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Records of opecoeline species *Pseudopecoelus* cf. *vulgaris* and *Anomalotrema koiae* Gibson & Bray, 1984 (Trematoda, Opecoelidae, Opecoelinae) from fish of the North Pacific, with notes on the phylogeny of the family Opecoelidae

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Abstract

Opecoelid species *Pseudopecoelus* cf. *vulgaris* and *Anomalotrema koiae* Gibson & Bray, 1984 were found in fish collected in the boreal waters of the North Pacific. *Pseudopecoelus* cf. *vulgaris* differs from *Pseudopecoelus vulgaris* (Manter, 1934) in terms of the egg size. This is the first record of *A. koiae* in the North Pacific, and the second of *Pseudopecoelus* cf. *vulgaris*. Previously, *A. koiae* was recorded only in North Atlantic fish. Partial sequences of 28S rDNA obtained for these two species and six other previously unsequenced representatives of the family Opecoelidae were analysed together with data from GenBank. Phylogenetic analysis supports the allocation of the six clades of opecoelids – Helicometrinae, Opecoelinae, Opistholebetinae, 'freshwater Plagioporinae', 'marine Plagioporinae B' and 'marine Plagioporinae C', and confirms the paraphyly of the group 'deep-sea Plagioporinae'. Our phylogeny does not support previous hypotheses about the monophyly of opecoelines with a uroproct.

Introduction

The family Opecoelidae Ozaki, 1925 is a large group of trematodes parasitic in marine and freshwater fish. The modern taxonomic model of this family, based on morphological (Cribb, 2005; Bakhoum *et al.*, 2017) and genetic data (Bray *et al.*, 2016; Martin *et al.*, 2018b, c), distributes opecoelids between six subfamilies: Opecoelinae, Helicometrinae, Opecoelininae, Opistholebetinae, Stenakrinae and Plagioporinae *s. lato*. Plagioporinae *s. lato* is the most problematic group of opecoelids from the taxonomic point of view, as phylogenetic reconstructions demonstrate the polyphyly of this subfamily (Andres *et al.*, 2014; Shedko *et al.*, 2015; Bray *et al.*, 2016; Fayton and Andres, 2016; Faltýnková *et al.*, 2017; Fayton *et al.*, 2017; Martin *et al.*, 2017, 2018b; Rima *et al.*, 2017; Fayton *et al.*, 2018).

In this study, we report on two opecoeline species, *Pseudopecoelus* cf. *vulgaris* and *Anomalotrema koiae* Gibson & Bray, 1984, from fish collected in the boreal waters of the North Pacific. Previously, *A. koiae* was recorded as a parasite only on North Atlantic fish (e.g. Gibson, 1996), and this is the second record of *Pseudopecoelus* cf. *vulgaris* in the North Pacific (see Machida and Araki, 1992). We detail the phylogeny of the family Opecoelidae, taking into account new partial sequences of 28S rDNA of these two species and six other previously unsequenced representatives of the family.

Materials and methods

Specimen collection

The trematodes *Pseudopecoelus* cf. *vulgaris* and *A. koiae* were collected during a parasitological examination of North Pacific fishes *Lycodes brunneofasciatus* Suvorov, 1935, *Microankathus fedorovi* (Mandrytsa, 1991) and *Sebastes* sp. (table 1). A total of 15 specimens of *Lycodes brunneofasciatus* (length 21.0–52.5 cm, weight 135–910 g), 30 specimens of *M. fedorovi* (length 7.5–17.0 cm, weight 19.3–45.0 g), and nine specimens of *Sebastes* sp. (length 30.2–45.0 cm, weight 415–1185 g) were examined in this research. All the specimens were caught by the fishing vessel *Anatoly Torchinov*, when it was fishing for *Pleurogrammus monopterygius* (Pallas, 1810) during 16–25 March 2017 at depths of 150–400 m in the environs of

Table 1. List of newly obtained sequences.

Species (consensus sequences)	Host species and site of infection	Number of specimens	Location	GenBank accession number	Local deposit number
Anomalotrema koiae	*Microankathus fedorovi* (Mandrytsa, 1991) (Actinopterygii: Cyclopteridae), intestine	1	Environs of the Simushir Island, North Pacific	MH161429	T-4-SS-(Op_1-806.1)
Discoverytrema gibsoni	*Muraenolepis marmorata* Günther, 1880 (Actinopterygii: Muraenolepididae), intestine	1	The Ross Sea	MH161430	T-7-SS-(Op_2-654.1)
Discoverytrema markowskii	*M. marmorata*, intestine	1	The Ross Sea	MH161431	T-8-SS-(Op_3-654.x)
Macvicaria muraenolepidis	*M. marmorata*, intestine	1	The Ross Sea	MH161432	T-9-SS-(Op_4-654.3)
Nicolla skrjabini	*Ponticola gorlap* (Iljin, 1949) (Actinopterygii: Gobidae), intestine	1	Obzhorovsky Channel in the Volga River Delta, Astrakhan Region, Russia	MH161438	T-6-SS-(Op_14-Nic96)
Podocotyle atomon	*Littorina saxatilis* (Olivi, 1792) (Gastropoda: Littorinidae)	1	Educational and research station 'Belomorskaia' of Saint Petersburg State University, White Sea, Russia	MH161437	T-10-SS-(Op_13-cerc)
Pseudopecoelus cf. *vulgaris* (sequences 1 and 2)	*Lycodes brunneofasciatus* Suvorov, 1935 (Actinopterygii: Zoarcidae), intestine	2	Environs of the Simushir Island, North Pacific	MH161433	T-1-SS-(Op_7-786.1)
				MH161434	T-2-SS-(Op_8-786.3)
Pseudopecoelus cf. *vulgaris* (sequence no. 3)	*Sebastes* sp. (Actinopterygii: Sebastidae), intestine	1	Environs of the Simushir Island, North Pacific	MH161436	T-3-SS-(Op_11-787)
Sphaerostoma bramae	*Abramis brama* (Linnaeus, 1758) (Actinopterygii: Cyprinidae), intestine	1	Obzhorovsky Channel in the Volga River Delta, Astrakhan Region, Russia	MH161435	T-5-SS-(Op_9-S.br)

Simushir Island. The fish nomenclature was adopted following Voskoboinikova (2015) and Froese and Pauly (2017).

Additionally, novel genetic data are provided for six other opecoelids, namely adult *Sphaerostoma bramae* (Müller, 1776), *Nicolla skrjabini* (Iwanitzky, 1928), *Discoverytrema gibsoni* Zdzitowiecki, 1990, *Discoverytrema markowskii* Gibson, 1976 and *Macvicaria muraenolepidis* Zdzitowiecki, 1990, and *Podocotyle atomon* (Rudolphi, 1802) cercariae (table 1).

The adult worms collected for morphological study were fixed in 70% ethanol at room temperature under a glass cover without additional pressure and stained with acetocarmine. Species identification was performed following Gibson and Bray (1984), Bykhovskaya-Pavlovskaya and Kulakova (1987), Sokolov and Gordeev (2013), and Blend *et al.* (2017). The identification of the *P. atomon* cercariae was carried out according to the descriptions given in Hunninen and Cable (1943) and Chubrik (1966). According to modern data, in littoral molluscs on the north-western coast of the White Sea, there is only one species of cercariae: *P. atomon* (Levakin *et al.*, 2013). In the White Sea, another species of the genus *Podocotyle* Dujardin, 1845 is also noted: *P. reflexa* (Creplin, 1825) (see Shulman and Shulman-Albova, 1953). However, the morphology of cercariae of this species differs sharply from those of the larvae of *P. atomon*: there are three pairs of penetration glands in *P. atomon*, and four in *P. reflexa* (Koie, 1981). All the lengths in morphological descriptions are in µm unless otherwise noted. Specimens destined for molecular analysis (table 1) were fixed in 96% ethanol and stored at 4°C.

DNA extraction, amplification and sequencing, and phylogenetic analysis

In order to obtain 28S rDNA sequence, total DNA was isolated using the ZymoBead Genomic DNA Kit (http://www.zymoresearch.com). Only single trematode specimens were used for each DNA extraction. The DNA fragment of *c.* 1200 bp localized at the 5′ end of 28 rDNA was amplified using the BIO-RAD C1000 Thermal Cycler. Polymerase chain reactions (PCR) were performed in a total volume of 20 µl (11.5 µl H_2O, 2.5 µl Taq buffer, 2 µl dNTP at a concentration of 10 pM, 0.5 µl of each primer at a concentration of 10 pM, 1 µl of Taq polymerase ('Syntol'), 1 µl of the DNA template).

The trematode-specific forward primer LSU-5 (5′-TAG GTC GAC CCG CTG AAY TTA AGC A-3′) and reverse primer 1500R (5′-GCT ATC CTG AGG GAA ACT TCG-3′) were used. GenBank numbers of sequences used in the analysis are provided in table 2. The thermal cycle parameters were as follows: initial denaturation at 95°C (3 minutes); 35 cycles of 20 s at 95°C; 20 s at 56°C; 120 s at 72°C; 5 minutes at 72°C for the final extension. Amplicons were purified using the Cleanup Mini Purification Kit (Eurogene). All amplicons were sequenced directly using equipment belonging to the Research Park of Saint Petersburg State University (Centre for Molecular and Cell Technologies). Sequences from both forward and reverse primers were assembled in Chromas Pro 1.7.4. Each sequence obtained was amplified from primers whose landing sites are at a great distance from each other. Consequently, the sequences obtained from single instances of the

Table 2. List of species incorporated into molecular analysis.

Species	Host species	Location	GenBank accession number	References
Clade 'freshwater Plagioporinae'				
Neoplagioporus ayu	*Plecoglossus altivelis* (Temminck & Schlegel, 1846) (Actinopterygii: Plecoglossidae)	River Asahi, Okayama City, Okayama Prefecture, Japan	KX553947	Fayton and Andres (2016)
Neoplagioporus zacconis	*Opsariichthys platypus* (Temminck & Schlegel, 1846) (Actinopterygii: Cyprinidae)	River Uji, Uji City, Kyoto Prefecture, Japan	KX553949	Fayton and Andres (2016)
Neoplagioporus elongatus	*Sarcocheilichthys variegatus microoculus* Mori, 1927 (Actinopterygii: Cyprinidae)	Lake Biwa, Takashima City, Shiga Prefecture, Japan	KX553948	Fayton and Andres (2016)
Plagioporus aliffi	*Etheostoma blennioides newmanni* (Agassiz, 1854) (Actinopterygii: Percidae)	Arkansas, USA	KX905056	Fayton *et al.* (2017)
Plagioporus boleosomi	*Percina maculata* (Girard, 1859) (Actinopterygii: Percidae)	River West Twin, Wisconsin, USA	KX553953	Fayton and Andres (2016)
Plagioporus carolini	*Cottus carolinae* (Gill, 1861) (Actinopterygii: Cottidae)	Flint Creek, Arkansas, USA	MG214680	Fayton *et al.* (2018)
Plagioporus chiliticorum	*Notropis chiliticus* (Cope, 1870) (Actinopterygii: Cyprinidae)	Basin Creek, North Carolina, USA	KX553943	Fayton and Andres (2016)
Plagioporuis hageli	*Oncorhynchus mykiss* (Walbaum, 1792) (Actinopterygii: Salmonidae)	River Yuba, California, USA	KX553950	Fayton and Andres (2016)
Plagioporus ictaluri	*Noturus lachneri* Taylor, 1969 (Actinopterygii: Ictaluridae)	Gulpha Creek, Arkansas, USA	MG214679	Fayton *et al.* (2018)
Plagioporus fonti	*Percina nigrofasciata* (Agassiz, 1854) (Actinopterygii: Percidae)	Florida, USA	KX905054	Fayton *et al.* (2017)
Plagioporus kolipinskii	*Gasterosteus aculeatus* Linnaeus, 1758 (Actinopterygii: Gasterosteidae)	Lobos Creek, California, USA	KX553952	Fayton and Andres (2016)
Plagioporus limus	*Etheostoma squamosum* (Agassiz, 1854) (Actinopterygii: Percidae)	Arkansas, USA	KX905055	Fayton *et al.* (2017)
Plagioporus loboides (as *Plagiocirrus loboides* in GenBank)	*Fundulus nottii* (Agassiz, 1854) (Actinopterygii: Fondulidae)	The Upper Pascagoula River Wildlife Management Area, George County, Mississippi, USA	EF523477	Curran *et al.* (2007)
Plagioporus shawi	*Oncorhynchus tshawytscha* (Walbaum, 1792) (Actinopterygii: Salmonidae)	River McKenzie, Oregon, USA	KX553951	Fayton and Andres (2016)
Plagioporus sinitsini	*Notemigonus crysoleucas* (Mitchill, 1814) (Actinopterygii: Cyprinidae)	St. Lawrence River, Montreal, Canada	KX553944	Fayton and Andres (2016)
Urorchis goro	*Rhinogobius kurodai* (Tanaka, 1908) (Actinopterygii: Gobiidae)	Metoba River, Nagano, Japan	LC149880	Shimazu (2016)
Group 'deep-sea Plagioporinae'				
Allopodocotyle margolisi	*Coryphaenoides mediterraneus* (Giglioli, 1893) (Actinopterygii: Macrouridae)	Rockall Trough, UK	KU320596	Bray *et al.* (2016)
Bathycreadium brayi	*Phycis blennoides* (Brünnich, 1768) (Actinopterygii: Phycidae)	Tarragona, Spain	KJ683740	Pérez-del-Olmo *et al.* (2014)
Bathycreadium elongatum	*Trachyrincus scabrus* (Rafinesque, 1810) (Actinopterygii: Trachyrincidae)	Spain	JN085948	Constenla *et al.* (2011)
Buticulotrema thermichthysi	*Thermichthys hollisi* (Cohen, Rosenblatt & Moser, 1990) (Actinopterygii: Bythitidae)	South East Pacific Rise, hydrothermal vent site Hobbs, Pacific Ocean	KF733984	Bray *et al.* (2014)
Gaevskajatrema halosauropsi	*Halosauropsis macrochir* (Günther, 1878) (Actinopterygii: Halosauridae)	UK	AY222207	Olson *et al.* (2003)
Neolebouria lanceolata	*Polymixia lowei* Günther, 1859 (Actinopterygii: Polymixiidae)	West Florida Slope, USA	KJ001210	Andres *et al.* (2014)

(*Continued*)

Table 2. (*Continued.*)

Species	Host species	Location	GenBank accession number	References
Podocotyloides brevis	*Conger esculentus* Poey, 1861 (Actinopterygii: Congridae)	Mona Passage, Puerto Rico	KJ001212	Andres *et al.* (2014)
Clade Opecoelinae				
Anomalotrema koiae	*Sebastes viviparous* Krøyer, 1845 (Actinopterygii: Sebastidae)	Shetland Islands	KU320595	Bray *et al.* (2016)
Dimerosaccus oncorhynchi	*Oncorhynchus masou* (Brevoort, 1856) (Actinopterygii: Salmonidae)	Kedrovaya River, Primorsky Territory, Russia	FR870254	Shedko *et al.* (2015)
Opecoeloides furcatus	*Mullus surmuletus* Linnaeus, 1758 (Actinopterygii: Mullidae)	Mediterranean Sea near Corsica	AF151937	Tkach *et al.* (2000)
Opecoeloides fimbriatus	*Micropogonias undulatus* (Linnaeus, 1766) (Actinopterygii: Sciaenidae)	Gulf of Mexico	KJ001211	Andres *et al.* (2014)
Pseudopecoeloides tenuis	*Priacanthus hamrur* (Forsskål, 1775) (Actinopterygii: Priacanthidae)	NewCaledonia	KU320605	Bray *et al.* (2016)
Clade 'marine Plagioporinae B'				
Allopodocotyle epinepheli	*Epinephelus cyanopodus* (Richardson, 1846) (Actinopterygii: Serranidae)	New Caledonia	KU320598	Bray *et al.* (2016)
Bentholebouria blatta	*Pristipomoides argyrogrammicus* (Valenciennes, 1832) (Actinopterygii: Lutjanidae)	New Caledonia	KU320608	Bray *et al.* (2016)
Bentholebouria colubrosa	*Pristipomoides aquilonaris* (Goode & Bean, 1896) (Actinopterygii: Lutjanidae)	West Florida Shelf, USA	KJ001207	Andres *et al.* (2014)
Cainocreadium labracis (cercariae)	*Gibbula adansonii* (Payraudeau, 1826) (Gastropoda: Trochidae)	Els Alfacs Lagoon (Ebro Delta), Spain	JQ694144	Born-Torrijos *et al.* (2012)
Cainocreadium lintoni	*Epinephelus morio* (Valenciennes, 1828) (Actinopterygii: Serranidae)	Caribbean Sea	KJ001208	Andres *et al.* (2004)
Hamacreadium cribbi	*Lethrinus miniatus* (Forster, 1801) (Actinopterygii: Lethrinidae)	New Caledonia	KU320603	Bray *et al.* (2016)
Hamacreadium mutabile	*Lutjanus fulviflamma* (Forsskål, 1775) (Actinopterygii: Lutjanidae)	New Caledonia	KU320601	Bray *et al.* (2016)
Macvicaria macassarensis	*L. miniatus* (Actinopterygii: Lethrinidae)	Heron Island, Coral Sea, Australia	AY222208	Olson *et al.* (2003)
Pedunculacetabulum inopinipugnus	*Plectorhinchus chrysotaenia* (Bleeker, 1855) (Actinopterigii: Haemulidae)	Lizard Island, Queensland, Australia	MF805700	Martin *et al.* (2018c)
Pacificreadium serrani	*Plectropomus leopardus* (Lacepède, 1802) (Actinopterygii: Serranidae)	New Caledonia	KU320602	Bray *et al.* (2016)
Podocotyloides australis	*Diagramma pictum* (Thunberg, 1792) (Actinopterigii: Haemulidae)	Dunwich, North Stradbroke Island, Australia	MF805696	Martin *et al.* (2018c)
Podocotyloides gracilis	*D. pictum* (Actinopterigii: Haemulidae)	Lizard Island, Queensland, Australia	MF805693	Martin *et al.* (2018c)
Clade '*Pedunculacetabulum inopinipugnus* + *Podocotyloides stenometra*'				
Podocotyloides stenometra	*Heniochus chrysostomus* Cuvier, 1831 (Actinopterygii: Chaetodontidae)	Mo'orea, French Polynesia	MF926406	Martin *et al.* (2018c)
Pseudopycnadena tendu	*Pseudobalistes fuscus* (Bloch & Schneider, 1801) (Actinopterygii: Balistidae)	New Caledonia	FJ788506	Bray *et al.* (2009)
Clade Opistholebetinae				
Gaevskajatrema perezi	Unidentified fish	Near Corsica	AF184255	Tkach *et al.* (2001)
Maculifer sp.	*Diodon hysterix* Linnaeus, 1758 (Actinopterygii: Diodontidae)	Australia	AY222211	Olson *et al.* (2003)

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Table 2. (*Continued.*)

Species	Host species	Location	GenBank accession number	References
Macvicaria bartolii	*Diplodus annularis* (Linnaeus, 1758) (Actinopterygii: Sparidae)	Bay of Bizerte, Tunisia	KR149464	Antar *et al*. (2015)
Macvicaria crassigula	*Diplodus vulgaris* (Geoffroy Saint-Hilaire, 1817) (Actinopterygii: Sparidae)	Bouzedjar, Algeria	MF166847	Rima *et al*. (2017)
Macvicaria dubia	*Oblada melanura* (Linnaeus, 1758) (Actinopterygii: Sparidae)	Bay of Bizerte, Tunisia	KR149470	Antar *et al*. (2015)
Macvicaria gibsoni	*D. vulgaris* (Actinopterygii: Sparidae)	Algiers, Algeria	MF166845	Rima *et al*. (2017)
Macvicaria maamouriae	*Lithognathus mormyrus* (Linnaeus, 1758) (Actinopterygii: Sparidae)	Bizerte Lagoon, Tunisia	KR149468	Antar *et al*. (2015)
Macvicaria mormyri	*Sparus aurata* Linnaeus, 1758 (Actinopterygii: Sparidae)	Bouzedjar, Algeria	MF166850	Rima *et al*. (2017)
Macvicaria obovata (metacercariae)	*Cyclope neritea* (Linnaeus, 1758) (Gastropoda: Nassariidae)	Els Alfacs Lagoon (Ebro Delta), Spain	JQ694147	Born-Torrijos *et al*. (2012)
Magnaosimum brooksae	*Tripodichthys angustifrons* (Hollard, 1854) (Actinopterygii: Tricanthidae)	Moreton Bay, Australia	MG813907.2	Martin *et al*. (2018b)
Opistholebes amplicoelus	*Tetractenos hamiltoni* (Richardson, 1846) (Actinopterygii: Tetraodontidae)	Australia	AY222210	Olson *et al*. (2003)
Peracreadium idoneum	*Anarhichas lupus* Linnaeus, 1758 (Actinopterygii: Anarchichadidae)	UK	AY222209	Olson *et al*. (2003)
Pseudopycnadena fischthali	*D. vulgaris* (Actinopterygii: Sparidae)	Bouzedjar, Algeria	MF166851	Rima *et al*. (2017)
Propycnadenoides philippinensis	*Gymnocranius grandoculis* (Valenciennes, 1830) (Actinopterygii: Lethrinidae)	New Caledonia	KU320604	Bray *et al*. (2016)
Clade 'marine Plagioporinae C'				
Macvicaria magellanica	*Patagonotothen longipes* (Steindachner, 1876) (Actinopterygii: Nototheniidae)	Antarctica	KU212191	Hildebrand *et al*. (2016)
Podocotyloides parupenei	*Mulloidichthys vanicolensis* (Valenciennes, 1831) (Actinopterygii: Mullidae)	Lizard Island, Queensland, Australia	MF926409	Martin *et al*. (2018c)
Trilobovarium parvvatis	*Lethrinus nebulosus* (Forsskål, 1775) (Actinopterygii: Lethrinidae)	Lizard Island, Queensland, Australia	KY551562	Martin *et al*. (2017)
Choerodonicola renko	*Evynnis tumifrons* (Temminck & Schlegel, 1843) (Actinopterygii: Sparidae)	Minabe fish market, Wakayama Prefecture, Japan	MG844421	Martin *et al*. (2018a)
Choerodonicola arothokoros	*Scarus ghobban* (Forsskål, 1775) (Actinopterygii: Scaridae)	Moreton Bay, Australia	MG844418	Martin *et al*. (2018a)
Clade Helicometrinae				
Helicometra boseli	*Sargocentron spiniferum* (Forsskål, 1775) (Actinopterygii: Holocentridae)	New Caledonia	KU320600	Bray *et al*. (2016)
Helicometra epinepheli (as *Helicometra fasciata* in GenBank)	*Epinephelus fasciatus* (Forsskål, 1775) (Actinopterygii: Serranidae)	New Caledonia	KU320597	Bray *et al*. (2016)
Outgroup Brachycladiidae				
Campula oblonga	*Phocoena phocoena* (Linnaeus, 1758) (Mammalia: Phocoenidae)	UK	KM258671	Fraija-Fernández *et al*. (2015)
Oschmarinella macrorchis	*Mesoplodon carlhubbsi* (Moore, 1963) (Mammalia: Ziphiidae)	Samani, Hokkaido, Japan	LC269095	Nakagun *et al*. (2018)
Zalophotrema hepaticum	*Zalophus californianus* (Lesson, 1828) (Mammalia: Otariidae)	USA	AY222255	Olson *et al*. (2003)

worms were manually corrected, and the sequences obtained were consensual. The voucher specimens of the studied species were deposited in the specimen collection at the Department of Invertebrate Zoology at Saint Petersburg State University.

The newly obtained sequences were included in a general alignment (table 1). Firstly, the sequences were automatically aligned using the MUSCLE algorithm (Edgar, 2004), as implemented in SeaView 4.0 (Gouy *et al*., 2010). Then the alignment was verified

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manually. The phylogenetic analysis was performed using the maximum likelihood (ML) method, as implemented in the RaxML program (Stamatakis, 2006) at CIPRES Science Gateway (www.phylo.org) (Miller *et al.*, 2010). About 1000 sites were selected for the analysis. The stability of the clades was assessed using a non-parametric bootstrap with 1000 pseudoreplicates. All estimations in RaxML were calculated using default parameters.

Bayesian inference (BI) analysis was performed using MrBayes 3.1.2, a general time reversible (GTR) model with gamma correction for intersite rate variation (eight categories), and the covarion model. Trees were run as two separate chains (default heating parameters) for 15 million generations, at which point they had ceased converging. The quality of chains was estimated using built-in MrBayes tools and, additionally, the Tracer 1.6 package (Rambaut *et al.*, 2014). Based on the estimates using Tracer, 250,000 generations were discarded for burn-in (the relative burn-in parameter was switched off). The names of the clades are given following Martin *et al.* (2018b, c).

Results

Systematics

Family Opecoelidae Ozaki, 1925
Subfamily Opecoelinae Ozaki, 1925
Genus *Pseudopecoelus* von Wicklen, 1946

Pseudopecoelus cf. *vulgaris* (fig. 1A)

Description

(Based on five gravid specimens from *L. brunneofasciatus*). Body elongate-oval, flattened dorso-ventrally, 1.90–3.81 × 0.52–0.95 mm. Tegument unarmed. Oral sucker subspherical, unspecialized, 186–285 × 174–242. Ventral sucker slightly protuberant, subellipsoidal, unspecialized, 248–446 × 397–521; aperture surrounded by lip-like folds of tegument. Ratio of oral sucker width to ventral sucker width 1 : 2.10–2.29. Forebody 19.5–30.1% of body length. Mouth subterminal. Prepharynx not visible. Pharynx muscular, 153–199 × 92–135; oesophagus 167–360. Intestinal bifurcation short distance anterior to ventral sucker. Caeca terminate blindly near posterior extremity. Testes two, in third quarter of body, median, tandem, contiguous, lobated; anterior testis 186–496 × 310–546, posterior testis 248–459 × 298–583. Post-testicular region 533–980. Cirrus-sac absent. Seminal vesicle tubular, convoluted, free in parenchyma, extending some distance into hindbody. Pars prostatica surrounded by prostatic gland-cells; ejaculatory duct thick-walled, short. Genital pore at level of pharynx, sinistro-submedian. Ovary 4-lobed, median, pretesticular, contiguous with anterior testis, 174–273 × 260–397. Oviduct arising from antero-medial lobe of ovary. Laurer's canal opening on dorsal side of body between sinistral caecum and sinistral body margin, at level of ovary. Seminal receptacle uterine. Mehlis' gland distinct. Uterus preovarian, intercaecal. Metraterm not visible. Vitellarium follicular; follicles in two longitudinal lateral fields extending from ventral sucker region or intestinal bifurcation level to posterior extremity of body, frequently one field slightly longer than other. Vitelline fields overlapping caeca ventrally and confluent in post-testicular region on ventral side of body. Dorsal part of each field covers caecum only in ventral sucker and inter-gonadal areas; in post-testicular region divided into two rows: extra-caecal and submedian intra-caecal. Eggs numerous, oval, operculate, with small knob at anopercular pole, 79–82 × 41–47. Excretory bladder tubular, reaching to ovary; pore terminal.

Taxonomic summary

Host. Lycodes brunneofasciatus (Perciformes: Zoarcidae) and *Sebastes* sp. (Scorpeniformes: Sebascidae).

Locality. North Pacific in the environs of Simushir Island, 46°28′S, 150°59′E, depth 160 m.

Date of collection. 20 March 2017.

Site of infection. Intestine.

Prevalence and intensity. L. brunneofasciatus – 40% (n = 15), 3–10 individuals; *Sebastes* sp. – 55.6% (n = 9), 1 individual.

Specimens deposited. Mount no. 14266 in the Museum of Helminthological Collections at the Centre for Parasitology of the A.N. Severtsov Institute of Ecology and Evolution (IPEE RAS), Moscow, Russia.

Systematics

Family Opecoelidae Ozaki, 1925
Subfamily Opecoelinae Ozaki, 1925
Genus *Anomalotrema* Zhukov, 1957

Anomalotrema koiae Gibson & Bray, 1984 (fig. 1B)

Description

(Based on two gravid specimens from *M. fedorovi*). Body elongate, flattened dorso-ventrally, 1.60–1.86 × 0.37–0.5 mm. Tegument unarmed. Oral sucker subellipsoidal, unspecialized, 122 × 147–171. Ventral sucker pedunculate, with two sublateral muscular lobes on anterior margin and single median lobe on posterior margin, 322–360 × 260–285. Ratio of oral sucker width to ventral sucker width 1 : 1.67–1.77. Mouth subterminal. Prepharynx 52, muscular pharynx 92–122 × 110–159, oesophagus 183. Caeca extending dorso-laterally to posterior extremity of body and opening through separate ani, which lie ventrally to excretory pore. Testes two, in third quarter of body, median, tandem, contiguous entire, oval; anterior testis 174–186 × 236–298, posterior testis 217–233 × 248–273. Post-testicular region 322–446. Vas deferens absent; vasa efferentia joined directly to internal seminal vesicle. Cirrus-sac membranous, containing convoluted internal seminal vesicle, tubular pars prostatica and ejaculatory duct. Distal region of seminal vesicle surrounded by prostatic gland-cells. Genital pore at level of oesophagus, sinistro-submedian. Ovary three-lobed median, pretesticular, contiguous with anterior testis 159 × 190–226. Laurer's canal opening dorsally to sinistral caecum at level of ovary. Seminal receptacle uterine. Mehlis' gland distinct. Uterus preovarian, intercaecal. Metraterm muscular. Vitellarium follicular in two longitudinal lateral fields; fields extending from proximal part of external seminal vesicle to posterior extremity of body, interrupted at level of gonads and confluent in post-testicular region. Vitelline reservoir dorsal to ovary. Eggs numerous, 64–66 in length (deformed in Canada balsam). Excretory bladder tubular, extending to ovarian zone; pore terminal.

Taxonomic summary

Host. Microancathus fedorovi (Scorpeniformes: Cyclopteridae).

Locality. North Pacific in the environs of Simushir Island, 47°13′S, 152°24′E, depth 210 m.

Date of collection. 25 March 2017.

Site of infection. Intestine.

Prevalence and intensity. 6.7% (n = 30), 1–3 individuals.

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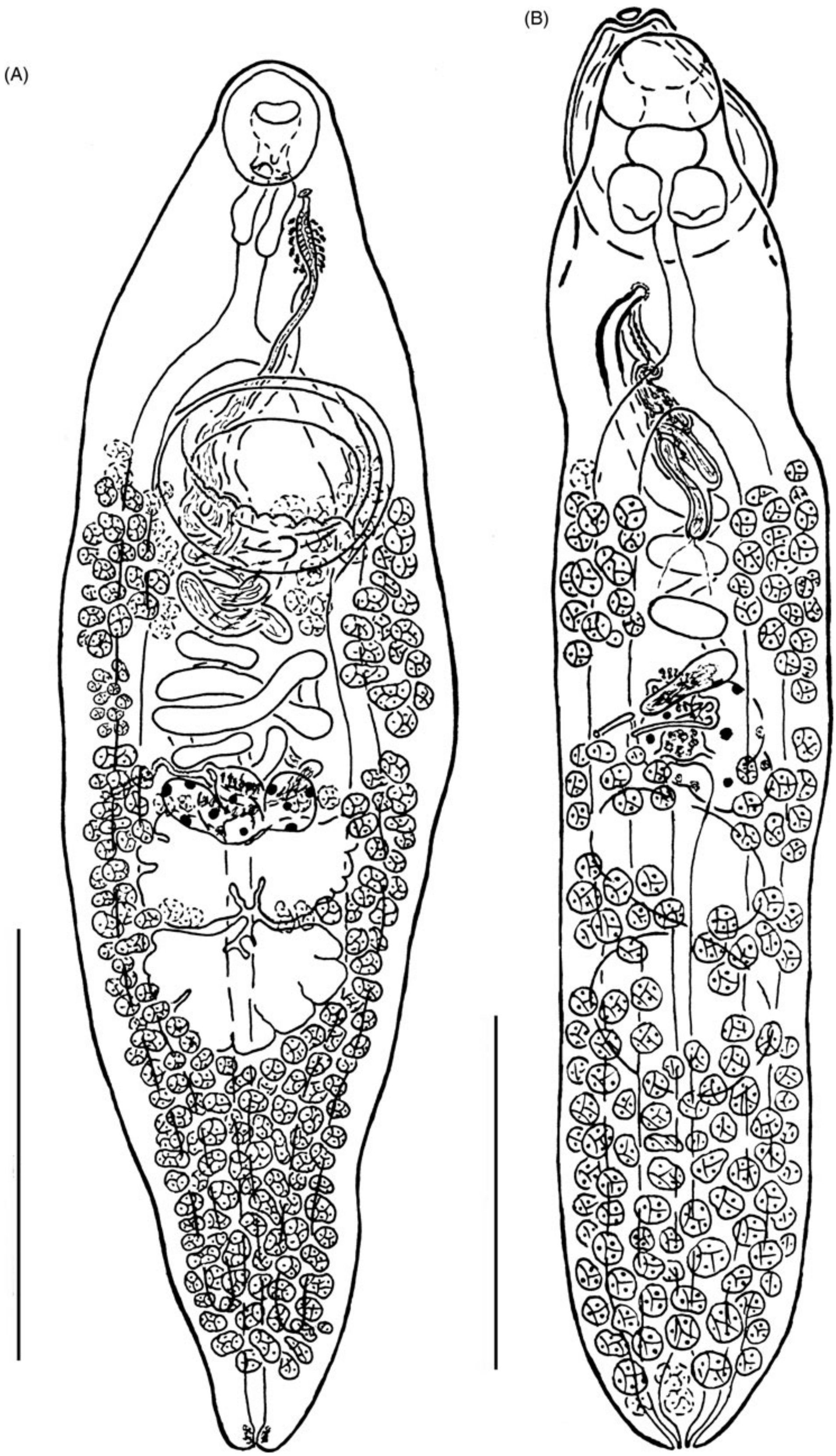

Fig. 1. (A) *Pseudopecoelus* cf. *vulgaris,* ventral view, and (B) *Anomalotrema koiae*, dorsal view (the basal part of the pedunculate ventral sucker is indicated by a dotted line). Scale bars: A, 1 mm; B, 0.5 mm.

Specimens deposited. Mounts no. 14264 and 14265 in the Museum of Helminthological Collections at the Centre for Parasitology of the A.N. Severtsov Institute of Ecology and Evolution (IPEE RAS), Moscow, Russia.

Phylogenetic data

The BI and ML trees were similar terms of general topology (figs 2 & 3). All opecoelids were distributed between six well-supported

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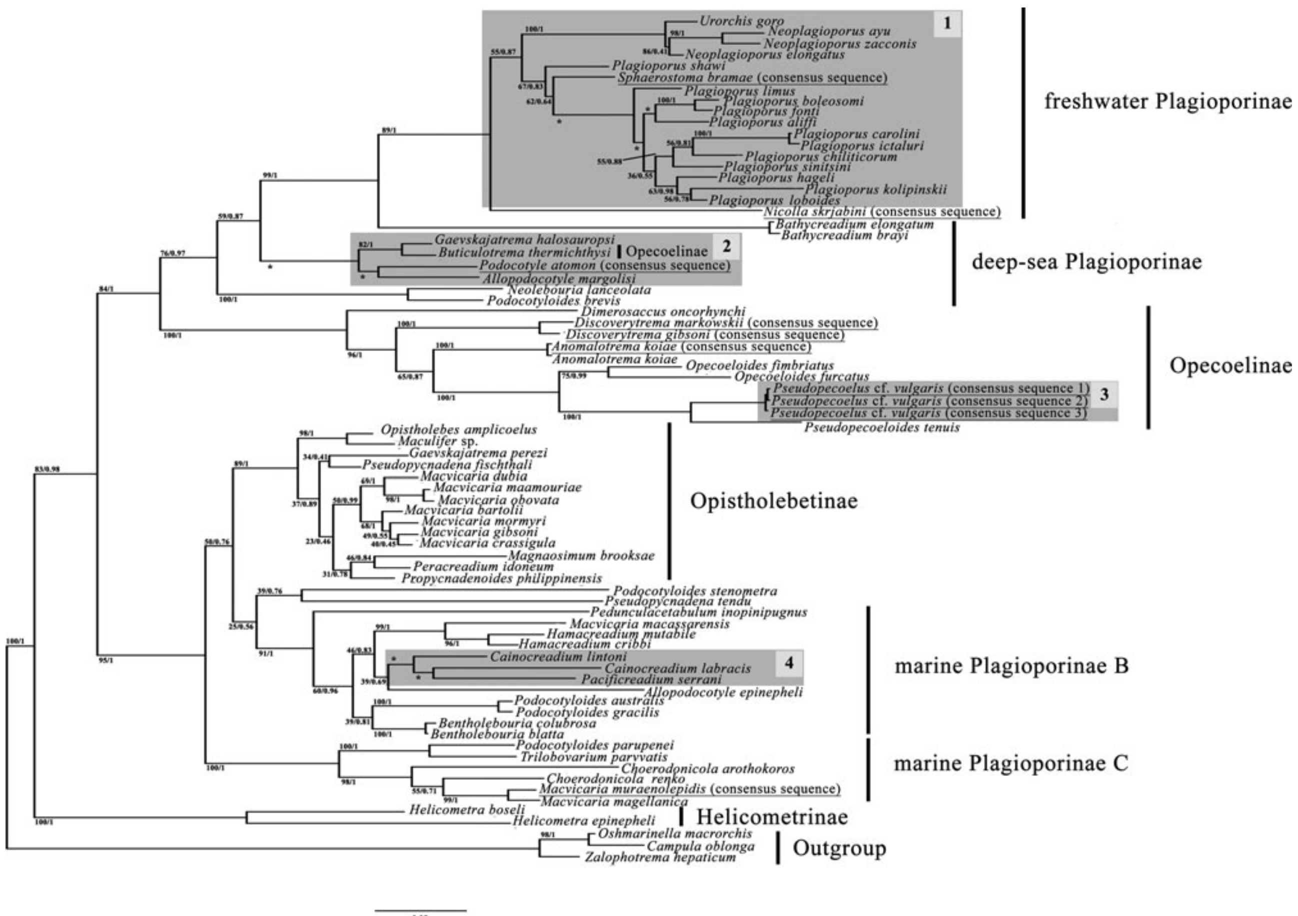

Fig. 2. Phylogenetic tree of the Opecoelidae based on the analysis of 28S rDNA partial sequences using ML and BI algorithms; nodal numbers indicate bootstraps/posterior probabilities. The brachycladiid species *Zalophotrema hepaticum*, *Oshmarinella macrorchis* and *Campula oblonga* were used as outgroups. Clades with grey shading have different supports in the BI and ML trees and are shown in fig. 3.

clades – Helicometrinae, Opecoelinae, Opistholebetinae, 'freshwater Plagioporinae', 'marine Plagioporinae B' and 'marine Plagioporinae C', and also the paraphyletic group 'deep-sea Plagioporinae' and the poorly supported clade '*Pedunculacetabulum inopinipugnus* +*Podocotyloides stenometra*' (fig. 2).

Pseudopecoelus cf. *vulgaris* and *A. koiae* were included in the clade Opecoelinae (fig. 2). Three sequences of 28S rDNA for *Pseudopecoelus* cf. *vulgaris* (GenBank no. MH161433 and MH161434 ex *L. brunneofasciatus,* and MH161436 ex *Sebastes* sp.) were identical. The newly obtained sequence of 28S rDNA in our own specimen of *A. koiae* was identical to that of *A. koiae* GenBank no. KU320595. Other species examined in this study were distributed across the tree as follows: *D. gibsoni* and *D. markowskii* were sister species to each other in the clade Opecoelinae; *S. bramae* and *N. skrjabini* were in the clade 'freshwater Plagioporinae', *M. muraenolepidis* was in the clade 'marine Plagioporinae C', and *P. atomon* was in the group 'deep-sea Plagioporinae'.

Pseudopecoelus cf. *vulgaris* is a well-supported sister taxon of *Pseudopecoeloides tenuis* Yamaguti, 1940 within the clade Opecoelinae, and the group they form combines with *Opecoeloides* spp. to constitute a well-supported terminal subclade. This subclade has a weakly supported sister relationship with *A. koiae.* The *Discoverytrema* spp. group shares a common ancestor with a marine opecoelines group, consisting of *A. koiae, Pseudopecoelus* cf. *vulgaris, Pseudopecoeloides tenuis* and *Opecoeloides* spp. (fig. 2).

Macvicaria muraenolepidis was combined with *Macvicaria magellanica* Laskowski, Jeżewski & Zdzitowiecki, 2013 with high support, within the clade 'marine Plagioporinae C'. Group *M. muraenolepidis* + *M. magellanica* was combined with *Choerodonicola renko* Machida, 2014 and *Choerodonicola arothokoros* Martin, Cribb, Cutmore, & Huston, 2018 into a very well-supported subclade, which is sister to the also strongly supported subclade *Podocotyloides parupenei* + *Trilobovarium parvvatis* (fig. 2).

Podocotyle atomon was clustered with the deep-sea plagioporines *Allopodocotyle margolisi* Gibson, 1995, *Buticulotrema thermichthysi* Bray, Waeschenbach, Dyal, Littlewood & Morand, 2014 and *Gaevskajatrema halosauropsi* Bray & Campbell, 1996. However, the strict interrelation between *P. atomon* and these species remains unresolved (figs 2 & 3). The position of *S. bramae* and *N. skrjabini* within the 'freshwater Plagioporinae' clade was poorly resolved (figs 2 & 3).

Discussion

The genus *Pseudopecoelus* von Wicklen, 1946 unites 39 species (Blend *et al.*, 2017). The specimens that we studied are undoubtedly conspecific with the *Pseudopecoelus* sp. collected by Machida and Araki (1992) from *Careproctus roseofuscus* Gilbert & Burke, 1912 in the Sea of Okhotsk. In turn, these parasites are most similar to *Pseudopecoelus vulgaris* (Manter, 1934) in terms of the size of the body, ratio of suckers, presence of lip-like folds around the

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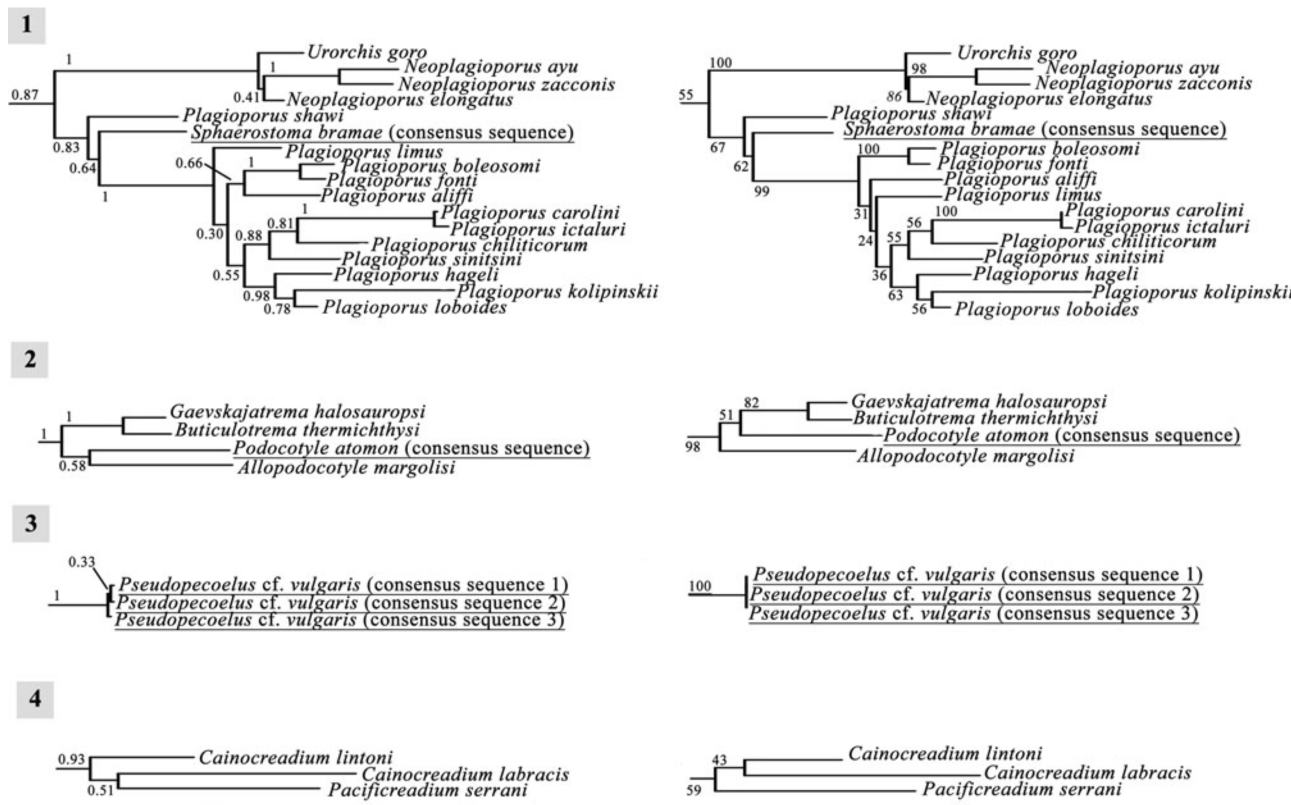

Fig. 3. Mismatches between phylogenetic trees inferred with the ML and BI algorithms. On the left are fragments of the Bayesian tree and on the right are fragments of the ML tree.

ventral sucker, position of the genital pore and the anterior edge of the vitelline fields, seminal vesicle and gonad shape, position of the gonads very close together and continuous (without discontinuity) arrangement of vitelline follicles opposite to the testes (see Blend *et al.*, 2017). Nevertheless, the egg length in *Pseudopecoelus* cf. *vulgaris* specimens (= *Pseudopecoelus* sp. Machida & Araki, 1992) is less than that of gravid *P. vulgaris* s. str.; 79–83 vs 90–127 µm (this study; Manter, 1934, 1954; Machida and Araki, 1992). For very young individuals of *P. vulgaris* s. str. with a body length of up to 1.39 mm, eggs of a smaller length, 78 µm, are indicated by Manter (1934). The trematodes examined by us cannot be classified as very young, based on both the length of the body (1.90–3.81) and the presence of numerous eggs in the uterus. The closest to the Simushir Island area that *P. vulgaris* s. str. have been discovered is Tosa Bay, off the Pacific coast of southern Japan (Kuramochi, 2001). Thus, molecular data on *P. vulgaris* s. str. are required for the clarification of the taxonomic relationship between these two parasites.

The genus *Anomalotrema* Zhukov, 1957 contains two species: *A. putjatini* Zhukov, 1957 (type species) and *A. koiae* (see Gibson and Bray, 1984; Gibson, 1996). Morphological differences between these species are manifested in the length of the body (2.38–7.38 mm vs 1.35–4.4 mm), shape of the ovary (indistinctly lobed or bell-shaped vs clearly 3–4 lobed) and sucker ratio (1 : 1.47–1.6 vs 1 : 1.8–2.4) (Zhukov, 1957, 1963; Gibson and Bray, 1984; Gibson, 1996). Until now, *A. putjatini* has been recorded from North Pacific scorpeniform and gadiform fish (Zhukov, 1957, 1963; Korotaeva, 1968), and *A. koiae* from North Atlantic scorpeniform, gadiform and pleuronectiform fish (Gibson, 1996; Gaevskaya, 2002). Our specimens correspond with the description of *A. koiae* in all features except the sucker ratio, and they occupy a position intermediate between *A. koiae* and *A. putjatini* in the indicated feature. However, given the identity of our sample of 28S rDNA sequences compared with those of *A. koiae* (see above), we consider them conspecific with this species.

The inclusion of *Pseudopecoelus* cf. *vulgaris*, *A. koiae* and *Discoverytrema* spp. in the clade Opecoelinae corresponds with the generally accepted point of view regarding the subfamilial affiliation of these trematodes (Zdzitowiecki, 1990a; Gibson, 1996; Cribb, 2005; Blend *et al.*, 2017). The molecular data we obtained support the morphologically based monophyly of the genus *Discoverytrema* Gibson, 1976 (see Zdzitowiecki, 1990a). The node support between *A. koiae* and the group that unites the representatives of the genera *Opecoeloides* Odhner, 1928, *Pseudopecoelus*, *Pseudopecoeloides* Yamaguti, 1940 is too low to be convincing (fig. 2). In this regard, it is impossible to ascertain yet which group among the mentioned opecoelines is the sister branch to the genus *Discoverytrema*. At the same time, present analysis demonstrates the sister position of the *Opecoeloides* spp. group with respect to the *Pseudopecoelus* cf. *vulgaris* + *Pseudopecoeloides tenuis* group. Previously published reconstructions of phylogeny of the opecoelids, which did not include *Pseudopecoelus* cf. *vulgaris*, have supported the view that *P. tenuis* is the sister taxon to the *Opecoeloides* spp. group (e.g. Bray *et al.*, 2016; Rima *et al.*, 2017; Fayton *et al.*, 2018; Martin *et al.*, 2018c). Based on these data, Bray *et al.* (2016) hypothesized monophyly of opecoelines with a uroproct. It is important to note that a uroproct is characteristic of *Pseudopecoeloides* spp. and *Opecoeloides* spp., but not *Pseudopecoelus* spp. (e.g. Cribb, 2005). Therefore, the data we have obtained do not support this hypothesis. It is difficult to identify reliable synapomorphies for the *Opecoeloides* spp. + (*Pseudopecoelus* cf. *vulgaris* + *Pseudopecoeloides tenuis*)

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subclade or the *Pseudopecoelus* cf. *vulgaris* + *Pseudopecoeloides tenuis* group, because of the limited number of opecoelines with a phylogenetic position verified using the molecular methods.

There are no clear morphological differences between the clades of the Plagioporinae sensu Cribb, 2005: 'freshwater Plagioporinae' 'marine Plagioporinae B', 'marine Plagioporinae C', Opistholebetinae, 'deep-sea Plagioporinae', and '*Pedunculacetabulum inopinipugnus* + *Podocotyloides stenometra*' (Bray *et al.*, 2016; Martin *et al.*, 2018b). The concept of these clades is supported mainly by phylogenetic and ecological evidence. The inclusion of freshwater representatives of the Plagioporinae *sensu* Cribb, 2005 (*S. bramae* and *N. skrjabini*) into the clade 'freshwater Plagioporinae' (fig. 2) was quite predictable, taking into account the last of the above-mentioned criteria.

The distribution of the remaining plagioporines studied – *M. muraenolepidis* and *P. atomon* – was difficult to predict. *Macvicaria muraenolepidis* appeared in the clade 'marine Plagioporinae C', as a sister to *M. magellanica* (fig. 2). At the same time, most *Macvicaria* Gibson & Bray, 1982 with known 28S rDNA sequences belonged to the clade Opistholebetinae (Rima *et al.*, 2017; Martin *et al.*, 2017, 2018a, b, c). Both species under consideration are in different morphological groups of *Macvicaria* spp. (by Aken'Ova *et al.*, 2008): group D (*M. muraenolepidis*) and group B (*M. magellanica*) (Zdzitowiecki, 1990b; Aken'Ova *et al.*, 2008; Laskowski *et al.*, 2013). However, both belong to the same zoogeographical group, namely the Southern Ocean fauna (Laskowski *et al.*, 2013).

Our analysis confirms the data provided by Martin *et al.* (2018a) on the close relationship between the Antarctic and Subantarctic *Macvicaria* spp. with representatives of the genus *Choerodonicola* Cribb, 2005. According to Martin *et al.* (2018a) the genus *Choerodonicola* is paraphyletic. According to our data, its paraphyly is unclear because of low support of the direct sister relationship of the *M. muraenolepidis* + *M. magellanica* group with *C. renko*. Like Martin *et al.* (2018a), we do not think there are enough data to put Antarctic and Subantarctic *Macvicaria* spp. into the genus *Choerodonicola* today. Firstly, revision of *Macvicaria* genus is impossible without molecular data for its type species *Macvicaria alacris* (Looss, 1901). The second reason is the uncertainty regarding the revealed relationships between the above-mentioned *Macvicaria* spp. and *C. renko*.

In general, our data indicate a polyphyly not only of the genus *Macvicaria,* but also three other genera of opecoelids: *Allopodocotyle* Pritchard, 1966, *Podocotyloides* Yamaguti, 1934 and *Gaevskajatrema* Gibson & Bray, 1982 (fig. 2), which is in full accordance with the data in the literature (Andres *et al.*, 2014; Shedko *et al.*, 2015; Bray *et al.*, 2016; Faltýnková *et al.*, 2017; Martin *et al.*, 2017, 2018c; Rima *et al.*, 2017).

According to Fayton and Andres (2016), and Martin *et al.* (2018b) three clades of the plagioporine species – 'marine Plagioporinae B', 'marine Plagioporinae C' and 'freshwater + deep-sea Plagioporinae' – require recognition as independent subfamilies. However, we should not forget that there are still no molecular data on indisputable representatives of the Stenakrinae (see Sokolov *et al.*, 2017), as well as for opecoelinines, which may change the topology of the tree for the Opecoelidae. Thus, the question about the taxonomic status of the three listed clades remains open.

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Conflict of interest. None.

Ethical standards. All applicable international, national and/or institutional guidelines for the care and use of animals were followed by the authors. All necessary permits for sampling and observational field studies were obtained by the authors from the competent authorities.

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