. Support probabilities less than 50% omitted. The scale-bar indicates the expected number of substitutions per site. Collapsed clade B includes *Macvicaria macassarensis*, *Pacificreadium serrani*, *Pedunculacetabulum inopinipugnus* and species of *Allopodocotyle*, *Bentholebouria*, *Cainocreadium*, *Hamacreadium* and *Podocotyloides*. Collapsed clade C includes *Macvicaria magellanica*, *M. pennelli*, *M. sp.* of Faltýnková *et al.* (2017), *Podocotyloides parupenei*, *Trilobovarium parvatis* and species of *Choerodonicola*. References for data retrieved from GenBank listed in Table 2.

below, one for each of these taxa, *Bathypodocotyle* gen. nov., *Halosaurotrema* gen. nov., *Mesobathylebouria* gen. nov. and *Abyssopedunculus* gen. nov., respectively. We also propose a new subfamilial arrangement for the deep-sea + freshwater clade, whereby the concept of the Plagioporinae is considered restricted to the freshwater taxa, *S. longistipes* + the new species of *Holsworthotrema* are recognized as representatives of the Stenakrinae, and *B. thermichthysi* is not considered to represent the Opecoelinae, because it is evidently closely related to species that possess a cirrus-sac and because there is no indication of a close relationship with species of *Opecoelina* Manter, 1943, the type-genus. Thus, recognition of three further subfamily concepts is required. On the basis of combined evidence from phylogeny, morphology and ecology, we propose one new subfamily here, the Bathycreadiinae, for *Bathycreadium*, and resurrection of the Podocotylinae Dollfus, 1960, for *Podocotyle* Dujardin, 1845, *Bathypodocotyle*, *Buticulotrema* Blend et al., 1993, *Halosaurotrema*, *Neolebouria* Gibson, 1976 and also *Macrourimegatrema* Blend et al., 2004 and *Tellervotrema* Gibson & Bray, 1982, two genera comprising species from deep-sea fishes for which no sequence data are yet available. We do not propose a new subfamily for *Abyssopedunculus* + *Mesobathylebouria*, because there is not yet a satisfactory morphological or ecological basis for uniting the two yet distinguishing such a concept from, in particular, that of the Podocotylinae and the Plagioporinae (s.s.).

PHYLUM PLATYHELMINTHES MINOT, 1876

CLASS TREMATODA RUDOLPHI, 1808

SUBCLASS DIGenea CARUS, 1863

ORDER PLAGIORCHIIDA LA RUE, 1957

FAMILY OPECOELIDAE OZAKI, 1925

SUBFAMILY STENAKRINAE YAMAGUTI, 1970

GENUS *SCORPIDOTREMA* AKEN'OVA & CRIBB, 2003

SCORPIDOTREMA LONGISTIPES AKEN'OVA & CRIBB, 2003

(FIG. 2; TABLE 3)

Type-host: *Scorpiis georgiana* Valenciennes (Perciformes: Scorpididae), banded sweep.

Type-locality: Off Point Peron, Western Australia.

Voucher material: One hologenophore, mounted dorso-laterally, from *S. georgiana* collected off Garden Island (32°12'46"S, 115°40'13"E), Western Australia (WAM V8938).

Site in host: Intestine.

Prevalence: 1 of 16 (6%).

Representative DNA sequences: One sequence generated and deposited at GenBank for each of three regions: partial 5.8S-ITS2-partial 28S rDNA (MK052933), partial 28S (MK052936), partial 18S (MK052939).

Description (based on a single, dorso-lateral hologenophore): Body elongate-oval, dorso-ventrally flattened, broadest in mid-hindbody, tapering slightly to bluntly rounded posterior. Tegument smooth in forebody, finely textured with small, regular projections in hindbody. Forebody short, less than one-fifth body length. Oral sucker subterminal, opens antero-ventrally, roughly spherical; body wall forms distinct pre-oral dorsal lobe. Ventral sucker pedunculate with exceptionally long peduncle with regular annular thickenings, exceptionally small, subspherical with length greater than depth. Prepharynx short. Pharynx ellipsoidal, smaller than oral sucker. Oesophagus very short. Intestine bifurcates in forebody at broad angle. Caeca thin, terminate blindly in close proximity near posterior extremity.

Testes smooth, ellipsoidal with length greater than width/depth, tandem, medial, intercaecal, contiguous, situated in posterior half of body, subequal with posterior testis larger; post-testicular zone about one-sixth body length. Cirrus-sac well developed, claviform, somewhat sinuous anteriorly, long, occupying more than one-quarter body length, extending well into hindbody. Seminal vesicle long, occupying more than half cirrus-sac length, broad, narrowing distally. Pars-prostatica indistinct. Ejaculatory duct long, thin, gently sinuous. Common genital atrium very short, simple. Genital pore small, slightly sinistro-submedial, bifurcal.

Ovary smooth, roughly spherical, medial, intercaecal, smaller than testes, separated from anterior testis by distinct gap, situated about one-half body length from anterior extremity. Seminal receptacle uterine. Laurer's canal present, short, opening antero-dorsal to ovary, widening proximally into small, receptacle-like chamber. Oviduct short. Oötype prominent. Uterus sinuous, entirely anterior to ovary, ascending ventral to cirrus-sac then becoming dorsal to ejaculatory duct. Metraterm thin to indistinct. Mehlis' gland prominent, extensive, antero-dorsal to ovary. Vitelline reservoir smaller than and antero-dorsal to ovary. Vitelline follicles numerous, regular, restricted to hindbody, reaching well beyond ovary but not to level of ventral sucker, distributed uninterrupted laterally and ventrally, mostly absent dorsally except patchily in post-testicular zone, becoming confluent ventrally in post-testicular zone and also in uterine and intergonadal zones. Eggs oval, operculate, tanned, unembryonated *in utero*, sometimes with slight polar protuberance at one end.

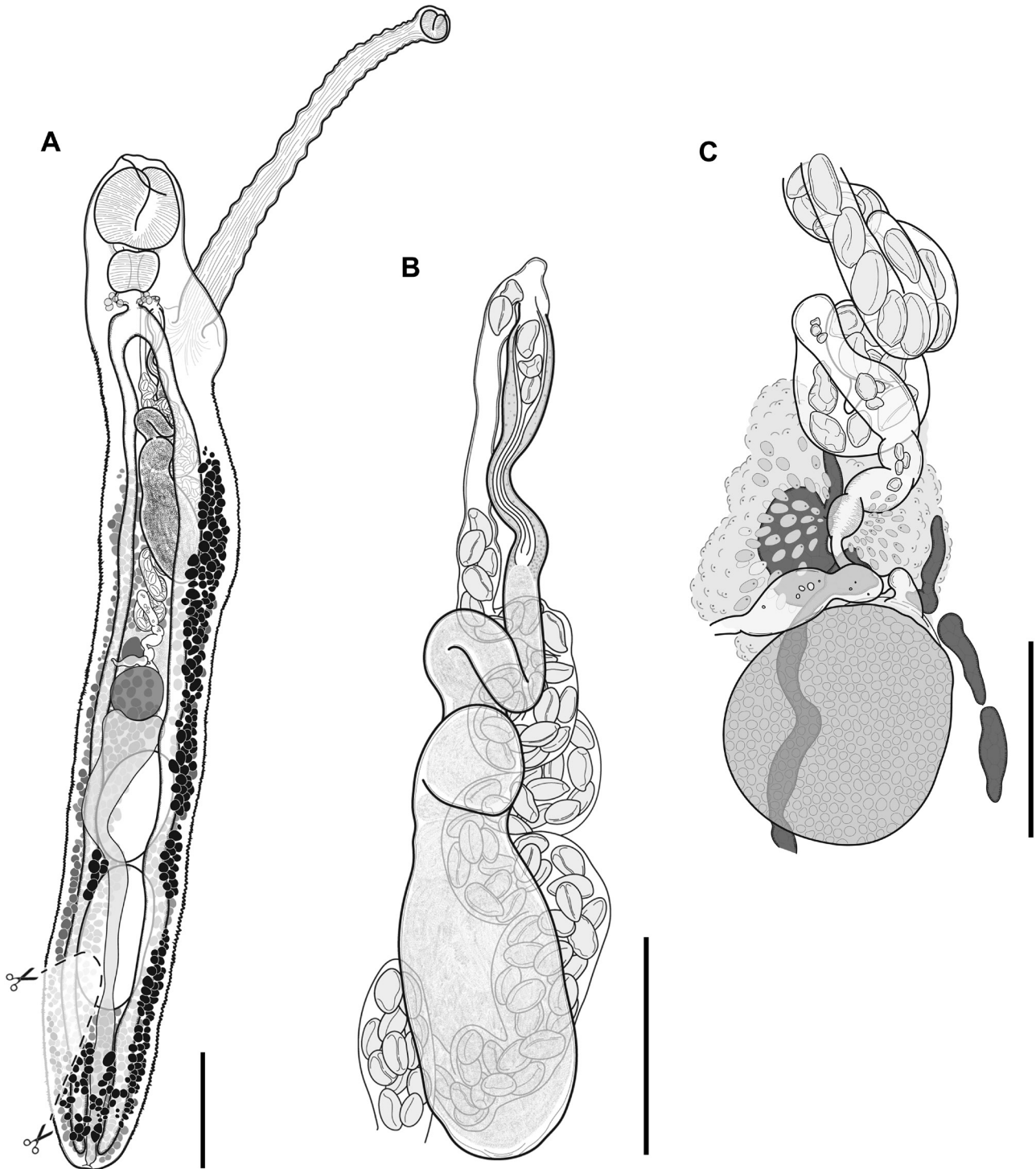


Figure 2. *Scorpionidotrema longistipes* Aken'Ova & Cribb, 2003, dorso-lateral perspective of voucher and hologenophore ex *Scorpiis georgiana*. A, whole worm (excised portion indicated by scissors symbol). B, terminal genitalia. C, ovarian complex. Scale-bars: A, 500 μ m; B, 300 μ m; C, 200 μ m.

Excretory vesicle tubular, extends to posterior margin of ovary, becoming more dilate anteriorly. Excretory pore terminal.

Remarks: Our single specimen of *S. longistipes* is consistent with the description provided by [Aken'Ova & Cribb \(2003\)](#) and was recovered from the same

Table 3. Morphometric data for *Scorpidotrema longistipes* and two new species of *Holsworthotrema* **gen. nov.**, expressed in micrometres, as percentages or as ratios. Egg measurements represent the average of multiple subsamples per specimen

	<i>S. longistipes</i>	<i>S. longistipes</i>	<i>H. enboubalichthys</i>	<i>H. chaoderma</i>
<i>N</i>	1 (this study)	8 (Aken'Ova & Cribb, 2003)	13	11
BL	4299	2786–3910 (3434)	2483–3990 (3179)	2132–2988 (2562)
BW	-	455–516 (474)	216–339 (264)	207–282 (252)
BD	521	-	248–318 (278)	157–296 (208)
FBL	756	748–1022 (905)	298–506 (376)	322–561 (413)
FBL/BL	18%	25–27 (26)%	10–14 (12)%	14–19 (16)%
OSL	349	250–302 (276)	119–178 (148)	116–153 (134)
OSW	-	243–283 (267)	156–175 (166)	156
OSD	349	-	136–185 (156)	127–164 (149)
OSD/OSL	1	-	0.98–1.17 (1.06)	0.96–1.38 (1.13)
VSL	131	89–120 (104)	148–204 (178)	179–206 (191)
VSW	-	90–117 (105)	169–227 (197)	-
VSD	110	-	150–200 (174)	189–229 (206)
PedL	1667	1153–1474 (1332)	90–223 (144)	146–288 (205)
PedL/BL	39%	37–42 (40)%	3–6 (5)%	5–11 (8)%
VSL/VSD	1.19%	-	0.91–1.11 (1)	0.85–1.02 (0.93)
VSL/OSL	0.38	-	1.08–1.35 (1.21)	1.2–1.62 (1.44)
VSW/OSW	-	0.36–0.42 (0.39)	0.96–1.11 (1.04)	-
VSD/OSD	0.31	-	0.97–1.24 (1.13)	1.25–1.6 (1.38)
PrePh	52	-	16–48 (24)	17–43 (32)
PhL	166	118–154 (144)	67–113 (81)	79–113 (89)
PhW	-	113–156 (137)	90, 99 (94)	96
PhD	221	-	73–109 (93)	84–108 (98)
PhD/PhL	1.33	-	0.8–1.35 (1.15)	0.89–1.29 (1.1)
OSL/PhL	2.1	-	1.58–2.09 (1.83)	1.28–1.8 (1.52)
OSW/PhW	-	1.8–2.2 (2)	1.73, 1.76 (1.75)	1.63
OSD/PhD	1.58	-	1.5–2.06 (1.69)	1.46–1.62 (1.53)
Oe/BL	2%	-	1–3 (2)%	2–3 (2)%
PreBif/BL	15%	-	8–11 (10)%	10–13 (12)%
PreVit/BL	31%	-	21–34 (26)%	28–26 (31)%
PostC/BL	2%	-	2–3 (2)%	1–2 (2)%
ATL	496	373–477 (423)	171–359 (290)	191–263 (226)
ATW	-	258–326 (283)	131–246 (187)	127–160 (145)
ATD	292	-	130–221 (178)	102–138 (120)
ATL/ATD	1.7	-	1.32–2.08 (1.7)	1.55–1.93 (1.81)
PTL	624	473–559 (516)	212–423 (340)	191–313 (248)
PTW	-	237–326 (275)	121–235 (184)	145–169 (160)
PTD	302	-	161–221 (195)	120–146 (131)
PTL/PTD	2.07	-	1.33–2.19 (1.79)	1.58–2.01 (1.77)
PTL/ATL	1.26	-	1.01–1.4 (1.18)	0.98–1.2 (1.09)
PTD/ATD	1.03	-	0.98–1.41 (1.11)	0.98–1.27 (1.09)
PreT/BL	59%	-	56–63 (60)%	60–66 (63)%
PostT/BL	16%	14–15 (14)%	16–22 (20)%	16–24 (19)%
CSL	1491	1019–1140 (1075)	1213–2033 (1626)	1265–2142 (1596)
CSØ	233	127–170 (149)	66–111 (94)	38–78 (67)
CSL/BL	35%	-	45–67 (51)%	45–74 (62)%
CS occupies	1189	-	921–1572 (1191)	802–1398 (1021)
CS occupies/BL	28%	-	33–41 (37)%	37–47 (40)%
SVL/CSL	73%	-	16–39 (29)%	15–38 (26)%
GA	54	-	20–57 (35)	18–34 (25)

Table 3. Continued

	<i>S. longistipes</i>	<i>S. longistipes</i>	<i>H. enboubalichthys</i>	<i>H. chaoderma</i>
PreGP/BL	15%	10–17 (14)%	9–12 (10)%	11–15 (13)%
OvL	228	163–205 (192)	100–191 (148)	74–140 (109)
OvW	-	155–215 (178)	86–172 (136)	95–110 (104)
OvD	233	-	111–170 (133)	56–87 (75)
PreOv/BL	51%	-	50–55 (53)%	54–61 (56)%
Ov to CS/BL	9%	-	3–8 (5)%	1–5 (3)%
EggL	59	43–61 (52)	52–60 (56)	53, 53
EggW	35	23–36 (28)	28–34 (31)	25, 30 (28)

Abbreviations: L, length; W, width; D, depth; B, body; FB, forebody; OS, oral sucker; VS, ventral sucker; Ped, ventral sucker peduncle; PrePh, prepharynx; Ph, pharynx; Oe, oesophagus; Bif, intestinal bifurcation; Vit, vitelline zone; C, caeca; AT, anterior testis; PT, posterior testis; T, testicular zone; CS, cirrus-sac; SV, seminal vesicle; GA, genital atrium; GP, genital pore; Ov, ovary.

host species and in the same region. Our specimen is significantly larger and has a relatively shorter forebody (Table 3). We confirm that there is no canalicular seminal receptacle in this species, although a small dilation of the Laurer's canal is apparent. We also confirm that the caeca do not unite to form a uroproct but terminate blindly close to one-another. Aken'Ova & Cribb (2003) did not mention texturing of the tegument, but we observed small, regular projections in the hindbody. In our specimen, a small number of eggs appear to have entered the distal region of the ejaculatory duct via the common genital atrium. We note that Aken'Ova & Cribb (2003) appear to have illustrated the same unusual but probably unimportant condition.

HOLSWORTHOTREMA GEN. NOV.

Diagnosis: Body elongate, subcylindrical. Forebody short, less than one-fifth body length. Tegument smooth in forebody, textured with fine projections in hindbody. Oral sucker unspecialized, terminal, opening antero-ventrally, roughly spherical. Ventral sucker larger than oral sucker, roughly spherical, pedunculate, papillate with 7–9 small, round papillae either side of aperture. Prepharynx very short. Pharynx unspecialized, smaller than oral sucker, subspherical. Oesophagus short, constricted. Intestine bifurcates at approximately 45° in forebody. Caeca thin, unite with excretory vesicle to form uroproct close to posterior extremity. Testes two, ellipsoidal, smooth, tandem, usually separate, intercaecal, situated more than 50% of body length from anterior extremity; post-testicular zone less than 25% of body length. Cirrus-sac well developed, exceptionally long, highly sinuous, extends well into hindbody, occupies more than 33% of body length. Internal seminal vesicle long, sinuous, broad, narrowing anteriorly. Pars-prostatica indistinct. Ejaculatory duct exceptionally long, tightly sinuous. Cirrus sometimes everted. Genital atrium short, simple. Genital pore sinistro-submedial, bifurcal or immediately post-bifurcal, small, often indistinct. Ovary smooth,

roughly spherical, medial, anterior to and separated by short distance from anterior testis, situated about 50% of body length from anterior extremity. Seminal receptacle uterine. Laurer's canal present, short, opening dorsal to ovary, widening proximally into small, receptacle-like chamber. Oviduct short. Oötype prominent. Uterus sinuous, preovarian, intercaecal, ascending dorsal to cirrus-sac. Metraterm thin to indistinct. Mehlis' gland prominent, extensive, antero-dorsal to ovary. Vitelline reservoir smaller than and antero-dorsal to ovary. Vitelline follicles numerous, regular, restricted to hindbody, distributed laterally and ventrally, sparse dorsally, interrupted at level of ovary and each testis. Eggs operculate, tanned, unembryonated *in utero*, without polar protuberances or filaments. Excretory vesicle tubular, extends to level of ovary. Excretory pore terminal or dorso-subterminal. In the digestive tract of herbivorous, marine fishes of the genus *Kyphosus* (Kyphosidae) in southern Australian waters.

Type-species: *Holsworthotrema enboubalichthys* sp. nov.

Other species: *Holsworthotrema chaoderma* sp. nov.

ZooBank registration LSID: <http://www.zoobank.org/urn:lsid:zoobank.org:act:30C5014D-2AF3-447E-900A-5861F640397A>

Etymology: The new genus is named in honour of and in gratitude to Dr William (Bill) and Carol Holsworth. The Holsworth foundation has generously supported Ph. D. candidatures of SBM and DCH, has directly enabled this study, and has contributed to various other student projects undertaken at the Marine Parasitology Research Laboratory, The University of Queensland.

Remarks: The two new species for which we propose *Holsworthotrema* occur in sympatry with *S. longistipes* in phylogenetically related and ecologically similar host fishes. They are clearly closely related to

that species, differing at 9, 16 to 17, and 8 to 9 base-positions in the ITS2, 28S and 18S rDNA regions, respectively. The two new species also share several morphological features with *S. longistipes*, including an elongate body, short forebody, specialized tegument, pedunculate ventral sucker, long cirrus-sac extending well into the hindbody, long seminal vesicle, entire ovary, prev ovarian uterus, uterine seminal receptacle and vitelline follicles restricted to the hindbody and not reaching to the level of the posterior margin of the ventral sucker. However, several important morphological distinctions are also apparent such that these two new species should be allocated to a distinct genus. Most importantly, the caeca form a uroproct in the new species but terminate blindly in *S. longistipes*, a distinction always considered of generic significance among the Opecoelidae (Cribb, 2005), and usually also among digeneans more generally. Additionally, in the new species the uterus runs dorsal vs. ventral to the cirrus-sac, the cirrus-sac and ejaculatory duct are much longer and prominently sinuous, the ventral sucker is larger than the oral sucker vs. smaller, the ventral sucker peduncle is relatively short and broad vs. exceptionally long and thin, and the ventral sucker has papillae.

The two new species are also similar morphologically to another stenakrine concept, *Pseudopecoelina* Yamaguti, 1942. Species of that genus do have a uroproct and essentially differ from the new species only in that the ventral sucker lacks papillae and is sessile vs. pedunculate, and in that the cirrus-sac and ejaculatory duct, although long and tubular, are never as long as in the new species and are simple vs. strongly sinuous. There has been no suggestion of any tegument specializations in species of *Pseudopecoelina*, although none has been examined with SEM. Species of *Pseudopecoelina* are known only from shallow-water carnivorous fishes in the Tropical Indo-West Pacific. The type-species, *P. dampieriae* Yamaguti, 1942, is known only from the firetail dottyback *Labracinus cyclophthalmus* (Müller & Troschell) (Perciformes: Pseudochromidae) [as *Dampiera hellmuthi* (Bleeker)] in Japanese waters (Yamaguti, 1942). Therefore, it seems likely that the two new species will prove to be more closely related to *S. longistipes* than to species of *Pseudopecoelina*.

HOLSWORTHOTREMA ENBOUBALICHTHYS SP. NOV.

(FIGS 3, 4; TABLE 3)

Type-host: *Kyphosus cornelii* (Whitley) (Perciformes: Kyphosidae), western buffalo bream.

Type-locality: Off Garden Island (32°12'46"S, 115°40'13"E), seaward side, Western Australia.

Other localities: Off Point Peron (32°15'59"S, 115°41'03"E), Rockingham, Western Australia.

Type-material: Holotype (WAM V8939) and 14 paratypes (WAM V8940–8953), including two ventral mounts (WAM V8950, V8950) and two hologenophores (WAM V8952, V8953).

Site in host: Pyloric caeca.

Prevalence: 6 of 7 (86%).

Representative DNA sequences: One sequence deposited for partial 5.8S-ITS2-partial 28S rDNA (MK052934), representative of two identical replicates; one sequence each for partial 28S (MK052937) and partial 18S (MK052940).

ZooBank registration LSID: <http://www.zoobank.org/urn:lsid:zoobank.org:act:762A377D-F8CF-4D78-9508-247DF7CAAF3E>

Etymology: The specific name is a compound word, to be treated as a noun in apposition, derived from Greek *en-*, in, *boubalos*, buffalo, and *ichthys*, fish. The name refers to the apparent specialization of this species for *Kyphosus cornelii*, the western buffalo bream, a relatively distinctive species among the otherwise similar *Kyphosus* fishes.

Description (based on 11 lateral mounts, 2 ventral mounts, 2 hologenophores and SEM photographs of 3 specimens): Body elongate, broadest in mid-hindbody, tapering slightly posteriorly. Tegument smooth in forebody, finely textured with regular annular ridges in hindbody. Forebody short, less than one-sixth body length. Oral sucker terminal, opens antero-ventrally, roughly spherical. Ventral sucker papillate with about eight papillae set a short distance from anterior and posterior margins of aperture, pedunculate with short broad peduncle, larger than oral sucker, ellipsoidal with depth greater than length. Prepharynx short. Pharynx ellipsoidal, smaller than oral sucker. Oesophagus short, sinuous. Intestine bifurcates in forebody at 45° angle. Caeca unite with excretory vesicle to form uroproct near posterior extremity.

Testes smooth, ellipsoidal with length greater than width and depth, tandem, medial, intercaecal, usually separated by distinct gap, situated in posterior half of body, subequal with posterior testis slightly larger; post-testicular zone about one-fifth body length. Cirrus-sac well developed, sinuous, exceptionally long, occupying at least one-third body length, extending well into hindbody. Seminal vesicle long, broad, narrowing distally. Pars-prostatica indistinct. Ejaculatory duct exceptionally long,

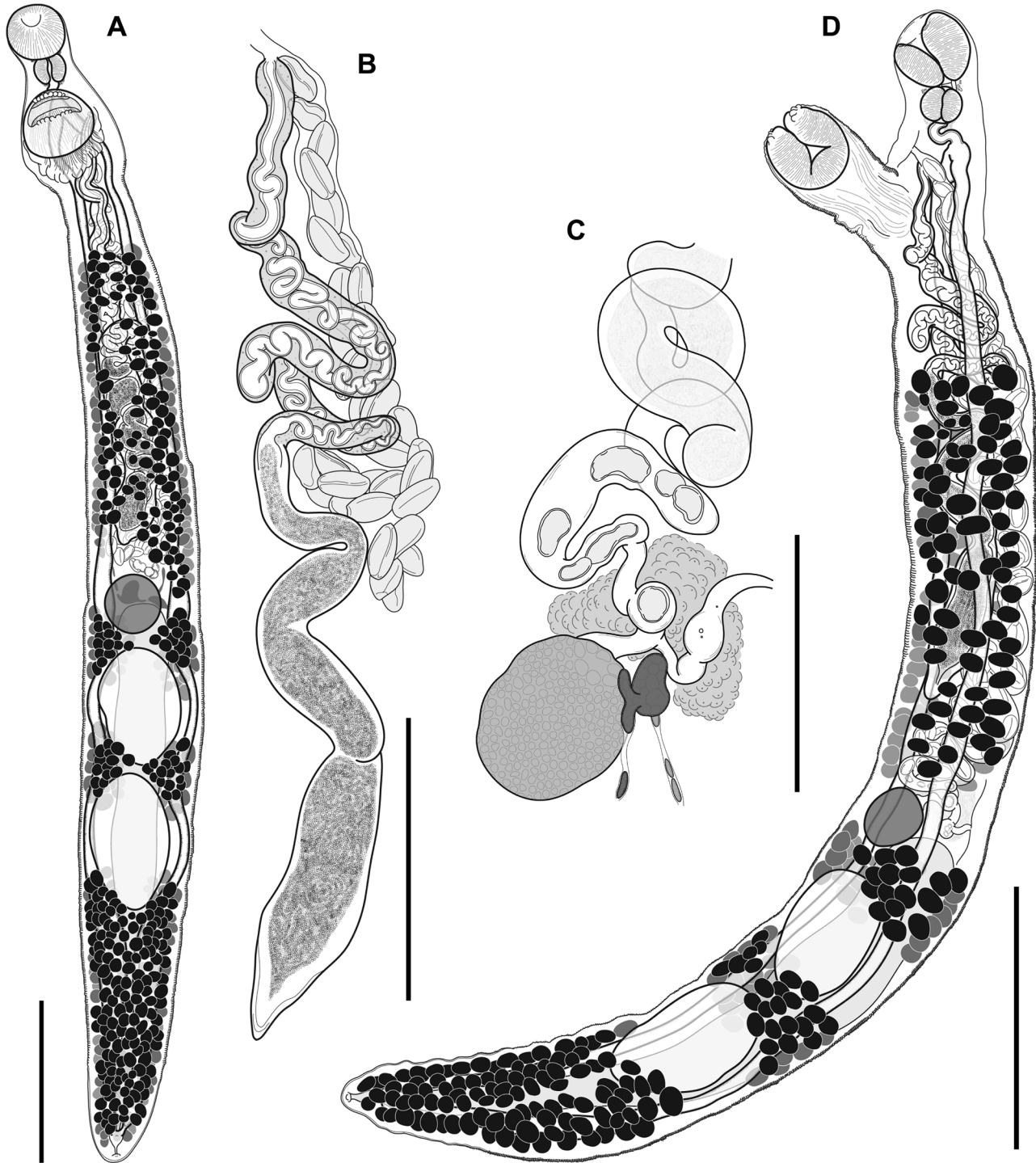


Figure 3. *Holsworthotrema enboubalichthys* gen. et sp. nov. ex *Kyphosus cornelii*. A, whole worm, paratype, ventral perspective. B, terminal genitalia of holotype, lateral perspective. C, ovarian complex of paratype, lateral perspective. D, whole worm, holotype, lateral perspective. Scale-bars: A, D, 500 µm; B, 300 µm; C, 200 µm.

strongly contorted. Cirrus sometimes everted. Common genital atrium very short, simple. Genital pore small, sinistro-submedial, bifurcal to immediately post-bifurcal.

Ovary smooth, roughly spherical, medial, intercaecal, smaller than testes, separated from anterior testis by distinct gap, situated at least one-half body

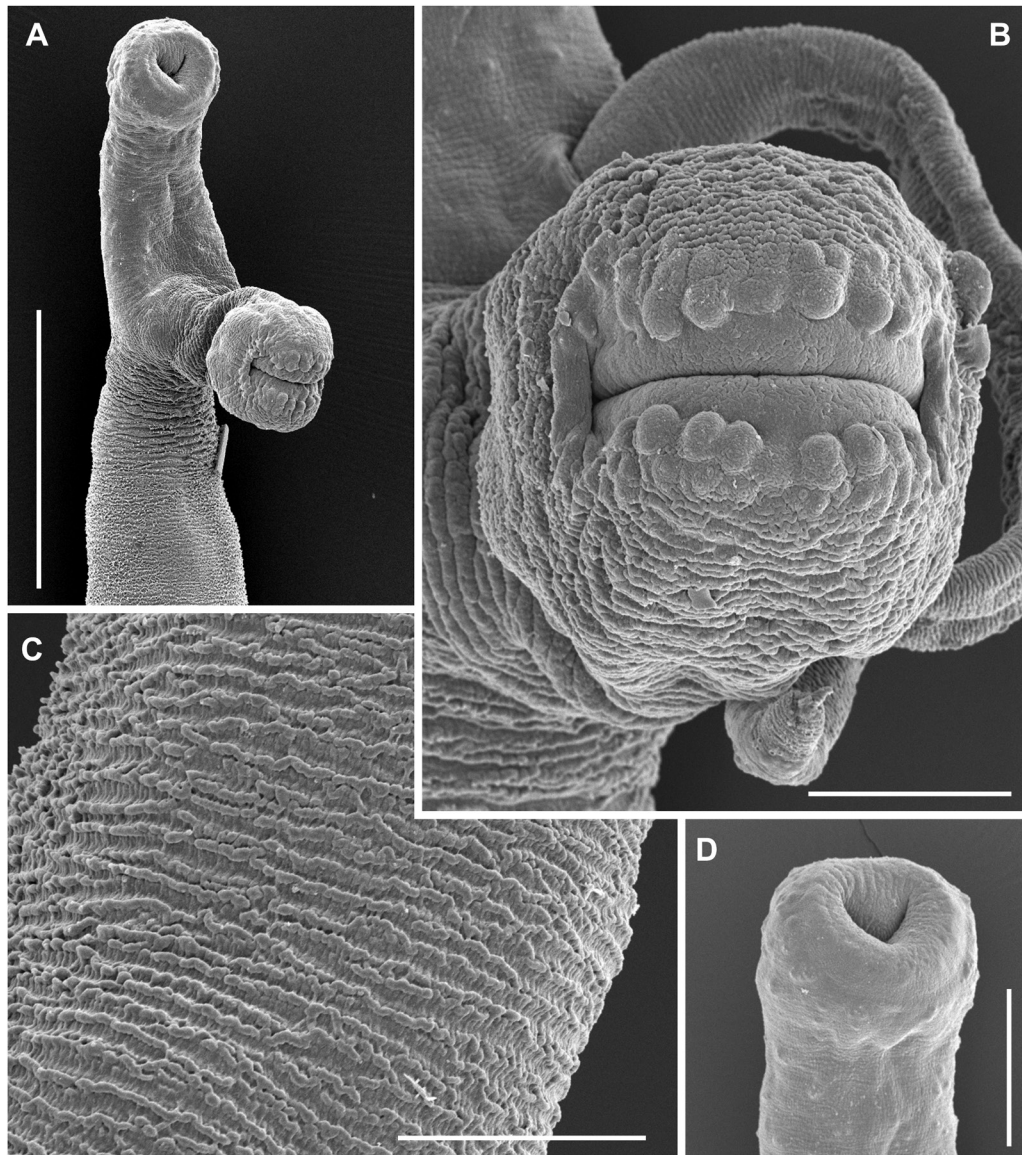


Figure 4. *Holsworthotrema enboubalichthys* gen. et sp. nov. ex *Kyphosus cornelii*, scanning electron micrographs. A, anterior portion, ventro-lateral perspective, note smooth tegument in forebody vs. textured in hindbody. B, ventral sucker, ventral perspective, with everted cirrus in background, note papillae set-back from aperture. C, tegument, mid-hindbody, note regularity of projections. D, oral sucker, ventral perspective. Scale-bars: A, 300 µm; B, C, 50 µm; D, 100 µm.

length from anterior extremity. Seminal receptacle uterine. Laurer's canal present, short, opening dorsal to ovary, widening proximally into small, receptacle-like chamber. Oviduct short. Oötype prominent. Uterus sinuous, entirely anterior to ovary, ascending dorsal to cirrus-sac. Metraterm thin to indistinct. Mehlis' gland prominent, extensive, antero-dorsal to ovary. Vitelline reservoir smaller than and antero-dorsal to ovary. Vitelline follicles numerous, regular, restricted to hindbody, distributed laterally and ventrally, sparse dorsally, interrupted at level of ovary

and each testis. Eggs operculate, tanned, unembryonated *in utero*, without polar protuberances or filaments. Excretory vesicle tubular, extends to level of ovary. Excretory pore dorso-subterminal.

Remarks: Based on our sampling of *Kyphosus* fishes and the relative distinctiveness of *K. cornelii*, it seems likely that *H. enboubalichthys* is oioxenous, i.e. restricted to a single definitive host species. *Kyphosus cornelii* is restricted to shallow rocky and reef flat habitats along the coast of Western Australia,

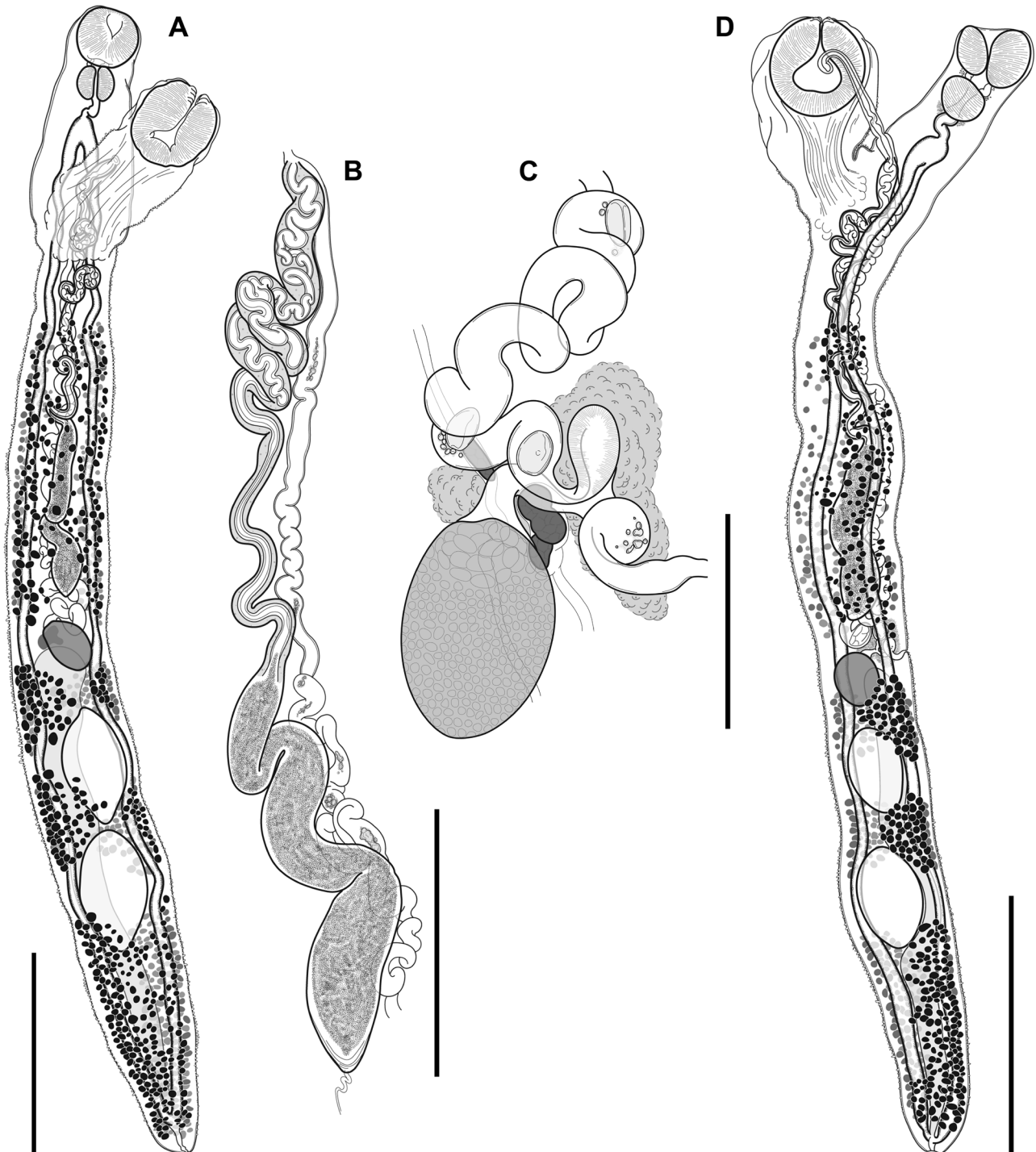


Figure 5. *Holsworthotrema chaoderma* gen. et sp. nov. A, whole worm, paratype ex *Kyphosus sydneyanus*, ventral perspective. B, terminal genitalia of paratype ex *Kyphosus gladius*, lateral perspective. C, ovarian complex of paratype ex *Kyphosus gladius*, lateral perspective. D, whole worm, holotype ex *Kyphosus gladius*, lateral perspective. Scale-bars: A, D, 500 µm; B, 300 µm; C, 100 µm.

ranging from Ningaloo Reef to Geographe Bay, south of Perth (Knudsen & Clements, 2013b). Thus, *H. enboubalichthys* is likely to be endemic to Western

Australia, although it is unclear whether this species exploits its host across the entirety of its range or is instead restricted to colder, southern waters.

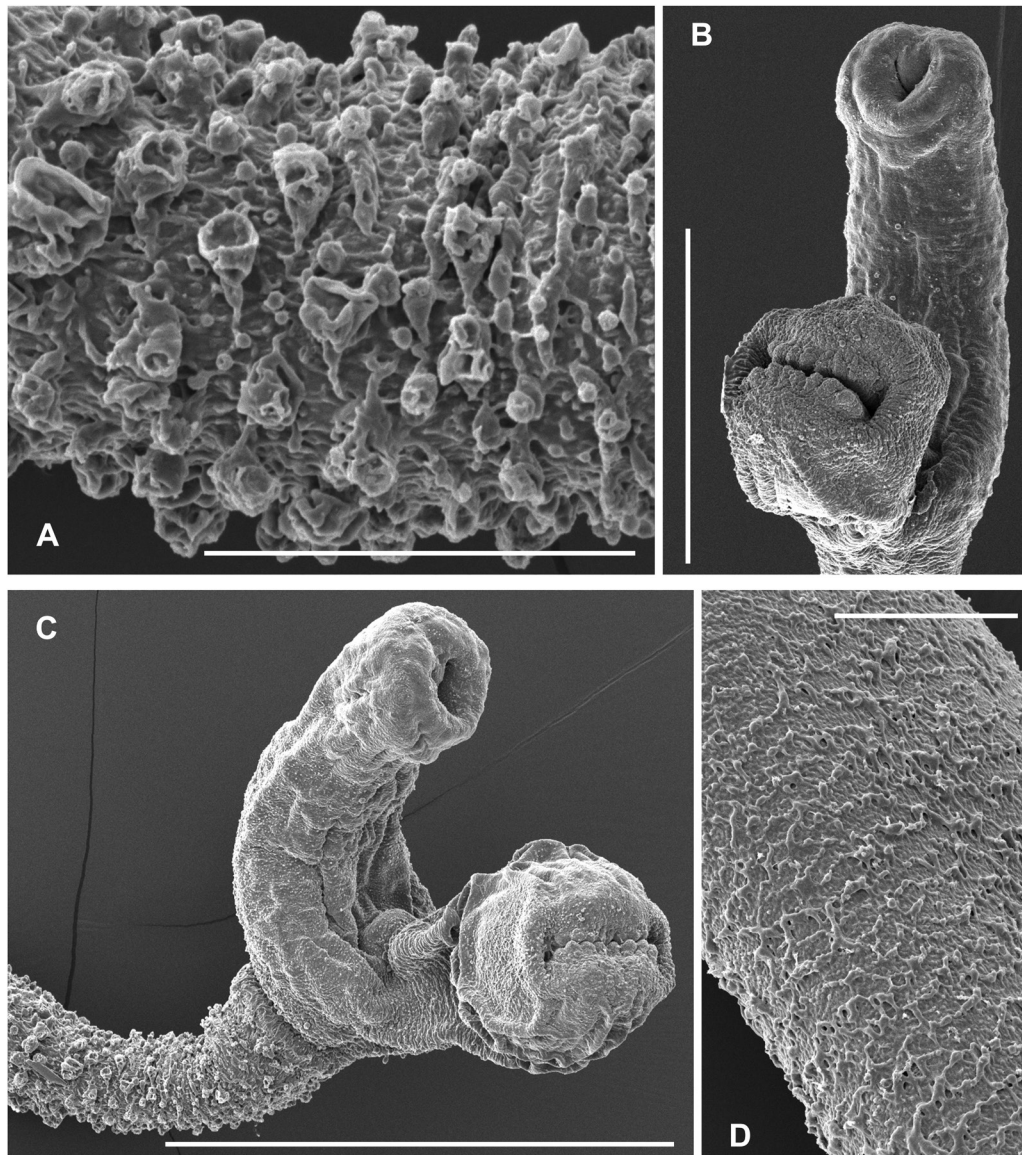


Figure 6. *Holsworthotrema chaoderma* gen. et sp. nov. ex *Kyphosus gladius*, scanning electron micrographs. A, tegument, mid-hindbody, note irregularity of projections. B, anterior portion, ventro-lateral perspective, note smooth tegument in forebody and ventral sucker papillae at aperture margin. C, anterior portion. Lateral perspective. D, tegument, mid-hindbody, note variation compared with A. Scale-bars: A, D, 50 µm; B, 200 µm; C, 300 µm.

HOLSWORTHOTREMA CHAODERMA SP. NOV.

(FIGS 5, 6; TABLE 3)

Type-host: *Kyphosus gladius* Knudsen & Clements, 2013 (Perciformes: Kyphosidae), gladius sea chub.

Type-locality: Off Point Peron (32°15'59"S, 115°41'03"E), Rockingham, Western Australia.

Other host–locality combinations: *Kyphosus sydneyanus* (Günther), silver drummer, Thomson Bay (31°59'51"S, 115°32'59"E), off Rottnest Island, Western Australia.

Type-material: Holotype (WAM V8954) and 11 paratypes (WAM V8955–V8965), including one ventral mount (WAM V8964) and one hologenophore (WAM V8965).

Site in host: Pyloric caeca.

Prevalence: 1 of 1 *K. gladius*; 2 of 3 (66%) *K. sydneyanus*.

Representative DNA sequences: One sequence deposited for partial 5.8S-ITS2-partial 28S rDNA (MK052935), representative of two identical replicates,

one each from specimens recovered from *K. gladius* and *K. sydneyanus*; one sequence each for partial 28S (MK052938) and partial 18S (MK052941).

ZooBank registration LSID: <http://www.zoobank.org/urn:lsid:zoobank.org:act:08607F03-6EA6-4671-84A9-96988A83812F>

Etymology: The specific name is a compound word to be treated as a noun in apposition, derived from Greek, *chaos*, meaning without order, and *derma*, the skin. The name refers to the irregularity of the tegumental projections in this species, especially in comparison with those of the type-species.

Description (based on 8 lateral mounts, 2 ventrolateral mounts, 1 ventral mount, 1 hologenophores and SEM photographs of 4 specimens): Body elongate, broadest in mid-hindbody, tapering slightly posteriorly. Tegument smooth in forebody, finely textured with irregular projections in hindbody. Forebody short, less than one-fifth body length. Oral sucker terminal, opens antero-ventrally, subspherical with depth usually greater than length. Ventral sucker papillate with about 8 papillae lining anterior and posterior margins of aperture, pedunculate with short broad peduncle surrounded by loose tegumental folds, larger than oral sucker, ellipsoidal with depth greater than length. Prepharynx short. Pharynx ellipsoidal, smaller than oral sucker. Oesophagus short, sinuous. Intestine bifurcates in forebody at 45° angle. Caeca unite with excretory vesicle to form uroproct near posterior extremity.

Testes smooth, ellipsoidal to fusiform with length greater than width and depth, tandem, medial, intercaecal, usually separated by distinct gap, situated in posterior half of body, subequal with posterior testis slightly larger; post-testicular zone about one-fifth body length. Cirrus-sac well developed, sinuous, exceptionally long, occupying more than one-third body length, extending well into hindbody. Seminal vesicle long, broad, narrowing distally. Pars-prostatica indistinct. Ejaculatory duct exceptionally long, strongly contorted. Cirrus sometimes everted. Common genital atrium very short, simple. Genital pore small, sinistro-submedial, bifurcal to immediately post-bifurcal.

Ovary smooth, ellipsoidal, medial, intercaecal, smaller than testes, separated from anterior testis by distinct gap, situated in posterior half of body. Seminal receptacle uterine. Laurer's canal present, short, opening dorsal to ovary, widening proximally into small, receptacle-like chamber. Oviduct short. Oötype prominent. Uterus sinuous, entirely anterior to ovary, ascending dorsal to cirrus-sac. Metraterm thin to indistinct. Mehlis' gland prominent, extensive,

antero-dorsal to ovary. Vitelline reservoir smaller than and antero-dorsal to ovary. Vitelline follicles numerous, regular, restricted to hindbody, distributed laterally and ventrally, sparse dorsally, interrupted at level of ovary and each testis. Eggs operculate, tanned, unembryonated *in utero*, without polar protuberances or filaments. Excretory vesicle tubular, extends to level of ovary. Excretory pore terminal.

Remarks: *Holsworthotrema chaoderma* is reliably distinguished from *H. enboubalichthys* based on the combination of several subtle qualitative traits and morphometric differences. In *H. choaderma*, the vitelline follicles are smaller and less dense, the ventral sucker peduncle tegument has the appearance of loose folds, the ventral sucker papillae are situated at the aperture vs. set back from the aperture, the tegumental projections are irregular vs. regular, the excretory pore is terminal vs. dorso-subterminal, and the eggs are consistently few, often none. Morphometrically, *H. chaoderma* is smaller, has a relatively longer forebody, less equal suckers, a relatively longer preovarian zone, and a relatively longer ventral sucker peduncle. In addition to morphological differences, *H. choaderma* is clearly distinct from *H. enboubalichthys* molecularly, differing at 2, 7 and 2 base-positions in ITS2, 28S and 18S rDNA, respectively.

GENERA OF UNDIAGNOSED SUBFAMILY

ABYSSOPEDUNCULUS GEN. NOV.

Diagnosis: Body elongate, subcylindrical. Forebody short, about one-sixth body length. Tegument smooth, thin. Oral sucker unspecialized, terminal. Ventral sucker, unspecialized, larger than oral sucker, pedunculate. Prepharynx short. Pharynx unspecialized, smaller than oral sucker. Oesophagus relatively long. Intestine bifurcates in forebody or dorsal to ventral sucker. Caeca blind, extending well beyond testes near to posterior extremity. Testes two, smooth, ellipsoidal, medial, separate; post-testicular zone occupies about one-quarter total body length. Cirrus-sac well developed, short, broad oval, mostly dorsal to ventral sucker, may protrude slightly beyond ventral sucker into hindbody. Seminal vesicle internal, occupying most of cirrus-sac, broad proximally, becoming looped distally. Pars prostatica indistinct. Ejaculatory duct short. Common genital atrium short, simple. Genital pore pre-bifurcal, sinistro-submedial. Ovary smooth, medial, anterior to and separated from anterior testis, situated about one-half body length from anterior extremity. Seminal receptacle canalicular, smaller than and antero-dorsal to ovary. Laurer's canal present, short, opens dorsal to ovary. Mehlis' gland present. Uterus preovarian, intercaecal, approaches genital atrium ventral to cirrus-sac. Vitelline follicles dense, restricted to hindbody, extending from near

to posterior extremity to well beyond ovary anteriorly but not reaching level of ventral sucker, distributed dorsally, laterally and ventrally, becoming confluent ventrally in post-testicular zone and preovarian zone. Eggs oval, operculate, unembryonated *in utero*. Excretory vesicle tubular, extends to level of ovary. Excretory pore terminal. In mesopelagic north-west Atlantic fishes, known only from Congridae (Anguilliformes).

Type and only species: *Abyssopedunculus brevis* (Andres & Overstreet, 2013) comb. nov. (syn. *Podocotyloides brevis*).

ZooBank registration LSID: <http://www.zoobank.org/urn:lsid:zoobank.org:act:8337048F-1E99-4C54-8C02-D651BC00A341>

Etymology: The generic name is composed from Latin *abyss*, the deep, and *pedunculus*, a peduncle or stem. The name is chosen because the concept is distinguishable among plagioprine (*s.l.*) type taxa in deep-sea fishes by a pedunculate ventral sucker. The genus is to be treated as feminine.

Remarks: *Podocotyloides* Yamaguti, 1934 was recently revised by Martin *et al.* (2018c). Genuine species are known mostly from Indo-West Pacific shallow-water fishes of the Haemulidae (Perciformes) and resolve to clade B in the major marine Plagioprinae (*s.l.*) clade. *Abyssopedunculus brevis* resolves among the deep-sea taxa and was described based on material from *Conger esculentus* (Anguilliformes: Congridae) collected at a depth of 200 m off Puerto Rico (Andres & Overstreet, 2013). Thus, it cannot be considered to belong in *Podocotyloides*. Morphologically, it differs most obviously from genuine *Podocotyloides* species in that its excretory vesicle reaches only to the level of the ovary vs. the level of the ventral sucker, its cirrus-sac is short and oval vs. long, tubular and sigmoid, its ejaculatory duct is short and simple vs. long and provided with petaloid appendages, and its metraterm is indistinct vs. well developed and provided with a prominent sphincter. Among the taxa represented in the deep-sea + freshwater clade, *A. brevis* is distinct in that it has both a pedunculate ventral sucker and a canalicular seminal receptacle.

MESOBATHYLEBOURIA GEN. NOV.

Diagnosis: Body oval to elongate-oval, dorso-ventrally flattened. Forebody about one-quarter to one-third body length. Tegument smooth, relatively thick. Oral sucker unspecialized, terminal or subterminal. Ventral sucker unspecialized, larger than oral sucker. Pharynx short. Pharynx unspecialized, smaller

than oral sucker. Oesophagus unspecialized. Intestine bifurcates in forebody. Caeca blind, extending near to posterior extremity. Testes two, smooth or irregularly lobed, medial, usually contiguous; post-testicular zone about one-tenth to over one-quarter body length. Cirrus-sac well developed, short, claviform, not extending into hindbody. Seminal vesicle internal, broad. Pars prostatica present. Ejaculatory duct relatively long. Common genital atrium short, simple. Genital pore pre-bifurcal, sinistro-submedial. Ovary irregularly lobed, medial to dextro-submedial, anterior to and contiguous with anterior testis, separated from ventral sucker. Seminal receptacle canalicular, smaller than ovary. Laurer's canal present, short, opens dorsal to ovarian complex. Mehlis' gland present. Uterus preovarian, usually intercaecal. Vitellarium follicular, dense, extending from near to posterior extremity to level of intestine bifurcation or beyond, may be interrupted at level of ventral sucker, may become confluent in post-testicular zone or forebody. Eggs oval, operculate, unembryonated *in utero*, <80 µm long. Excretory vesicle tubular, extends to level of ovary.

In Indo-West Pacific marine fishes of several orders, mainly from the mesopelagic zone.

Type-species: *Mesobathylebouria lanceolata* (Price, 1934) comb. nov. [syn. *Podocotyle lanceolata*, *Neolebouria lanceolata* (Price, 1934) Reimer, 1987].

Other species: *Mesobathylebouria acanthogobii* (Yamaguti, 1951) comb. nov. [syn. *Plagioporus acanthogobii*, *Neolebouria acanthogobii* (Yamaguti, 1951) Gibson, 1976]; *M. lobata* (Yamaguti, 1934) comb. nov. [syn. *Lebouria lobata*, *Neolebouria lobata* (Yamaguti, 1934) Gibson, 1976]; *M. tinkerbella* (Thompson & Margolis, 1987) comb. nov. (syn. *Neolebouria tinkerbella*); *M. tohei* (Yamaguti, 1970) comb. nov. [syn. *Plagioporus tohei*, *Neolebouria tohei* (Yamaguti, 1970) Gibson, 1976].

ZooBank registration LSID: <http://www.zoobank.org/urn:lsid:zoobank.org:act:9752A24C-CBFD-48F9-AEA3-A349CDBAE7F4>

Etymology: The generic name is composed from Greek *meso*, middle, and *bathos*, deep, combined with the genus *Lebouria* Nicoll, 1909, named after Dr Marie Lebour and the stem for *Neolebouria*, but now a junior synonym of *Peracreadium* Nicoll, 1909. The name is chosen because these species are morphologically similar to those of *Neolebouria* and are generally found in fishes from the lower epipelagic to mesopelagic zone. The genus is to be treated as feminine.

Remarks: *Neolebouria* currently comprises 11 species [plus *N. maorum* (Allison, 1966) Gibson,

1976 *incertae sedis*], but these must be considered to represent at least two genera because *N. georgiensis* Gibson, 1976, the type-species, and *N. lanceolata* do not resolve together in molecular phylogenetic analyses (Faltýnková *et al.*, 2017; Martin *et al.*, 2017). *Neolebouria georgiensis* is almost certainly congeneric with *N. antarctica* (Szidat & Graefe, 1967) Zdzitowiecki, 1990 and *N. terranovaensis* Zdzitowiecki *et al.*, 1993. These three species are known only from Antarctic and sub-Antarctic waters, in notothenioid perciform fishes (Artedraconidae, Bathydraconidae, Channichthyidae and Nototheniidae) and also species of *Paraliparis* (Scorpaeniformes: Liparidae) (Gibson, 1976; Zdzitowiecki, 1987, 1991, 1997; Zdzitowiecki *et al.*, 1993; Palm *et al.*, 2007). As per Martin *et al.* (2017), we consider one other nominal species, *Neolebouria merretti* Gibson & Bray, 1982, to be more convincingly congeneric with *N. georgiensis* than *N. lanceolata*. It is known from an ipnopid (Aulopiformes) collected at 2985–3311 m deep south-west of the Canary Islands (Gibson & Bray, 1982). These four species have large (1800–4800 µm long), robust bodies, thick tegument, medial and irregularly lobed gonads and large eggs, greater than 80 µm long. In *N. georgiensis*, *N. merretti* and *N. terranovaensis* the cirrus-sac reaches to about the level of the posterior margin of the ventral sucker or a little beyond into the hindbody, although in *N. antarctica* it is much shorter. The original descriptions for *N. georgiensis* and *N. merretti* are highly detailed (Gibson, 1976; Gibson & Bray, 1982) and thus two potentially significant characters serve to convincingly unite these species. First, in each species, the entirety of the oesophagus, and also the anterior part of the caeca in *N. merretti*, is surrounded by especially dense gland cells, and second, the seminal vesicle is broad and dilate proximally but then becomes long, thin, coiled and duct-like prior to the pars-prostatica and ejaculatory duct.

We propose *Mesobathylebouria* for *N. lanceolata* and four of the remaining six species of *Neolebouria*: *N. acanthogobii*, *N. lobata*, *N. tinkerbella* and *N. tohei*. Collectively, these five species occur in a broad range of fishes from mainly the lower epipelagic to mesopelagic zone, but not including notothenioids, other Antarctic/sub-Antarctic fishes or indeed other perciform fishes: Anguilliformes (Nettastomatidae, Congridae), Aulopiformes (Ipnopidae), Lophiiformes (Chaunacidae), Ophidiiformes (Ophidiidae), Polymixiiformes (Polymixiidae), Scorpaeniformes (Hoplichthyidae, Triglidae) and Stomiiformes (Phosiichthyidae) (Martin *et al.*, 2017 and references therein). Four of these species have Indo-Pacific distributions, *N. acanthogobii* and *N. lobata* are both known from off Japan and Mozambique, *N. tohei* is known from Hawaii and *N. tinkerbella* is known from off British Columbia,

whereas *N. lanceolata* is known from the Gulf of Mexico and the Caribbean and also from off Mozambique. Species included here in *Mesobathylebouria* can be distinguished most objectively from those retained in *Neolebouria* (s.s.) by smaller eggs, measuring less than 80 µm long. These species are also less robust and some have a dextro-submedial ovary or smooth testes. In *N. lanceolata* the cirrus-sac almost reaches to the posterior margin of the ventral sucker, but in the other four species it is either entirely anterior to the ventral sucker or overlaps only the anterior portion.

Two nominal *Neolebouria* species are unaccounted for, *N. leiognathi* (Wang *et al.*, 1992) Bray, 2002 and *N. pentacerotis* Machida & Araki, 2002. *Neolebouria leiognathi* is known from the deep pugnose ponyfish *Secutor ruconius* (Perciformes: Leiognathidae), a small coastal fish, off China (Wang *et al.*, 1992). It has almost equal suckers, a neatly trilobed ovary, eggs greater than 90 µm long and possibly a medial genital pore. It is unlikely to belong in either *Neolebouria* or *Mesobathylebouria*, but its placement elsewhere is difficult. Few opecoelids are known from leiognathids and none bear particular resemblance to *N. leiognathi*. The original description is lacking in detail and thus, for the present, we consider *N. leiognathi* a species *incertae sedis*. *Neolebouria pentacerotis* is known from the Japanese armourhead *Pentaceros japonica* (Perciformes: Pentacerotidae), a lower epipelagic to mesopelagic fish, collected at depth in Japanese waters (Machida & Araki, 2002). It has exceptionally small eggs, 27–33 µm long, and thus is consistent with the concept of *Choerodonicola* Cribb, 2005, species of which are known only from perciform fishes, including in Japanese waters. *Choerodonicola* may be polyphyletic (Martin *et al.*, 2018a), based on sequence data available for two species: one, *C. arothokoros* Martin *et al.*, 2018, in which the ovary is entire and the other, *C. renko* Machida, 2014, in which it is lobed. In the type-species, *C. choerodontis* (Yamaguti, 1934) Cribb, 2005, the ovary has an intermediate, irregularly lobed condition. Of the two sequenced representative species, *N. pentacerotis* is probably more closely related to *C. renko* Machida, 2014, which is known from two Japanese sparids: the yellowfin seabream *Dentex abei* Iwatsuki *et al.* and the yellowback seabream *D. hypselosomus* Bleeker, which reportedly range from depths of 50 to 200 m (Iwatsuki *et al.*, 2007). Thus, we propose *Choerodonicola pentacerotis* (Machida & Araki, 2002) comb. nov. It is distinguishable within the genus by a distinct interruption in the vitelline field at the level of the ventral sucker; *C. interruptus* (Manter, 1954) Martin *et al.*, 2018 also has an interrupted vitelline field, but the interruption is at the level of the testes in that species and it is known only from a labrid (Perciformes) endemic to New Zealand.

BATHYCREADIINAE SUBF. NOV.

Diagnosis: Body elongate-oval to elongate, dorso-ventrally flattened, large. Tegument smooth. Forebody short, occupies less than one-fifth total body length. Oral sucker unspecialized. Ventral sucker larger than oral sucker, sessile to protuberant. Pharynx unspecialized. Prepharynx short. Oesophagus unspecialized. Intestine bifurcates in forebody or dorsal to ventral sucker. Caeca unite to form cyclocoel near posterior extremity. Testes two, smooth, medial, usually separate. Cirrus-sac well developed, extending into hindbody. Seminal vesicle, internal. Pars-prostatica present. Ejaculatory duct long. Common genital atrium simple. Genital pore pre-bifurcal, sinistro-submedial to sinistral. Ovary smooth, medial, smaller than, anterior to and usually separate from testes. Seminal receptacle canalicular. Laurer's canal present. Uterus preovarian. Vitelline follicles restricted to hindbody, extending anteriorly beyond ovary, sometimes reaching posterior margin of ventral sucker. Eggs operculate, unembryonated *in utero*. Excretory vesicle tubular, extends to level of ovary. Excretory pore terminal. In demersal and bathydemersal, mesopelagic, northern Atlantic marine fishes of Gadiformes (Lotidae, Macrouridae, Moridae, Phycidae)

Type and only genus: *Bathycreadium* Kabata, 1961.

Remarks: The Bathycreadiinae is proposed for *Bathycreadium*. Analyses of molecular data available for *B. brayi* demonstrate that it is phylogenetically distinctive among the deep-sea and freshwater clade and consistently resolves as sister to the freshwater taxa (Fig. 1). *Bathycreadium* currently comprises six species, five of which are known only from mesopelagic gadiform fishes in the north Atlantic, collected from depths between 340 and 665 m [no depth reported for *B. elongata* (Maillard, 1970) Bray, 1973]. These species agree closely in morphology, most significantly in that in each the caeca form a cyclocoel. Among opecoelids with a canalicular seminal receptacle, only one other genus, *Nicolla* Wisniewski, 1934, is defined for species with a cyclocoel. The two concepts are distinguished by the presence vs. absence of vitelline follicles in the forebody. *Nicolla* comprises both freshwater and marine species. Sequence data generated from specimens identified as the type-species, *N. skrjabini* (Iwantitzky, 1928) Dollfus, 1960 [= *N. ovata* (Pigulewsky, 1931) Wisniewski, 1934], a freshwater form, indicate that it resolves external to all other freshwater plagiopore taxa, thus providing a link between the freshwater genus concepts and that of *Bathycreadium*. *Nicolla skrjabini* is separated by

relatively long branch lengths from the remaining freshwater taxa represented in the analyses, but *B. brayi* is separated by even greater distinction (Fig. 1). Moreover, like the other freshwater taxa, species of *Nicolla* have a short excretory vesicle, reaching only to the testes, as opposed to the ovary in species of *Bathycreadium*. Therefore, considering the ecological, morphological and phylogenetic distinctions relative to the freshwater taxa, we think it is most informative to recognize *Bathycreadium* in a separate subfamily.

The sixth species of *Bathycreadium*, *B. mullii* Abdel-Gaber *et al.*, 2018, probably does not belong in the genus. It was recently described based on specimens recovered from *Mullus surmeletus* (Perciformes: Mullidae) in the south-east Mediterranean (Abdel-Gaber *et al.*, 2018), a frequently reported combination for both *Opecoeloides furcatus* (Bremser in Rudolphi, 1819) Odhner, 1928 and *Poracanthium furcatum* Dollfus, 1948, two elongate opecoeline taxa in which the caeca form a uroproct in the former and unite and open via a common anus in the latter.

PODOCOTYLINAE DOLLFUS, 1959

Diagnosis: Body oval, fusiform, pyriform or elongate-oval, dorso-ventrally flattened, medium to large. Tegument smooth. Forebody less than one-half body length. Oral sucker unspecialized or funnel-shaped. Ventral sucker larger than oral sucker, sessile to protuberant. Pharynx unspecialized. Prepharynx short. Oesophagus unspecialized. Intestine bifurcates in forebody or dorsal to ventral sucker. Caeca blind, terminate near posterior extremity. Testes two, smooth or lobed, medial or diagonal, separate or contiguous. Cirrus-sac usually well developed, may be absent, restricted to forebody or overlapping ventral sucker or extending into hindbody. Seminal vesicle, usually internal, sometimes naked, usually long, thin and sinuous distally. Pars-prostatica present, may be inconspicuous. Ejaculatory duct often short. Common genital atrium simple. Genital pore bifurcal or pre-bifurcal, sinistro-submedial. Ovary smooth to regularly or irregularly lobed, medial to dextro-submedial, smaller than and anterior to testes, contiguous with or separate from anterior testis, separate from ventral sucker. Seminal receptacle canalicular. Laurer's canal present. Mehlis' gland present. Uterus preovarian. Vitelline follicles always extending beyond testes posteriorly, entering forebody or restricted to hindbody, may be distributed ventrally and dorsally or absent dorsally. Eggs oval, operculate, unembryonated *in utero*, without filaments or protuberances. Excretory vesicle tubular, extends to level of ovary. Excretory pore

terminal. In Antarctic, sub-Antarctic and deep-sea, especially bathypelagic, fishes, several families.

Type-genus: *Podocotyle* Dujardin, 1845

Other genera: *Bathypodocotyle* gen. nov., *Buticulotrema* Dronen & McEachran, 1993; *Halosaurotrema* gen. nov.; *Macrourimegatrema* Blend *et al.*, 2004; *Neolebouria* Gibson, 1976; *Tellervotrema* Gibson & Bray, 1982.

Remarks: Dollfus (1959) proposed the Podocotyliidae and Podocotyloidea, but most subsequent authors have considered *Podocotyle*, the type-genus, to belong in the Opecoelidae. Nevertheless, as per the principle of coordination, Dollfus (1959) also established the subfamily name, the Podocotylinae. Here, this subfamily concept is resurrected for *Podocotyle*, as well as *Bathypodocotyle*, *Buticulotrema*, *Halosaurotrema* and *Neolebouria*, each of which is represented in phylogenetic analyses by a single species and together form a strongly supported clade (Fig. 1). We also include *Macrourimegatrema* and *Tellervotrema* in this concept. These genera comprise two and three species, respectively, none of which are represented by sequence data. Collectively, podocotyline taxa are almost all known from deep-sea and/or temperate to polar fishes. Species of *Bathypodocotyle*, *Buticulotrema*, *Macrourimegatrema* and *Tellervotrema* occur in gadiform fishes, specifically of the Macrouridae and mainly in the north Atlantic; one species is known from the north Pacific (Gibson & Bray, 1982; Blend *et al.*, 2004, 2007; Kuramochi, 2009). The sole species of *Halosaurotrema* is known from a north Atlantic halosaurid (Notocanthiformes) (Bray & Campbell, 1996), and species of *Neolebouria*, as recognized here, are known mainly from Antarctic and sub-Antarctic notothenioid perciform fishes; two are also known from a liparid (Scorpaeniformes), and a third is known only from an Atlantic ipnoid (Aulopiformes) (Gibson, 1976; Gibson & Bray, 1982; Zdzitowiecki, 1987, 1991, 1997; Zdzitowiecki *et al.*, 1993; Palm *et al.*, 2007). The many, ecologically diverse species of *Podocotyle* probably represent multiple distinct lineages, but five nominal species, and one unnamed species discussed by Blend *et al.* (2017), are known only from deep-sea fishes in the north-west Atlantic: four, *P. harrissae* Bray & Campbell, 1996, *P. nimoyi* Blend, Dronen, & Armstrong, 2016, *P. pearsei* Manter, 1934 and the unnamed species are from macrourids, one, *P. shistotesticulata* Bray & Campbell, 1996, is from a morid, another gadiform fish, and one, *P. bathyhelminthos* Blend & Dronen, 2015, from an ophidiid (Ophidiiformes) (Manter, 1934; Bray & Campbell, 1996; Blend & Dronen, 2015b; Blend *et al.*, 2016, 2017). These species are almost certainly

closely related to species of *Bathypodocotyle* and *Tellervotrema*, differing from the former by a lobed vs. entire ovary and from the latter by restriction of the vitelline field to the hindbody. Perhaps most compellingly, like species of *Bathypodocotyle* and *Tellervotrema*, all six species of *Podocotyle* have a short cirrus-sac (in *P. bathyhelminthos* it may protrude a little into the hindbody) and a short to exceptionally short ejaculatory duct. *Podocotyle atomon*, the only sequenced representative of the genus, is known from a variety of fishes, mainly from the north Atlantic, Arctic and north Pacific, including Gadiformes (Gadidae), but also Anguilliformes, Gasterosteiformes, Perciformes, Pleuronectiformes, Scorpaeniformes and Sygnathiformes. It resolved as sister to *B. margolisi* in the phylogenetic analyses (Fig. 1) and is more consistent with the type species, *P. angulata* Dujardin, 1845, than with the *Podocotyle* species from deep-sea macrourids. *Podocotyle angulata* is also known from fishes of the Anguilliformes, Gasterosteiformes and Pleuronectiformes in the north Atlantic.

Morphologically, the taxa included here in the Podocotylinae are mostly highly conserved, distinguished from one-another mainly by the distribution of the vitelline follicles, lobation and arrangement of the gonads, and relative lengths of the forebody and hindbody. Thus, similar to the distinctions made for *Podocotyle* above, the concept of *Halosaurotrema* differs from that of *Neolebouria* (s.s) in hindbody length and arrangement of the gonads, *Bathypodocotyle* differs from *Neolebouria* (s.s) and *Tellervotrema* mainly in restriction of the vitelline follicles to the hindbody, and *Neolebouria* (s.s) and *Tellervotrema* essentially differ only in the presence vs. absence of a dorsal vitelline field and perhaps in the regularity and degree of ovary lobation. Specialized conditions serve to distinguish two genera: species of *Macrourimegatrema* have a funnel-shaped oral sucker, and, most significantly, species of *Buticulotrema* lack a cirrus-sac. *Buticulotrema* is, therefore, usually considered to belong to the Opecoelininae, but the concept of that subfamily is dubious. Species of *Buticulotrema* bear no significant resemblance to those of *Opecoelina*, the type-genus, or *Bartoliella* Aken'Ova, 2003, the only other nominal genus. Neither includes species known from bathypelagic fishes and both concepts require the caeca to unite and open into a common anus, whereas in species of *Buticulotrema* the caeca are blind. Sequence data have not yet been generated from specimens related to species of either *Opecoelina* or *Bartoliella*, but it seems unlikely that these will prove to be closely related to those of *Buticulotrema*.

The concept of the Podocotylinae can be distinguished from the Bathycreadinae by blind caeca vs. caeca forming a cyclocoel and from the Opecoelininae and Stenakrinae by a canalicular vs. uterine seminal receptacle. Distinction

from the Plagioporidae (s.s.), i.e. the freshwater clade, is more subtle, but we think two characters provide a potentially reliable basis. In the Podocotylinae, the excretory vesicle is longer, always reaching to the level of the ovary vs. at most to the level of the testes. Second, in some freshwater plagioporine taxa, the uterus becomes extracaecal and/or post-testicular, whereas in the Podocotylinae it is (almost) always intercaecal and preovarian. Only in one species from deep-sea fishes, *Podocotyle nimoyi*, is the uterus not strictly preovarian. In this species it extends to between the ovary and the anterior testis and partially dorsal to the anterior testis, but it does not enter the post-testicular zone. Additionally, a short cirrus-sac with a seminal vesicle becoming long or even duct-like distally and, in particular, a short ejaculatory duct occupying less than half the length of the cirrus-sac, appears to unite several podocotyline taxa, specifically species of *Bathypodocotyle*, *Neolebouria*, *Tellervotrema* and some *Podocotyle*.

BATHYPODOCOTYLE GEN. NOV.

Diagnosis: Body elongate-oval, dorso-ventrally flattened, large. Tegument smooth. Oral sucker, unspecialized, terminal or subterminal. Ventral sucker unspecialized, larger than oral sucker, may be protuberant. Prepharynx short. Pharynx unspecialized, slightly smaller than oral sucker. Oesophagus unspecialized. Intestine bifurcates in forebody. Caeca blind, extending beyond testes near to posterior extremity. Testes two, smooth, round or wedge shaped, medial, contiguous; post-testicular zone occupies at least one-quarter total body length. Cirrus-sac well developed, short, retrorse oval, restricted to forebody or overlapping anterior portion of ventral sucker. Seminal vesicle internal, occupying most of cirrus-sac, sinuous, broad proximally, becoming long, sinuous and narrow, almost duct-like, distally. Pars prostatica prominent. Ejaculatory duct exceptionally short. Common genital atrium simple. Genital pore pre-bifurcal, sinistro-submedial. Ovary smooth, medial or dextro-submedial, contiguous with anterior testis. Seminal receptacle canalicular, smaller than ovary, retrorse. Laurer's canal present, opens dorsal to ovary. Mehlis' gland present. Uterus preovarian, intercaecal. Vitellarium follicular, dense, restricted to hindbody, becoming confluent in post-testicular zone, extending near to posterior extremity. Eggs oval, operculate, unembryonated *in utero*. Excretory vesicle tubular, extends to level of ovary. Excretory pore terminal. In mesopelagic and bathypelagic north Atlantic and north Pacific fishes of the Macrouridae (Gadiformes).

Type-species: *Bathypodocotyle margolisi* (Gibson, 1995) comb. nov. (syn. *Allopodocotyle margolisi*).

Other species: *Bathypodocotyle enkaimushi* (Blend *et al.*, 2015) comb. nov. (syn. *Allopodocotyle enkaimushi*).

ZooBank registration LSID: <http://www.zoobank.org/urn:lsid:zoobank.org:act:097A860F-3892-413A-805E-790D00548435>

Etymology: The generic name is composed from Greek *bathos*, deep and the existing opecoelid genus *Podocotyle*, itself from *podo-*, relating to the foot and *kotyle*, a cup. The name is chosen because the new genus is comprised of deep-sea taxa previously recognized in *Allopodocotyle*, which itself is based on similarity to the concept of *Podocotyle*.

Remarks: *Allopodocotyle* is loosely defined, accommodates many species and is evidently polyphyletic. The genus currently comprises about 24 species from a broad range of marine fishes, united, among typically 'plagioporine' genera, by an entire ovary and restriction of the vitelline follicles to the hindbody (Cribb, 2005). The type-species, *A. plectropomi* (Manter, 1963), Pritchard, 1966, and its most convincing congeners, are known from Indo-West Pacific groupers (Perciformes: Serranidae: Epinephelinae). Sequence data have been published, by Bray *et al.* (2016), for four species considered to belong in *Allopodocotyle*: *A. epinepheli* (Yamaguti, 1942) Pritchard, 1966, *A. margolisi* and two species identified only to genus. Data for *A. epinepheli* and one of the unidentified species, both collected from Indo-West Pacific groupers, resolve together in the large marine Plagioporidae (s.l.) clade. We consider these data to represent the phylogenetic position of genuine *Allopodocotyle* species. *Allopodocotyle margolisi* is phylogenetically distant from these taxa and is known from the Mediterranean grenadier *Coryphaenoides mediterraneus* (Giglioli) (Gadiformes: Macrouridae), collected at 1745–2195 m deep from the Rockall Trough in the north-east Atlantic (Gibson, 1995). Compared with *A. epinepheli*, *A. plectropomi* and also *A. serrani* (Yamaguti, 1952) Pritchard, 1966, another convincing species known from Indo-West Pacific groupers, *A. margolisi* is most distinguishable by the configuration of its terminal genitalia. It has a short cirrus-sac not extending into the hindbody and an exceptionally short ejaculatory duct vs. a relatively long cirrus-sac extending well into the hindbody with a long ejaculatory duct occupying at least half the length of the cirrus-sac. *Allopodocotyle margolisi* can further be distinguished from these species in that it has medial and contiguous vs. diagonal and separate testes and in that the vitelline follicles extend anteriorly to the posterior margin of the ventral sucker vs. only to the level of the ovary or a little beyond. One other nominal species of *Allopodocotyle* agrees more closely in its

morphology and ecology with *A. margolisi* than with species from Indo-West Pacific groupers: *A. enkaimushi* has an exceptionally short ejaculatory duct and cirrus-sac, medial and contiguous testes, and vitelline follicles restricted to the hindbody but extending anteriorly to the posterior margin of the ventral sucker. It is also known from macrourids, collected at depths between 681 and 1061 m off Japan (Blend *et al.*, 2015). We propose *Bathypodocotyle* for these two species. We think that these species are probably closely related to other taxa known from deep-sea macrourid fishes, specifically species of *Macrourimegatrema*, *Tellervotrema* and some species of *Podocotyle*, as well as species of *Neolebouria* (s.s). However, none of these concepts can adequately accommodate the taxa included here in the new genus: *Macrourimegatrema* is defined for species with a funnel-shaped oral sucker and a more elongate body, species of *Tellervotrema* and *Podocotyle* have lobed ovaries, and species of *Tellervotrema* and *Neolebouria* have vitelline follicles entering the forebody.

HALOSAUREOTREMA GEN. NOV.

Diagnosis: Body pyriform. Tegument smooth. Oral sucker unspecialized, subterminal, subspherical. Ventral sucker unspecialized, larger than oral sucker, transversely ellipsoidal. Prepharynx short. Pharynx unspecialized, slightly smaller than oral sucker. Oesophagus distinct, unspecialized. Intestine bifurcates in forebody. Caeca blind, extending near to posterior extremity. Testes two, irregularly lobed, diagonal, contiguous, filling much of hindbody; post-testicular zone very short, roughly equal to post-caecal zone. Cirrus-sac well developed, overlapping ventral sucker, not extending into hindbody. Seminal vesicle internal, occupying about one-half cirrus-sac, broad proximally, narrowing abruptly prior to pars prostatica. Pars prostatica narrow, continuous with ejaculatory duct. Common genital atrium simple. Genital pore pre-bifurcal, sinistro-submedial. Ovary irregularly lobed, dextro-submedial, contiguous with anterior testis. Seminal receptacle canalicular, smaller than ovary. Laurer's canal present, opens dorsal to ovary. Mehlis' gland present. Uterus preovarian. Vitellarium follicular, distributed length of caeca, becoming confluent dorsally in forebody. Eggs operculate, unembryonated *in utero*. Excretory vesicle tubular, extends to level of ovary. Excretory pore terminal. In bathypelagic halosaurid fishes (Notocanthiformes) of the north Atlantic.

Type and only species: *Halosaureotrema halosaurepsi* (Bray & Campbell, 1996) comb. nov. (syn. *Gaevskajatrema halosaurepsi*).

ZooBank registration LSID: <http://www.zoobank.org/urn:lsid:zoobank.org:act:3C89E842-E7C6-482D-B24A-21F536D9EF86>

Etymology: *Halosaureotrema* is named for the host family of the only species, the Halosauridae. The name is derived from Greek *halos*, salt, *sauros*, lizard and *trema* for trematode.

Remarks: *Gaevskajatrema halosaurepsi* requires a new genus. It was described based on material recovered from *Halosaureopsis macrochir* (Günther) (Notocanthiformes: Halosauridae) collected between 1908 and 2570 m deep in the north Atlantic (Bray & Campbell, 1996); it is the only opecoelid known from a notocanthiform fish. Conversely, *G. perezi* (Mathias, 1926) Gibson & Bray, 1982, the type-species, and *G. pontica* (Koval, 1966), Machkevsky, 1990, the only other member of the genus, are known from shallow-water wrasses (Perciformes: Labridae) from the eastern Mediterranean to the Black Sea and from off Roscoff, north-western France (Gibson & Bray, 1982). Phylogenetic analyses show that *G. perezi* and *G. halosaurepsi* are only distantly related, with the type-species resolving among the Opistholebetinae (Martin *et al.*, 2018b). The morphological characters considered to be most important for defining *Gaevskajatrema* are caeca terminating lateral to the testes and vitelline follicles not extending beyond the testes posteriorly. Strictly, *G. halosaurepsi* exhibits both conditions, but not in a form consistent with that seen in *G. perezi*. In that species, the caeca are short such that the post-caecal zone occupies more than one-quarter of body length, and there is an appreciable post-testicular zone from which the vitelline follicles are excluded. In *G. halosaurepsi*, the caeca and vitelline follicles reach near to the posterior extremity, as is typical for most opecoelids, but the hindbody is relatively short and the testes large, such that the post-testicular zone is roughly equivalent to the post-caecal and post-vitelline zones. Furthermore, in *G. halosaurepsi* the ovary and testes are irregularly lobed vs. smooth and the excretory vesicle extends to the level of the ovary vs. the posterior testis. Thus, we propose *Halosaureotrema* gen. nov. and *H. halosaurepsi* comb. nov. The new concept is similar to *Neolebouria* (s.s), but differs in general body shape, the relatively short hindbody and post-testicular zone, and in that the testes are diagonal and the ovary dextro-submedial vs. all gonads medial.

DISCUSSION

Among the Opecoelidae, *Scorpidotrema longistipes* and species of *Holsworthotrema* are interesting for several reasons. These taxa are the only opecoelid genera for

which species are known only from herbivorous fishes, the only opecoelids known from *Kyphosus* and *Scorpiis* fishes in the Indo-Pacific, and are also likely endemic to southern Australian waters. We have not encountered any opecoelids in any species of *Kyphosus* other than from off south-west Western Australia. There are two records of opecoelids from *Kyphosus* fishes in the west Atlantic. Dyer-Williams & Bunkely-Williams (1992) reported *Hamacreadium mutabile* Linton, 1910, without description or illustration, from off Puerto Rico, and Gomes *et al.* (1974) reported *Plagioporus dollfusi* Gomes *et al.*, 1974 from off Brazil. Both reports probably represent the same or similar species, but almost certainly not belonging to either *Hamacreadium* or *Plagioporus*, species of which are restricted to carnivorous lutjanid and lethrinid fishes and freshwater Holarctic fishes, respectively (Cribb, 2005; Martin *et al.*, 2016). Only a few other opecoelids, and no stenakrines, are reliably known from herbivorous fishes; examples include species of *Choerodonnicola* Cribb, 2005 in scarids, species of *Diplobulbus* Yamaguti, 1934 in acanthurids and scarids, species of *Coitocaecum* Nicoll, 1915 in acanthurids, scarids and siganids, species of *Opecoelus* Ozaki, 1925 in girellids and species of *Helicometra* Odhner, 1902 in microcanthids. Girellids and microcanthids are closely related to kyphosids and thus it seems that opecoelids have independently exploited this particular group of fishes at least three times, as well as switching from carnivorous to herbivorous fishes on multiple occasions more generally. Perhaps most pertinently, species of *Holsworthotrema* and *S. longistipes* are the first opecoelids morphologically consistent with the concept of the Stenakrinae to be represented by sequence data in phylogenetic analyses [we do not consider species of *Biospeedotrema* here because broader phylogenetic analyses suggest they are not opecoelids (Shedko *et al.*, 2015; Sokolov *et al.*, 2017)]. Thus, determination of their phylogenetic position has important ramifications for organizing the Opecoelidae into an adequate subfamilial classification.

The presence vs. absence of a well-developed cirrus-sac and a canalicular seminal receptacle are useful and important characters to be considered for subfamilial classification among the Opecoelidae. However, increasingly improved phylogenetic resolution for the family is gradually revealing a more complex narrative of diversification with respect to these characters than had been envisioned in the morphology-based view developed prior to substantial phylogenetic exploration (Gibson & Bray, 1982, 1984; Cribb, 2005). That classification hypothesis divided the Opecoelidae into the Plagioporinae and the Opecoelinae, for taxa possessing and lacking both features, respectively. The minor subfamilies, the Stenakrinae and

Opecoelininae, were established to accommodate the relatively few taxa possessing a well-developed cirrus-sac but lacking a canalicular seminal receptacle, or lacking a well-developed cirrus-sac but possessing a canalicular seminal receptacle, respectively. It has been speculated that the Stenakrinae may represent the basal opecoelid condition (Gibson & Bray, 1984), a view supported by some broader phylogenetic analyses, which resolved the Opecoelidae as sister to the Acanthocolpidae Lühe, 1906 and Brachycladiidae Odhner, 1905 (Littlewood *et al.*, 2015), both of which comprise species with a well-developed cirrus-sac and without a canalicular seminal receptacle. However, the most parsimonious interpretation of the current phylogeny for the Opecoelidae suggests that basal opecoelids possessed both a well-developed cirrus-sac and a canalicular seminal receptacle, with the cirrus-sac being lost or reduced at least twice, once in the Opecoelinae and once in *Buticulotrema*, and, likewise, with the canalicular seminal receptacle being replaced with a uterine receptacle at least three times, once in the Opecoelinae, once among the marine plagiopore (s.l.) clade in *Podocotyloides stenometra* Pritchard 1966 (Martin, 2018c) and once in *Holsworthotrema* + *Scorpidotrema*. Thus, the absence of both characters reliably serves to distinguish the Opecoelinae, but the presence of either or both characters is not, on its own, diagnostic of subfamily.

In this context, subfamilial placement of *Holsworthotrema* and *Scorpidotrema* is difficult. Given that the canalicular seminal receptacle has evidently been lost independently at least three times in the Opecoelidae, its absence should not be considered sufficient to place these taxa in the Stenakrinae. The morphological concept of *Holsworthotrema* is similar to that of *Pseudopecoelina*. However, Cribb (2005) considered *S. longistipes* the least convincing member of the Stenakrinae and we agree that there are few similarities between the concepts of *Holsworthotrema* + *Scorpidotrema* and *Stenakron*, the type-genus. Species of *Stenakron* are characterized by a smaller fusiform body, longer forebody, sessile ventral sucker, medial genital pore, submedial and lobed ovary, short caeca terminating at the level of the testes and extension of the vitelline follicles into the forebody but also not beyond the testes posteriorly.

Although species of *Holsworthotrema* and *S. longistipes* are known only from shallow-water, coastal fishes, they resolve among deep-sea taxa and the Stenakrinae are well represented in deep-sea and cold-water fishes, by species of three genera: *Stenakron*, *Hexagrammia* Baeva, 1965 and *Caudotestis* Issaïtschikov, 1928. *Stenakron* comprises six species known mainly from fishes in the Arctic, north Atlantic and north Pacific, especially Pleuronectiformes

(Pleuronectidae), Scorpaeniformes (Cottidae and Liparidae), Gadiformes (Gadidae, Phycidae) and zoarcoid perciforms (Stichaeidae, Zoarcidae). Likewise, *Hexagrammia* comprises just two species, both known only from hexagrammids (Scorpaeniformes) in the north Pacific. Finally, like species of *Stenakron* and *Hexagrammia*, *Caudotestis nicolli* Issaïtschikov, 1928 and *C. opisthorchis* (Polyanski, 1955) Cribb, 2005 are known from fishes in the Arctic, north Atlantic and north Pacific, including of the Cottidae, Gadidae, Hexagrammidae and Liparidae, but others, *C. glacialis* (Zdzitowiecki, 1989) Cribb, 2005, *C. kerguelensis* (Prudhoe & Bray, 1973) Cribb, 2005 and *C. patagonensis* Cantatore *et al.*, 2012, are known from cold and deep-sea fishes in Antarctic and sub-Antarctic regions, specifically notothenioid perciforms (Arteidraconidae, Bathydraconidae, Channichthyidae and Nototheniidae), scorpaeniforms (Congiopodidae and Liparidae) and ophidiiforms (Ophidiidae). Of these, *C. kerguelensis* is most significant, because it is known from off Macquarie Island, Heard Island and the Kerguelen Islands, thus establishing a plausible biogeographical connection with species of *Holsworthotrema* and *S. longistipes* from southern Australian waters. Therefore, in the absence of sequence data for any other stenakrine taxa, we think it is reasonable to predict that *Stenakron*, *Hexagrammia* and *Caudotestis*, and thus the Stenakrinae itself, will prove to resolve among the deep-sea clade. Thus, even though species of *Holsworthotrema* and *S. longistipes* occur in shallow-water fishes, plausible biogeographical and ecological links exist such that it is most parsimonious to assume that these taxa belong to the Stenakrinae.

Accepting *Holsworthotrema* and *Scorpidotrema* as representative of the Stenakrinae is problematic phylogenetically. The proximity of these taxa to *Mesobathylebouria lanceolata* and *Abyssopedunculus brevis* appears to require one of two unsatisfactory concessions: either also incorporating *M. lanceolata* and *A. brevis* into the Stenakrinae and thus expanding the concept to include species with a canalicular seminal receptacle, or recognizing another new subfamily, at present just for those concepts, without any compelling morphological or ecological basis. This issue is part of the broader problem of establishing adequate definitions for clades of the former catch-all taxon, the Plagioporinae. It is now well demonstrated that none of the taxa within the major marine Plagioporinae (s.l.) clade can be considered to belong to the subfamily. Recent efforts (Martin *et al.*, 2018b) have made progress towards organizing these taxa into an informative classification, but the status of taxa belonging to the deep-sea clade is more problematic. We think that the simplest and default solution, considering the Plagioporinae to represent the

union of all taxa resolving to the deep-sea and freshwater clade, is inadequate, because such a group would unite substantial morphological and phylogenetic diversity, including the concept of the Stenakrinae, and would ignore the obvious ecological distinction between deep-sea and freshwater systems. Restriction of the Plagioporinae to the freshwater clade is appealing because it aligns with a clear ecological basis and appears to encapsulate a phylogenetically distinct and morphologically conserved, yet distinguishable, clade. Thus far, sequence data place species of *Plagioporus*, *Neoplagioporus* Shimazu, 1990, *Nicolla*, *Sphaerostomum* Rudolphi, 1809 and *Urorchis* Ozaki, 1927 in the clade, but we expect that it will probably also prove to include the following opecoelid concepts that likewise accommodate taxa infecting mainly Nearctic or Palearctic freshwater fishes: *Multivitellina* Schell, 1974, *Nezpercella* Schell, 1974, *Pseudurorchis* Yamaguti, 1971 and *Pseudosphaerostomum* Koval & Shevchenko, 1970. Morphologically, all these concepts are united by an elongate-oval to fusiform body, a sub-medial genital pore and an unusually short excretory vesicle. Additionally, in all except *Neoplagioporus*, *Nicolla* and *Plagioporus*, the uterus extends into the post-testicular and/or extracaecal regions. It is worth noting that the Sphaerostomatinae Poche, 1926 has priority over the Plagioporinae, but, as per article 35.5 of the ICZN, the Plagioporinae should be retained due to its ubiquitous application in the literature.

If the Plagioporinae is to be restricted to freshwater forms, and *Holsworthotrema* and *Scorpidotrema* are to be considered representative of the Stenakrinae, then it would appear from analyses of the available sequence data that the deep-sea taxa require organization into three new subfamilies. We think that species of *Bathycreadium* are sufficiently distinctive morphologically and phylogenetically to propose the Bathycreadiinae, and likewise that sufficient morphologically and ecologically conserved taxa can be grouped to resurrect the Podocotylineae. This arrangement leaves only species of *Mesobathylebouria* and *Abyssopeduncula brevis* inadequately accommodated. On the basis of phylogeny, we think that these taxa probably require a new subfamily, but that such a proposal is premature, because, unlike the other subfamilies discussed here, neither a morphological nor an ecological basis is yet clear. It is apparent that there is limited overlap between the range of fishes exploited by species of *Mesobathylebouria* + *A. brevis* and the taxa we consider to belong to the Podocotylineae or Stenakrine. Podocotyline and stenakrines occur mainly in mesopelagic to bathypelagic gadiforms and Antarctic/sub-Antarctic notothenioid perciforms, with stenakrines also occurring in liparid, cottid and hexagrammid scorpaeniforms and pleuronectiforms.

In contrast, species of *Mesobathylebouria* + *A. brevis* occur in a broad range of fishes collected mainly from the lower epipelagic to mesopelagic zones. We speculate that these ecological distinctions may prove to be significant and might relate to differences in life cycle, particularly the range of second intermediate hosts exploited. However, among the deep-sea taxa represented by sequence data, life-cycle details are known only for *P. atomon*, which exploits amphipods as second intermediate hosts (Hunninen & Cable, 1943). Finally, determination of the phylogenetic position of one genus in particular, the monotypic *Mesocreadium* Reimer, 1987, may be especially useful in understanding the bounds between the Stenakrinae and the clade represented by *M. lanceolata* and *A. brevis*. Like some stenakrine taxa, *Mesocreadium hoplichthys* Reimer, 1987 has a medial genital pore and short caeca, but has a canalicular seminal receptacle and is known only from a hoplichthyid scorpaeniform from off Mozambique, a host group also recorded for *Mesobathylebouria lobata* but not known for any stenakrine or podocotyline taxa. Further investigation, in particular better representation of taxa with molecular data, is required to determine an appropriate classification for the clade represented by *Mesobathylebouria* + *A. brevis*.

Following the new taxonomic hypotheses proposed here, eight opacoelid subfamilies are recognized, with the requirement for at least a further three suggested by current phylogenetic analyses: one for *Mesobathylebouria* + *A. brevis* and at least two for taxa within the major marine Plagioporidae (*s.l.*) clade. This represents a significant proliferation from the four accepted by most modern authors prior to the landmark phylogenetic study by Bray *et al.* (2016). The purpose of a subfamily classification system is to distinguish groups of similar and related genera in such a way as to be useful and informative, yet reflective of relationships. Thus, it might appear that there is some risk to utility by dividing the Opacoelidae into too many subfamilies, on the basis of finer and finer morphological distinctions, such that straightforward diagnosis is no longer possible. This threat is perhaps best exemplified here by the prospect of distinguishing the Podocotylinae from a concept comprising *Mesobathylebouria* or *Allopodocotyle*, where species of *Neoleobouria* and *Bathypodocotyle* have previously been closely implicated with taxa from those concepts, respectively, and are apparently distinguishable morphologically only on the basis of fine, subtle characters. However, in our opinion, these examples are symbolic of the problems and historical legacy left by the broad and, ultimately, unsatisfactory concept of the Plagioporidae. Most opacoelids lack specialized features, and so many genera are diagnosed based on

a combination of characters such as shape and alignment of the gonads, extent of the vitelline follicles, and the position of the genital pore. These characters are useful, but evidently highly plastic, and yet, historically, have dictated classification despite substantial differences in biogeographic distribution or host ecology and/or phylogeny. Thus, it is not surprising that two similar, morphologically unspecialized, but ecologically distinct opacoelid concepts, such as *Bathypodocotyle* and *Allopodocotyle*, should be distantly related.

A system of complexity comparable to that of the Opacoelidae is seen in that of the taxa within the Hemiuroidea Looss, 1899. This group represents a large assemblage with richness similar to that of the Opacoelidae (both comprise over 1000 species). The Hemiuroidea is currently organized into 14 families; some, such as the Hemiuridae Looss, 1899, Didymozoidae Monticelli, 1888 and Lecithasteridae Odhner, 1905, are large, and others, such as the Bathycotylidae Dolfus, 1932, Gonocercidae Skrjabin & Guschanskaja, 1955, Isoparorchidae Travassos, 1922, Ptychogonimidae Dolfus, 1937 and Sclerodistomoididae Gibson & Bray, 1979 comprise far fewer taxa. However, unlike the opacoelids, these hemiuroid taxa are diverse in morphology and in their ecological specialization, collectively exploiting numerous sites within their fish hosts, including the stomach, gills, swimbladder and tissues, in addition to the intestines. In contrast, only a few exceptional opacoelids exploit the stomach instead of the intestines. Thus, the Opacoelidae is remarkable for its huge richness yet low diversity. We think it inevitable and desirable that increasingly finer generic and subfamilial concepts will be required to adequately reflect phylogenetic relationships as they continue to be uncovered.

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REFERENCES

- Abdel-Gaber R, Abdel-Ghaffar F, Mehlhorn H, Al Quraishy S, Morsy K, Maher S. 2018.** Light microscopic study of four plagiiorchiid trematodes infecting marine fish in the south-eastern Mediterranean Sea, Alexandria City, with descriptions of two new species. *Parasitology Research* **117**: 1341–1356.
- Aken'Ova TOL, Cribb TH. 2003.** *Scorpidotrema longistipes* n. g., n. sp. (Digenea: Opecoelidae) from *Scorpiis georgiana* (Teleostei: Scorpididae) from southern Western Australia. *Systematic Parasitology* **54**: 97–102.
- Andres MJ, Overstreet RM. 2013.** A new species of *Podocotyloides* (Digenea: Opecoelidae) from the grey conger eel, *Conger esculentus*, in the Caribbean Sea. *Journal of Parasitology* **99**: 619–623.
- Andres MJ, Pulis EE, Overstreet RM. 2014a.** New genus of opecoelid trematode from *Pristipomoides aquilonaris* (Perciformes: Lutjanidae) and its phylogenetic affinity within the family Opecoelidae. *Folia Parasitologica* **61**: 223–230.
- Andres MJ, Ray CL, Pulis EE, Curran SS, Overstreet RM. 2014b.** Molecular characterization of two opecoelid trematodes from fishes in the Gulf of Mexico, with a description of a new species of *Helicometra*. *Acta Parasitologica* **59**: 405–412.
- Antar R, Georgieva S, Gargouri L, Kostadinova A. 2015.** Molecular evidence for the existence of species complexes within *Macvicaria* Gibson & Bray, 1982 (Digenea: Opecoelidae) in the western Mediterranean, with descriptions of two new species. *Systematic Parasitology* **91**: 211–229.
- Blend CK, Dronen NO. 2015a.** A review of the genus *Helicometra* Odhner, 1902 (Digenea: Opecoelidae: Plagioporinae) with a key to species including *Helicometra overstreeti* n. sp. from the cuck-eel *Luciobrotula corethromycter* Cohen, 1964 (Ophidiiformes: Ophidiidae) from the Gulf of Mexico. *Marine Biodiversity* **45**: 183–270.
- Blend CK, Dronen NO. 2015b.** Description of a new species of *Podocotyle* Dujardin, 1845 (Digenea: Opecoelidae: Plagioporinae) from the cusk-eel, *Luciobrotula corethromycter* Cohen, 1964 (Ophidiiformes: Ophidiidae), from the Gulf of Mexico and Caribbean Sea. *Acta Parasitologica* **60**: 234–243.
- Blend CK, Dronen NO, Armstrong HW. 2004.** *Macrourimegatrema brayi* n. gen., n. sp. (Digenea: Opecoelidae) from four species of deep-sea macrourid fishes from the Gulf of Mexico and Caribbean Sea, with a list of endohelminths reported from species of *Bathygadus* and *Gadomus* (Macrouridae). *Zootaxa* **566**: 1–18.
- Blend CK, Dronen NO, Armstrong HW. 2007.** *Macrourimegatrema gadoma* n. sp. (Digenea: Opecoelidae) from the doublethread grenadier *Gadomus arcuatus* (Goode & Bean) (Macrouridae) in the Gulf of Mexico and Caribbean Sea. *Systematic Parasitology* **67**: 93–99.
- Blend CK, Kuramochi T, Dronen NO. 2015.** *Allopodocotyle enkaimushi* n. sp. (Digenea: Opecoelidae: Plagioporinae) from the short-tail grenadier, *Nezumia proxima* (Gadiformes: Macrouridae), from Sagami Bay, Japan, with a key to species of this genus and a checklist of parasites reported from this host. *Comparative Parasitology* **82**: 219–230.
- Blend CK, Dronen NO, Armstrong HW. 2016.** *Podocotyle nimoyi* n. sp. (Digenea: Opecoelidae: Plagioporinae) and a re-description of *Podocotyle pearsei* Manter, 1934 from five species of deep-sea macrourids from the Gulf of Mexico and Caribbean Sea. *Zootaxa* **4117**: 491–512.
- Blend CK, Dronen NO, Racz GR, Gardner SL. 2017.** *Pseudopecoelus mccauleyi* n. sp. and *Podocotyle* sp. (Digenea: Opecoelidae) from the deep waters off Oregon and British Columbia with an updated key to the species of *Pseudopecoelus* von Wicklen, 1946 and checklist of parasites from *Lycodes cortezi* (Perciformes: Zoarcidae). *Acta Parasitologica* **62**: 231–254.
- Born-Torrijos A, Kostadinova A, Raga JA, Holzer AS. 2012.** Molecular and morphological identification of larval opecoelids (Digenea: Opecoelidae) parasitising prosobranch snails in a Western Mediterranean lagoon. *Parasitology International* **61**: 450–460.
- Bray RA. 2004.** The bathymetric distribution of the digenean parasites of deep-sea fishes. *Folia Parasitologica* **51**: 268–274.
- Bray RA, Campbell RA. 1996.** New plagioporines (Digenea: Opecoelidae) from deep-sea fishes of the North Atlantic Ocean. *Systematic Parasitology* **33**: 101–113.
- Bray RA, Justine J-L. 2009.** *Neolebouria blatta* n. sp. (Digenea: Opecoelidae) from *Pristipomoides argyrogrammicus* (Valenciennes) and *Etelis carbunculus* Cuvier (Perciformes: Lutjanidae) off New Caledonia. *Systematic Parasitology* **74**: 161–167.
- Bray RA, Justine J-L. 2016.** *Hamacreadium cribbi* n. sp. (Digenea: Opecoelidae) from *Lethrinus miniatus* (Forster) (Perciformes: Lethrinidae) from New Caledonian waters. *Systematic Parasitology* **93**: 761–770.
- Bray RA, Waeschenbach A, Dyal P, Littlewood DTJ, Morand S. 2014.** New digeneans (Opecoelidae) from hydrothermal vent fishes in the south eastern Pacific Ocean, including one new genus and five new species. *Zootaxa* **3768**: 73–87.
- Bray RA, Cribb TH, Littlewood DTJ, Waeschenbach A. 2016.** The molecular phylogeny of the digenean family Opecoelidae Ozaki, 1925 and the value of morphological characters, with the erection of a new subfamily. *Folia Parasitologica* **63**: 1–11.
- Constenla M, Carrassón M, Moyà CM, Fernández-Chacón A, Padrós F, Repullés-Albelda A, Montero FE. 2011.** Parasitisation by *Bathycreadium elongatum* (Digenea, Opecoelidae) in pyloric caeca of *Trachyrincus scabrus* (Teleostei, Macrouridae). *Diseases of Aquatic Organisms* **96**: 239–247.
- Cribb TH. 2005.** Family Opecoelidae Ozaki, 1925. In: Jones A, Bray RA, Gibson DI, eds. *Keys to the Trematoda*, Vol. 2. Wallingford: CABI Publishing and The Natural History Museum, 443–531.
- Cribb TH, Bray RA. 2010.** Gut wash, body soak, blender and heat-fixation: approaches for the effective collection,

- fixation and preservation of trematodes of fishes. *Systematic Parasitology* **76**: 1–7.
- Cribb TH, Bray RA, Littlewood DTJ, Pichelin S, Herniou EA. 2001.** The Digenea. In: Littlewood DTJ, Bray RA, eds. *Interrelationships of the Platyhelminthes*. London: Taylor & Francis, 186–185.
- Curran SS, Overstreet RM, Tkach VV. 2007.** Phylogenetic affinities of *Plagiocirrus* Van Cleave and Mueller, 1932 with the description of a new species from the Pascagoula River, Mississippi. *Journal of Parasitology* **93**: 1452–1458.
- Dollfus RP. 1959.** Recherches expérimentales sur *Nicolla gallica* (R.-Ph. Dollfus 1941) R.-Ph. Dollfus 1958, sa cercaire cotylicerque et sa métacercare progénétique. Observations sur la famille des Coitocaecidae Y. Ozaki 1928, s.f. Coitocaecinae F. Poche 1926 Trematoda Podocotyloidea et sure les cercaires cotylicerques d'eau douce et marines. *Annales de Parasitologie Humaine et Comparée* **34**: 595–622.
- Edgar RC. 2004.** MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**: 1792–1797.
- Faltýnková A, Georgieva S, Kostadinova A, Bray RA. 2017.** Biodiversity and evolution of digeneans of fishes in the Southern Ocean. In: Klimpel S, Kuhn T, Mehlhorn H, eds. *Biodiversity and evolution of parasitic life in the Southern Ocean*. Switzerland: Springer International Publishing, 49–74.
- Fayton TJ, Andres MJ. 2016.** New species of *Plagioporus* Stafford, 1904 (Digenea: Opcoelidae) from California, with an amendment of the genus and a phylogeny of freshwater plagioporines of the Holarctic. *Systematic Parasitology* **93**: 731–748.
- Fayton TJ, Choudhury A, McAllister CT, Robison HW. 2017.** Three new species of *Plagioporus* Stafford, 1904 from darters (Perciformes: Percidae), with a redescription of *Plagioporus boleosomi* (Pearse, 1924) Peters, 1957. *Systematic Parasitology* **94**: 159–182.
- Fayton TJ, McAllister CT, Robison HW, Connior MB. 2018.** Two new species of *Plagioporus* (Digenea: Opcoelidae) from the Ouchita madtom, *Noturus lachneri*, and the banded sculpin, *Cottus caroliniae*, from Arkansas. *Journal of Parasitology* **104**: 145–156.
- Gibson DI. 1976.** Monogenea and Digenea from fishes. *Discovery Reports* **36**: 179–266.
- Gibson DI. 1995.** *Allopodocotyle margolisi* n. sp. (Digenea: Opcoelidae) from the deep-sea fish *Coryphaenoides (Chalinura) mediterraneus* in the northeastern Atlantic. *Canadian Journal of Fisheries and Aquatic Science* **52**: 90–94.
- Gibson DI, Bray RA. 1982.** A study and reorganization of *Plagioporus* Stafford, 1904 (Digenea: Opcoelidae) and related genera, with special reference to forms from European Atlantic waters. *Journal of Natural History* **16**: 529–559.
- Gibson DI, Bray RA. 1984.** On *Anomalotrema* Zhukov, 1957, *Pellamyzon* Montgomery, 1957, and *Opcoelina* Manter, 1934 (Digenea: Opcoelidae), with a description of *Anomalotrema koiae* sp. nov. from North Atlantic waters, *Journal of Natural History* **18**: 949–964.
- Hildebrand J, Sitko J, Zaleśny G, Jeżewski W, Laskowski Z. 2016.** Molecular characteristics of representatives of the genus *Brachylecithum* Shtrom, 1940 (Digenea, Dicrocoeliidae) with comments on lifecycle and host specificity. *Parasitology Research* **115**: 1417–1425.
- Hunninen AV, Cable RM. 1943.** The life history of *Podocotyle atomon* (Rudolphi) (Trematoda: Opcoelidae). *Transactions of the American Microscopical Society* **62**: 57–68.
- ICZN. 2012.** *International Commission on Zoological Nomenclature*. Amendment of articles 8, 9, 10, 21 and 78 of the International Code of Zoological Nomenclature to expand and refine methods of publication. *Bulletin of Zoological Nomenclature* **69**: 161–169.
- Iwatsuki Y, Akazaki M, Taniguchi N. 2007.** Review of the species of the genus *Dentex* (Perciformes: Sparidae) in the western Pacific defined as the *D. hypselosomus* complex with the description of a new species, *Dentex abei* and a redescription of *Evynnis tumifrons*. *Bulletin of the National Museum of Nature and Science Series A* **1**: 29–49.
- Kabata Z. 1961.** A new genus and species of trematode parasitic in *Macrurus fabricii* (Sundevall), a deep-sea fish. *Proceedings of the Zoological Society of London* **136**: 285–292.
- Keller A, Schleicher T, Schultz J, Muller T, Dandekar T, Wolf M. 2009.** 5.8S–28S rRNA interaction and HMM-based ITS2 annotation. *Gene* **430**: 50–57.
- Knudsen SW, Clements KD. 2013a.** *Kyphosus gladius*, a new species of sea chub from Western Australia (Teleostei: Kyphosidae), with comments on *Segutilum klunzingeri* Whitley. *Zootaxa* **3599**: 1–18.
- Knudsen SW, Clements KD. 2013b.** Revision of the fish family Kyphosidae (Teleostei: Perciformes). *Zootaxa* **3751**: 1–101.
- Knudsen SW, Clements KD. 2016.** World-wide species distributions in the family Kyphosidae (Teleostei: Perciformes). *Molecular Phylogenetics and Evolution* **101**: 252–266.
- Kuramochi T. 2009.** Digenean trematodes of fishes from deep-sea areas off the Pacific Coast of Northern Honshu, Japan. In: Fujita T ed. *Deep-sea fauna and pollutants off Pacific Coast of northern Japan*. National Museum of Nature and Science Monographs **39**: 25–37.
- Lanfear R, Calcott B, Ho SYW, Guindon S. 2012.** PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* **29**: 1695–1701.
- Littlewood DTJ, Bray RA, Waeschenbach A. 2015.** Phylogenetic patterns of diversity in cestodes and trematodes. In: Morand S, Krasnov BR, Littlewood DTJ, eds. *Parasite diversity and diversification. Evolutionary ecology meets phylogenetics*. Cambridge, UK: Cambridge University Press, 304–319.
- Machida M, Araki J. 2002.** Three new species of digenean trematodes found in deep-sea fishes of Japan and adjacent waters. *Bulletin of the National Science Museum, Tokyo Series A, Zoology* **24**: 195–200.
- Martin SB, Cutmore SC, Ward S, Cribb TH. 2017.** An updated concept and revised composition for *Hamacreadium* Linton, 1910 (Opcoelidae: Plagioporidae) clarifies a previously obscured pattern of host-specificity among species. *Zootaxa* **4254**: 151–187.
- Martin SB, Cutmore SC, Cribb TH. 2017.** Revision of *Neolebouria* Gibson, 1976 (Digenea: Opcoelidae), with *Trilobovarium* n. g., for species infecting tropical and subtropical shallow-water fishes. *Systematic Parasitology* **94**: 307–338.

- Martin SB, Cribb TH, Cutmore SC, Huston DC. 2018a.** The phylogenetic position of *Choerodonicola* Cribb, 2005 (Digenea: Opecoelidae) with a partial life cycle for a new species from the blue-barred parrotfish *Scarus ghobban* Forsskål (Scaridae) in Moreton Bay, Australia. *Systematic Parasitology* **95**: 337–352.
- Martin SB, Crouch K, Cutmore SC, Cribb TH. 2018b.** Expansion of the concept of the Opistholebetinae Fukui, 1929 (Digenea: Opecoelidae Ozaki, 1925), with *Magnosimum brooksae* n. g., n. sp. from *Tripodichthys angustifrons* (Hollard) (Tetradontiformes: Triacanthidae) in Moreton Bay, Australia. *Systematic Parasitology* **95**: 121–132.
- Martin SB, Cutmore SC, Cribb TH. 2018c.** Revision of *Podocotyloides* Yamaguti, 1934 (Digenea: Opecoelidae), resurrection of *Pedunculacetabulum* Yamaguti, 1934 and the naming of a cryptic opecoelid species. *Systematic Parasitology* **95**: 1–31.
- Manter HW. 1934.** Some digenetic trematodes from deep-water fish of Tortugas, Florida. *Papers from Tortugas Laboratory* **28**: 257–345.
- Miller MA, Pfeiffer W, Schwartz T. 2010.** *Creating the CIPRES Science Gateway for inference of large phylogenetic trees*. Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans, LA, 1–8.
- Olson PD, Cribb TH, Tkach VV, Bray RA, Littlewood DTJ. 2003.** Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *International Journal for Parasitology* **33**: 733–755.
- Palm HW, Klimpel S, Walter T. 2007.** Demersal fish parasite fauna around the South Shetland Islands: high species richness and low host specificity in deep Antarctic waters. *Polar Biology* **30**: 1513–1522.
- Palumbi SR, Martin A, Romano S, McMillan WO, Stice L, Grabowski G. 1991.** *The Simple Fool's Guide to PCR*. Department of Zoology, University of Hawaii, Honolulu, Hawaii.
- Pérez-del-Olmo A, Dallarés S, Carrassón M, Kostadinova A. 2014.** A new species of *Bathycreadium* Kabata, 1961 (Digenea: Opecoelidae) from *Phycisblennoides* (Brünnich) (Gadiformes: Phycidae) in the western Mediterranean. *Systematic Parasitology* **88**: 233–244.
- Rima M, Marzoug D, Pérez-del-Olmo A, Kostadinova A, Bouderbala M, Georgieva S. 2017.** New molecular and morphological data for opecoelid digeneans in two Mediterranean sparid fishes with descriptions of *Macvicaria gibsoni* n. sp. and *M. crassigula* (Linton, 1910) (*sensu stricto*). *Systematic Parasitology* **94**: 739–763.
- Ronquist F, Teslenko M, van der Mark P, Ayres D, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012.** MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**: 539–542.
- Shedko MB, Sokolov SG, Atopkin DM. 2015.** The first record of *Dimerosaccus oncorhynchi* (Trematoda: Opecoelidae) in fishes from rivers of Primorsky Territory, Russia, with a discussion on its taxonomic position using morphological and molecular data. *Parazitologiya* **49**: 171–189.
- Sokolov SG, Lebedeva DI, Gordeev II, Khasanov FK. 2017.** *Zdzitowieckitrema incognitum* gen. et sp. nov. (Trematoda: Xiphidiata) from the Antarctic fish *Muraenolepis marmorata* Günther, 1880 (Gadiformes: Muraenolepidae): ordinary morphology but unclear family affiliation. *Marine Biodiversity*. doi:10.1007/s12526-017-0830-0.
- Sokolov SG, Shchenkov SV, Gordeev II. 2018.** Records of opecoeline species *Pseudopecoelus* cf. *vulgaris* and *Anolmalotrema koiae* Gibson & Bray, 1984 (Trematoda, Opecoelidae, Opecoelinae) from fish of the North Pacific, with notes on the phylogeny of the family Opecoelidae. *Journal of Helminthology*. doi:10.1017/S0022149X18000573.
- Stamatakis A. 2014.** RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**: 1312–1313.
- Tamura K, Stecher G, Peterson D, Filipowski A, Kumar S. 2013.** MEGA6: Molecular Evolutionary Genetics Analysis v.6.0. *Molecular Biology and Evolution* **30**: 2725–2729.
- Tkach VV, Pawlowski J, Mariaux J, Swiderski Z. 2001.** Molecular phylogeny of the suborder Plagiorchiata and its position in the system of Digenea. In: Littlewood DTJ, Bray RA, eds. *Interrelationships of the Platyhelminthes*. London: Taylor and Francis, 186–193.
- Wang Y-Y, Wang P-Q, Zhang W-H. 1992.** Opecoelid trematodes of marine fishes from Fujian Province. *Wuyi Science Journal* **9**: 67–89. [In Chinese.]
- Zdzitowiecki K. 1987.** Digenetic trematodes from the alimentary tract of fishes off South Shetlands (Antarctica). *Acta Parasitologica Polonica* **32**: 219–232.
- Zdzitowiecki K. 1991.** Occurrence of digeneans in open sea fishes off the South Shetland Islands and South Georgia, and a list of fish digeneans in the Antarctic. *Polish Polar Research* **12**: 55–72.
- Zdzitowiecki K. 1997.** Digenea of fishes of the Weddell Sea. IV. Three opecoelid species of the genera *Neolebouria*, *Helicometra* and *Stenakron*. *Acta Parasitologica* **42**: 138–143.
- Zdzitowiecki K, Pisano E, Vacchi M. 1993.** Antarctic representatives of the genus *Neolebouria* Gibson, 1976 (Digenea, Opecoelidae), with description of one new species. *Acta Parasitologica* **38**: 11–14.