

Research Paper

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**Author for correspondence:**

D.M. Atopkin, Fax.: +7 4232310193

E-mail: [atop82@gmail.com](mailto:atop82@gmail.com)

# Morphological and molecular data for species of *Lecithaster* Lühe, 1901 and *Hysterolecithoides* Yamaguti, 1934 (Digenea: Lecithasteridae) from fish of East Asia and phylogenetic relationships within the Hemiuroidea Looss, 1899

D.M. Atopkin<sup>1,2</sup>, M. Nakao<sup>3</sup>, V.V. Besprozvannykh<sup>1</sup>, N.D. Ha<sup>4</sup>, H.V. Nguyen<sup>4</sup> and M. Sasaki<sup>3</sup>

<sup>1</sup>Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of Russian Academy of Sciences, Vladivostok, Russia; <sup>2</sup>Department of Cell Biology and Genetics, Far Eastern Federal University, Vladivostok, Russia; <sup>3</sup>Department of Parasitology, Asahikawa Medical University, Midorigaoka-Higashi 2-1, Hokkaido 078-8510, Japan and <sup>4</sup>Institute of Ecology and Biodiversity, Vietnamese Academy of Sciences and Technology, Hanoi, Vietnam

## Abstract

Four representatives of the genus *Lecithaster* and one representative of the genus *Hysterolecithoides* were found during investigation of the trematode fauna of fish species in Vietnamese, Japanese and eastern coastal waters of the Russian Far East. Based on morphometric data, adult trematodes from Vietnamese *Strongylura strongylura* and Russian *Acanthogobius flavimanus* were identified as *Lecithaster confusus*, trematodes from Vietnamese *Hemirhamphus marginatus* as *L. sayori* and from osmerid fishes as *L. salmonis*. Further, a single specimen of *Lecithaster* sp. and representatives of *Hysterolecithoides epinepheli* were found in Vietnamese *Siganus fuscescens*. Morphological and molecular data, including 18S ribosomal DNA (rDNA) V4 fragment, 28S rDNA D1-D3 fragment, internal transcribed spacers (ITS) and a mitochondrial COI gene fragment were analysed for *Lecithaster* spp. The results revealed that *L. sayori* and *L. salmonis* are not synonyms of *L. stellatus* and *L. gibbosus*, respectively, but that *Hysterolecithoides frontilatus* and *H. guangdongensis* are junior synonyms of *H. epinepheli*. The 28S-rDNA-based phylogenetic tree of Hemiuroidea showed a distinct position for the genus *Lecithaster* with internal differentiation into three subclades, including *L. confusus*, *L. sayori* and *Lecithaster* sp. within the first subclade, *L. mugilis* and *L. sudzuhensis* within the second subclade and *L. salmonis* and *L. gibbosus* within the third subclade. Bayesian phylogenetic reconstructions of Hemiuroidea showed four clades for members of Hemiuridae and Lecithasteridae. The first clade consisted of Hemiuridae representatives and the second clade represented the genus *Lecithaster*. The third clade included genera *Aponurus* and *Lecithophyllum* (Lecithasteridae) and the fourth clade combined members of lecithasterid Quadrifoliovariinae and Hysterolecithinae and hemiurid Opisthadeninae and Bunocotyliidae with high statistical support.

## Introduction

The genus *Lecithaster* Odhner, 1905 contains more than 30 parasite species that infect marine and euryhaline fish species (WoRMS Editorial Board, 2014). Among these, the following eight species have been reported in East Asian and Australian coastal waters from fishes of the order Perciformes Bleeker, 1859: *L. atropi* Shen, 1987, *L. fusiformis* Wang, 1991, *L. stellatus* Looss, 1907 and *L. xiamenensis* Liu, 1995; of the order Clupeiformes Bleeker, 1959: *L. confusus* Odhner, 1905, *L. setipinnae* Qiu and Liang, 1995 and *L. chupanodona* Liu, 1995; and of the order Mugiliformes Günther, 1880: *L. mugilis* Yamaguti, 1970 and *L. sudzuhensis* Besprozvannykh, Atopkin, Ngo, Ermolenko, Ha, Tang, Beloded, 2017 (Yamaguti, 1934, 1970; Pan, 1984; Bray *et al.*, 1993; Liu *et al.*, 2010; Besprozvannykh *et al.*, 2017). *Lecithaster sayori* Yamaguti, 1938 and *L. tylosuri* Li, Qiu and Zang, 1989, which have been described from the order Beloniformes Berg, 1937 from East Asia, were synonymized with *L. stellatus* (Manter and Pritchard, 1960; Shen and Qiu, 1995), and *L. salmonis* Yamaguti, 1934 from Salmoniformes Bleeker, 1859, Siluriformes Cuvier, 1817 and Perciformes from Japan (Yamaguti, 1934, 1940) was synonymized with *L. gibbosus* by Margolis and Boyce (1969). Only morphological data were used to validate most *Lecithaster* species, including the species mentioned above. Molecular data were obtained only for *L. stellatus*, *L. mugilis*, *L. gibbosus* and *L. sudzuhensis* (Anderson and Barker, 1998 (direct submission); Cribb *et al.*, 2001; Olson *et al.*, 2003; Besprozvannykh *et al.*, 2017).

Adult worms from three *Lecithaster* species were found in the intestine of *Strongylura strongylura* (van Hasselt, 1823), *Hemirhamphus marginatus* (Forsskål, 1775) (order Beloniformes) from coastal waters off Cat Ba Island, Vietnam, *Osmerus mordax* (Mitchill, 1814) and *Hypomesus japonicus* (Brevoort, 1856) of the order Osmeriformes (Nelson, Grande and Wilson, 2016) from Japan and *Acanthogobius flavimanus* Temminck and Schlegel, 1845 (order Perciformes) from the southern Russian Far East. Moreover, one unidentified specimen, *Lecithaster* sp., along with *Hysteroleicithoides epinepheli* Yamaguti, 1934 were found in Vietnamese *Siganus fuscescens* (Houttuyn, 1782). Morphological and molecular data for these worms, and discussions about species validity and phylogenetic relationships within family Lecithasteridae Odhner, 1905 are provided below.

## Materials and methods

### Specimen collection

Adult worms consistent with the genus *Lecithaster* were found in the intestines of *Strongylura strongylura*, *Hemirhamphus marginatus* and *Siganus fuscescens* in coastal waters off Cat Ba Island, Halong Bay, Vietnam. *Osmerus mordax* and *Hypomesus japonicus* were found in Akkeshi Gulf, Hokkaido, Japan and *A. flavimanus* was found in Lake Lebedinoe, Chasansky district, Primorsky Region, Russia. Adult worms consistent with the genus *Hysteroleicithoides* were found in *S. fuscescens* in coastal waters off Cat Ba Island, Halong Bay, Vietnam. Worms from fish were rinsed in distilled water, killed in hot distilled water and preserved in 70% ethanol. After fixation, flukes were transferred to 96% ethanol. Whole mounts were prepared for morphometric and metric descriptions by staining the specimens with alum carmine, dehydrating the specimens in a graded ethanol series and cleaning them in clove oil, followed by mounting in Canada balsam under a coverslip on a slide. All sizes are presented in mm. This material is held in the parasitological collection of the Zoological Museum (Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of the Russian Academy of Sciences, Vladivostok, Russia; e-mail: [petrova@ibss.dvo.ru](mailto:petrova@ibss.dvo.ru)).

### Molecular analysis

Adult specimens of *Lecithaster confusus* (n = 8), *L. sayori* (n = 1), *L. salmonis* (n = 3), *Hysteroleicithoides epinepheli* (n = 3) and *Lecithaster* sp. (n = 1) were used for molecular analysis (table 1). Total DNA was extracted from flukes, which were fixed in 96% ethanol, using a "hot shot" technique (Truett, 2006).

Polymerase chain reaction (PCR) was used to amplify 18S ribosomal DNA (rDNA) with the primers 18S-8 (5'-GCA GCC GCG GTA ACT CCA GC-3') and 18S-A27 (5'-CCA TAC AAA TGC CCC CGT CTG-3') as described previously (Littlewood and Olson, 2001). The initial PCR reaction was performed in a total volume of 20 µl and contained 0.25 mM of each primer, approximately 10 ng of total DNA in water, 10X Dream Taq buffer, 1.25 mM dNTPs and 1 unit of Dream Taq polymerase (Thermo Scientific, USA). Amplification of a 2000 base pair (bp) fragment of 18S rDNA was performed in a GeneAmp 9700 (Applied Biosystems, USA) with a 5-minute denaturation at 96°C, 35 cycles of 1 minute at 96°C, 20 s at 58°C and 5 minutes at 72°C and a 10-minute extension at 72°C. Negative and positive controls using both primers were included.

28S rDNA was amplified with the primers DIG12 (5'-AAG CAT ATC ACT AAG CGG-3') and 1500R (5'-GCT ATC CTG AGG GAA ACT TCG-3') as described previously (Tkach *et al.*, 2003). The master mix for the PCR reaction was identical to that described above for 18S rDNA. Amplification of a 1200 bp fragment of 28S rDNA was performed in a GeneAmp 9700 (Applied Biosystems, USA) with a 3-minute denaturation at 94°C, 40 cycles of 30 s at 94°C, 30 s at 55°C and 2 minutes at 72°C and a 7-minute extension at 72°C. Negative and positive controls using both primers were included.

A ribosomal ITS1-5.8S-ITS2 fragment was amplified with primers BD1 (5'-GTC GTA ACA AGG TTT CCG TA-3') and BD2 (5'-TAT GCT TAA ATT CAG CGG GT-3') (Luton *et al.*, 1992) with an annealing temperature of 54°C. Negative and positive controls using both primers were included. A mitochondrial COI gene fragment was amplified and directly sequenced with primers Trema-cox1/F (5'-TTCGGTCATCCTGAGGTTTATG TT-3') and Trema-cox1/R (5'-CAGCAAATCATGATGCA AAAGGTA-3').

PCR products were directly sequenced using the ABI Big Dye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, USA), as recommended by the manufacturer, with the internal sequencing primers described by Littlewood and Olson (2001) for 18S rDNA, Tkach *et al.* (2003) for 28S rDNA and Luton *et al.* (1992) for the ITS2 rDNA fragment. PCR products were analysed using an ABI 3130xl genetic analyser at the Department of Cell Biology, Far Eastern Federal University. Sequences were submitted to GenBank of the NCBI database with the accession numbers listed in table 1.

rDNA sequences were assembled with SeqScape v. 2.6 software. Alignments and estimation of the number of variable sites and sequence differences were performed using MEGA 7.0 (Kumar *et al.*, 2016). Phylogenetic analyses of the nucleotide sequences were performed using the Bayesian algorithm with MrBayes v. 3.1.2 software (Huelsenbeck *et al.*, 2001). The best nucleotide substitution models, TIM3+G, TPM3uf+I+G and TPM1uf+G for ribosomal 28S, ITS2 and mitochondrial COI gene fragment, respectively, were estimated with jModeltest v. 2.1.5 software (Darriba *et al.*, 2012). Bayesian analysis was performed using 10,000,000 generations, with two independent runs. Summary parameters and the phylogenetic tree were calculated with a burnin of 1,500,000 generations. The significance of the phylogenetic relationships was estimated using posterior probabilities (Huelsenbeck *et al.*, 2001). The phylogenetic relationships among the species of Lecithasteridae were inferred from our data, along with the nucleotide sequences of the 18S rDNA, 28S rDNA, ITS2 rDNA and COI gene fragment of mitochondrial DNA of other trematode specimens obtained from the NCBI GenBank database (tables 1 and 2).

## Results

### *Lecithaster confusus* Odhner, 1905

*Host.* *Strongylura strongylura* (van Hasselt, 1824), Belonidae.

*Locality.* Coastal water off Cat Ba Island, Halong Bay, northern Vietnam (20°84'N, 106°59'E).

*Intensity of infection.* 1–17 worms per fish.

*Other host.* *Acanthogobius flavimanus* (Temminck & Schlegel, 1845), Gobiidae Cuvier, 1816.

**Table 1.** List of Lecithasteridae incorporated into molecular analysis.

Species	Subfamily	N	Definitive host	Source	GenBank accession number			
					V4 18S	28S	ITS2	COI
<i>Lecithaster confusus</i>	Lecithasterinae	5	<i>Strongylura strongylura</i>	This study	–	MH625968-MH625972	MH625982-MH625986	MH625996-MH626000
<i>L. confusus</i>	Lecithasterinae	4*	<i>Acanthogobius flavimanus</i>	This study	–	MH625973-MH625976	MH625987-MH625990	MH626001-MH626003
<i>L. salmonis</i>	Lecithasterinae	2*	<i>Osmerus mordax</i>	This study	–	MH625979-MH625980	MH625993	MH626006-MH626007
<i>L. salmonis</i>	Lecithasterinae	1	<i>Hypomesus japonicus</i>	This study	–	MH625981	MH625994	MH626008
<i>L. sayori</i>	Lecithasterinae	1	<i>Hemirhamphus marginatus</i>	This study	–	MH625977	MH625991	MH626004
<i>L. species</i>	Lecithasterinae	1	<i>Siganus fuscescens</i>	This study	–	MH625978	MH625992	MH626005
<i>L. gibbosus</i>	Lecithasterinae	1	<i>Merlangius merlangus</i>	Olson <i>et al.</i> (2003)	–	AY222199	–	–
<i>L. stellatus</i>	Lecithasterinae	1	<i>Acanthurus triostegus</i>	Anderson and Barker (1998)	–	–	AJ224749	–
<i>Lecithaster mugilis</i>	Lecithasteridae, Lecithasterinae	1	<i>Valamugil seheli</i>	Besprozvannykh <i>et al.</i> (2017)	–	LN865016	n/a	n/a
<i>L. sudzuhensis</i>	Lecithasteridae, Lecithasterinae	1	<i>Mugil cephalus</i>	Besprozvannykh <i>et al.</i> (2017)	–	LN865022	n/a	n/a
<i>Aponurus laguncula</i>	Lecithasterinae	1	<i>Rhomboplites aurorubens</i>	Claxton <i>et al.</i> (2017)	–	–	KU527430	–
<i>Aponurus</i> sp.	Lecithasterinae	1	<i>Mullus surmuletus</i>	Pankov <i>et al.</i> (2006);	–	DQ354368	–	–
<i>Aponurus</i> sp.	Lecithasterinae	1	<i>Mullus barbatus</i>	Carreras-Aubets <i>et al.</i> (2011)	–	HQ713441	HQ713442	–
<i>Lecithophyllum botryophorum</i>	Lecithasterinae	1	<i>Alepocephalus bairdii</i>	Olson <i>et al.</i> (2003) (as <i>Lecithophyllum botryophorum</i> )	–	AY222205	–	–
<i>Hysterolecithoides epinepheli</i>	Hysterolecithinae	3*	<i>Siganus fuscescens</i>	This study	–	MH625962- MH625964	MH625965- MH625967	MH625995
<i>H. frontilatum</i>	Hysterolecithinae	1	Unknown	Blair <i>et al.</i> (1998)	AF029813	–	–	–
<i>H. dandongensis</i>	Hysterolecithinae	1	<i>Siganus fuscescens</i>	Wang <i>et al.</i> (2010, unpublished)	HM545901	–	HM545900	–
<i>Bilacinia australis</i>	Lecithasteridae, Quadrifoliovariinae	1	<i>Apolemichthys arcuatus</i>	Chambers and Cribb (2006)	–	AY897568	AY897562	–
<i>Quadrifoliovarium maceria</i>	Lecithasteridae, Quadrifoliovariinae	1	<i>Naso tonganus</i>	Chambers and Cribb (2006)	–	AY897566	AY897560	–
<i>Q. pritchardae</i>	Lecithasteridae, Quadrifoliovariinae	1	<i>Naso unicornis</i>	Chambers and Cribb (2006)	–	AY897567	AY897561	–
<i>Q. quattuordecim</i>	Lecithasteridae, Quadrifoliovariinae	1	<i>Naso tonganus</i>	Chambers and Cribb (2006)	–	AY897565	AY897559	–
<i>Q. simplex</i>	Lecithasteridae, Quadrifoliovariinae	1	<i>Naso tonganus</i>	Chambers and Cribb (2006)	–	AY897564	AY897558	–
<i>Unilacinia asymmetrica</i>	Lecithasteridae, Quadrifoliovariinae	1	<i>Naso annulatus</i>	Chambers and Cribb (2006)	–	AY897569	AY897563	–

\*n = 3 for COI of *Lecithaster confusus* ex *Acanthogobius flavimanus*; n = 1 for ITS of *Lecithaster salmonis* ex *Osmerus mordax*; n = 1 for COI of *Hysterolecithoides epinepheli*  
n/a, accession numbers will be received later

**Table 2.** List of trematodes of Hemiuroidea, incorporated into molecular analysis of 28S rDNA from GenBank. Molecular data for Lecithasteridae are presented in table 1.

Species	Family/subfamily	Number of specimens	Source	GenBank accession number
<i>Gonocerca crassa</i>	Gonocercidae	1	Sokolov <i>et al.</i> (2018a)	KY197012
<i>G. muraenolepisi</i>	Gonocercidae	3	Sokolov <i>et al.</i> (2016)	HF543941; LN650651; LN865025;
<i>G. oshoro</i>	Gonocercidae	1	Sokolov <i>et al.</i> (2018a)	KY197013
<i>G. phycidis</i>	Gonocercidae	1	Sokolov <i>et al.</i> (2018a)	KY197009
<i>Hemipera manteri</i>	Gonocercidae	1	Olson <i>et al.</i> (2003) (as <i>Hemiperina manteri</i> )	AY222196
<i>Derogenes varicus</i>	Derogenidae, Derogeninae	1	Olson <i>et al.</i> (2003)	AY222189
<i>Thometrema lotzi</i>	Derogenidae, Halipeginae	1	Calhoun <i>et al.</i> (2013)	KC985236
<i>Aphanurus mugilis</i>	Hemiuridae, Aphanurinae	1	Atopkin <i>et al.</i> (2017)	LT607807
<i>Dinurus longisinus</i>	Hemiuridae, Dinurinae	1	Olson <i>et al.</i> (2003)	AY222202
<i>Lecithochirium microstomum</i>	Hemiuridae, Lecithochiriinae	1	Calhoun <i>et al.</i> (2013)	KC985235
<i>Lecithocladium excisum</i>	Hemiuridae, Elytrophallinae	1	Olson <i>et al.</i> (2003)	AY222203
<i>Plerurus digitatus</i>	Hemiuridae, Plerurinae	1	Olson <i>et al.</i> (2003)	AY222201
<i>Merlucciotrema praeclarum</i>	Family unknown	1	Olson <i>et al.</i> (2003)	AY222204
<i>Opisthadena dimidia</i>	Bunocotylidae, Opisthadeninae	1	Olson <i>et al.</i> (2003)	AY222198
<i>Machidatrema chilostoma</i>	Bunocotylidae, subfamily unknown	1	Olson <i>et al.</i> (2003)	AY222197
<i>Bunocotyle progenetica</i>	Bunocotylidae, Bunocotylinae	1	Pankov <i>et al.</i> (2006)	DQ354365
<i>Robinia aurata</i>	Bunocotylidae, Bunocotylinae	1	Pankov <i>et al.</i> (2006)	DQ354367
<i>Saturnius gibsoni</i>	Bunocotylidae, Bunocotylinae	1	Marzoug <i>et al.</i> (2014)	KJ010542
<i>Saturnius minutus</i>	Bunocotylidae, Bunocotylinae	1	Pankov <i>et al.</i> (2006) (as <i>Saturnius</i> sp.)	DQ354366
<i>Accacoelium contortum</i>	Accacoeliidae	1	Olson <i>et al.</i> (2003)	AY222190
<i>Copiatestes filiferus</i>	Syncoeliidae	1	Olson <i>et al.</i> (2003)	AY222188
<i>Didymocystis scomberomori</i>	Didymozoidae	1	Schrandt <i>et al.</i> (2016)	KU341979
<i>Didymozoid</i> sp.	Didymozoidae	2	Olson <i>et al.</i> (2003)	AY222192, AY222194
<i>Didymozoon scombri</i>	Didymozoidae	1	Olson <i>et al.</i> (2003)	AY222195
<i>Hirudinella ahi</i>	Hirudinellidae	1	Calhoun <i>et al.</i> (2013)	KC985238
<i>Hirudinella ventricosa</i>	Hirudinellidae	1	Calhoun <i>et al.</i> (2013)	KC985232
<i>Prosogonotrema bilabiatum</i>	Sclerodistomidae	1	Olson <i>et al.</i> (2003)	AY222191
<b>Outgroup</b>				
<i>Transversotrema haasi</i>	Transversotrematidae	1	Olson <i>et al.</i> (2003)	AY222186
<i>Crusziella formosa</i>	Transversotrematidae	1	Olson <i>et al.</i> (2003)	AY222185
<i>Prototransversotrema steeri</i>	Transversotrematidae	1	Olson <i>et al.</i> (2003)	AY222184
<i>Otodistomum cestoides</i>	Azygiidae	1	Olson <i>et al.</i> (2003)	AY222187

**Locality.** Lake Lebedinoe, Chasansky district, Primorsky Region, Russia (42°34'N, 130°41'E).

**Intensity of infection.** 15 worms per fish.

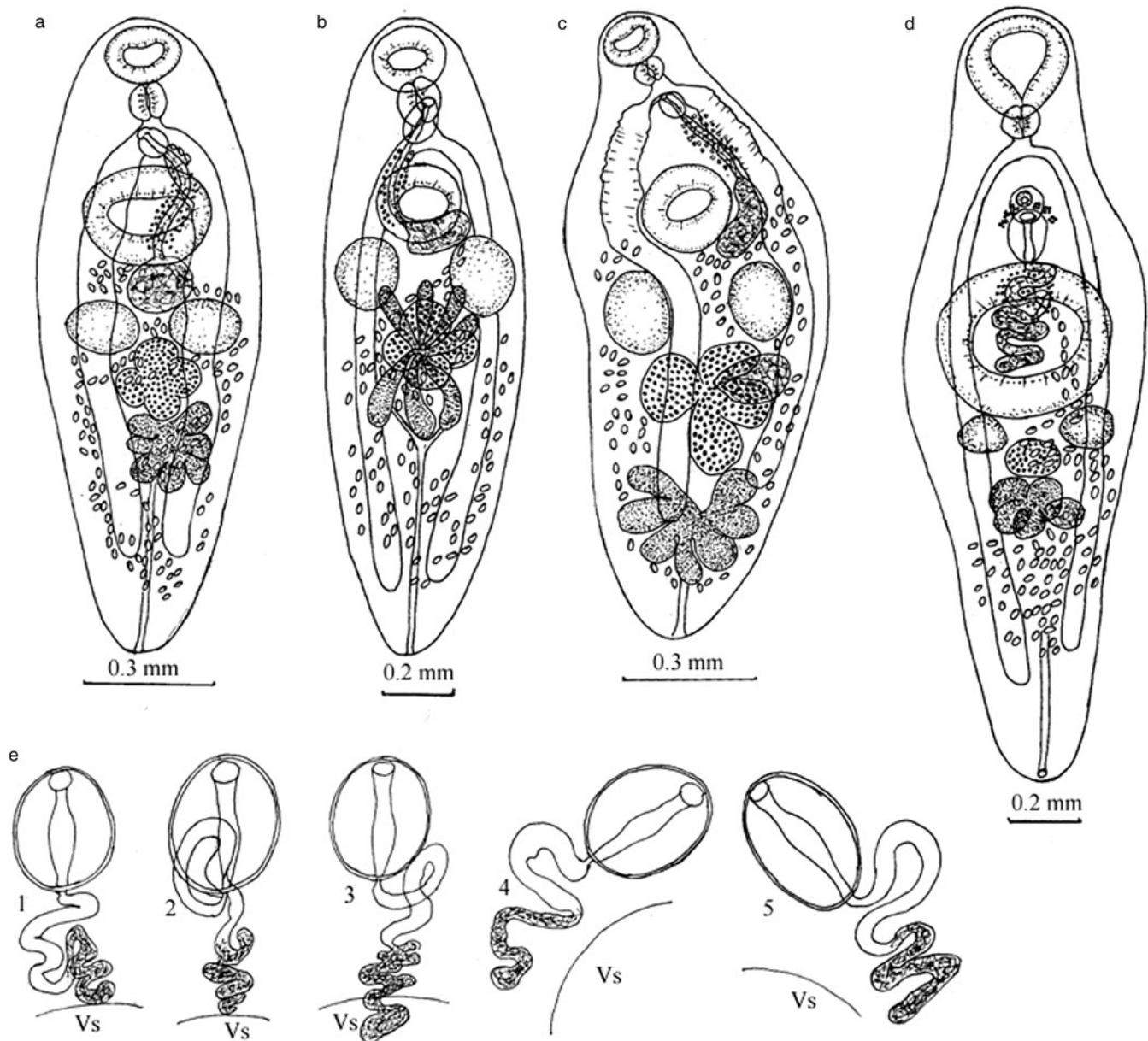
**Site.** Intestine.

**Description (based on 8 specimens; fig. 1a; table 3)**

Body fusiform, smooth. Pre-oral lobe present. Oral sucker sub-terminal, prepharynx absent, pharynx spherical, oesophagus short. Intestinal bifurcation immediately anterior or 0.065–0.154 mm from ventral sucker. Caeca reach level of middle vitellarium to middle postvitelline region. Ventral sucker large, on

border of anterior and middle thirds of body. Testes round, symmetrical and located in middle of body close to ventral sucker. Seminal vesicle saccate, between level of middle of ventral sucker and anterior margin of testes. Pars prostatica elongated, lined with vesicular cells and surrounded by numerous prostatic cells. Sinus sac oval, between anterior edge of ventral sucker and intestinal bifurcation, penetrated by hermaphroditic duct. Genital pore median, located at level of intestinal bifurcation. Pit connected to thin-walled sac located on median line posterior to genital pore. Ovary consists of four round lobes, between posterior border of testis and anterior margin of vitellarium. Seminal receptacle round, dorsal to ovary. Vitellarium consists of seven drop-shaped,





**Fig. 1.** Adult worms of Lecithasterinae Odhner, 1905 and Hysteroleicithinae Yamaguti, 1958. (a) *Lecithaster confusus* Odhner, 1905. (b) *L. sayori* Yamaguti, 1938. (c) *L. salmonis* Yamaguti, 1934. (d) *Hysteroleicithoides epinepheli* Yamaguti, 1934. (e) Terminal genitalia *H. epinepheli*: 1–3 ventral; 4 & 5 lateral. Vs, ventral sucker.

oval or elongated oval lobe located immediately posteroventral to ovary. Anterior lobes of vitellarium partially overlap ovary. Uterine loops located between posterior margin of ventral sucker and posterior end of body. Eggs small, oval and operculated. Excretory bladder Y-shaped, excretory pore terminal.

#### *Lecithaster sayori* Yamaguti, 1938

*Host.* *Hemirhamphus marginatus* (Fosskåi, 1775), Hemiramphidae Gill, 1859.

*Locality.* Coastal water off Cat Ba Island, Halong Bay, northern Vietnam (20°84'N, 106°59'E).

*Intensity of infection.* 2 worms per fish.

*Site.* Intestine.

#### *Description (based on 1 specimen; fig. 1b; table 3)*

Body is fusiform, smooth. Pre-oral lobe present. Oral sucker sub-terminal, prepharynx absent, pharynx spherical, oesophagus short. Intestinal bifurcation immediately anterior to ventral sucker. Caeca reach to posterior third of post-vitelline region. Ventral sucker in middle of anterior half of body. Testes round, symmetrical, adjacent or close to ventral sucker. Seminal vesicle transversely oval, between level of middle of ventral sucker and anterior margin of testes. Pars prostatica elongated, passes to right of median line of body, lined with vesicular cells and surrounded by numerous prostatic cells. Sinus sac oval and located anterior of ventral sucker, at level of pharynx-intestinal bifurcation, penetrated by hermaphroditic duct. Genital pore median, at pharynx level. Ovary consists of four round lobes, median line in middle third of body. Seminal receptacle round, dorsally

**Table 3.** Measurements for adult worms Lecithasteridae.

	<i>L. confusus</i> (Overstreet, 1971)									
	<i>Lecithaster. confusus</i> present study	<i>L. confusus</i> (on Odhner, 1905 from Olmo et al., 2006)	Host <i>Micropogon undulatus</i>	Host <i>Alosa chrysochloris</i>	<i>L. confusus</i> (Olmo et al., 2006)	<i>L. stelatus</i> (on Loos 1907 from Skrjabin, 1954)	<i>L. stelatus</i> (Yamaguti, 1934, 1953)*	<i>L. stelatus</i> (Bray et al., 1993)	<i>L. sayori</i> present study	<i>L. sayori</i> (Yamaguti, 1938)
Body	0.970–1.478 x 0.323–0.539	1.000–1.500 x 0.300–0.500	0.379–1.299 x 0.155–0.485	1.282–1.695 x 580–754	0.469–0.515 x 0.117–0.146	0.780–1.300 x 0.320–0.470	0.750–1.420 x 0.200–0.550	0.542–1.100 x 0.161–0.296	1.833 x 0.508	1.25–2.1 x 0.3–0.55
Oral sucker	0.081–0.131 x 0.086–0.177	130–160	0.049–0.126 x 0.055–0.122	0.136–0.162 x 0.139–0.171	0.058–0.075 x 0.054–0.72	0.080–0.120 x 0.126	0.075–0.123 x 0.084–0.138	0.060–0.096 x 0.067–0.111	0.185 x 0.208	0.14–0.18 in diameter
Pharynx	0.062–0.096 x 0.057–0.085	0.070–0.085	0.032–0.073 x 0.033–0.073	0.070–0.090 x 0.073–0.087.	0.035 x 0.030	0.073–0.07?	0.038–0.070 x 0.042–0.072	0.040–0.076 x 0.039–0.067	0.139 x 0.123	0.078–0.100 x 0.072–0.090
Ventral sucker	0.154–0.231 x 0.200–0.293	0.230–0.300	0.110–0.252 x 0.102–0.232	0.291–0.360 x 0.261–0.316.	0.082–0.096 x 0.085–0.096	0.150–0.260 x 0.170–0.380	0.130–0.210 x 0.130–0.230	0.110–0.167 x 0.142–0.196	0.227 x 0.235	0.2–0.3 in diameter
Oesophagus length	0–0.081	–	–	–	–	–	–	–	0.012	–
Forebody (% from body length)	0.235–0.435 (23.2–34.7)	–	(21.0–37.0)	–	0.162–0.179 (34.5–34.8)	–	–	(25–33)	0.447 24.4%	–
Sinus sac	0.042–0.104 x 0.039–0.077	0.080–0.110	0.029–0.067 x 0.019–0.049	0.055–0.110 x 0.046–0.073.	0.048 x 0.014–0.019	0.074–0.110 x 0.068–0.074	0.040–0.080	0.044–0.073 x 0.031–0.055	0.116 x 0.096	0.080–0.093 x 0.050–0.063
Pars prostatica	0.154–0.262	–	–	–	0.048–0.128	–	–	–	0.250	–
Left testis	0.096–0.200 x 0.096–0.212	–	0.038–0.157 x 0.036–0.116	0.116–0.218 x 0.106–0.220.	0.050 x 0.040	0.084 x 0.130 0.100–0.120 x 0.130–0.140	0.070–0.210 x 0.070–0.150	0.048–0.101 x 0.049–0.076	0.231 x 0.185 0.193 x 0.185	0.12–0.21 x 0.09–0.125
Right testis	0.116–0.200 x 0.104–0.193	–	0.044–0.139 x 0.038–0.133	0.128–0.197 x 0.102–0.212.	0.043–0.051 x 0.045–0.046	–	–	–	–	–
Ovary	0.096–0.243 x 0.085–0.250	–	0.067–0.255 x 0.057–0.177	0.194–0.316 x 0.093–0.249	0.048–0.083 x 0.040–0.058	0.130 x 0.150	0.24–0.31 x 0.18	0.095–0.208 x 0.088–0.143	0.270 x 0.258	–
Vitellarium	0.142–0.270 x 0.123–0.270	width 0.250	–	–	0.074–0.077 x 0.056–0.074	–	–	0.155–0.253 x 0.093–0.142	0.493 x 0.289	–
Eggs	0.0154–0.0169 x 0.0092–0.0108	0.015–0.017 x 0.007	0.015–0.023 x 0.009–0.015	0.015–0.022 x 0.009–0.014	0.018–0.019 x 0.010–0.012	0.015–0.018 x 0.010–0.012	0.015–0.018 x 0.009–0.012	0.011–0.016 x 0.008–0.010	0.015–0.019 x 0.0077–0.012	0.018 x 0.012
Sucker length ratio	1.44–2.05	1.75	1.7–2.3	1.8–2.0	1.33–1.57	–	–	1.77–2.12	1.23	–
Sucker width ratio	1.50–2.47	–	–	–	–	–	–	–	1.13	–
Postvitellin region length, (%)	0.162–0.474 (16.2–32.1)	–	(6.0–28.0)	–	(7.8–13.6)	–	–	(20–30)	0.620 (33.8)	–

\*Generalized data

to ovary. Vitellarium ventral to ovary, consists of seven elongated lobes; central part of vitellarium at level of central part of ovary. Uterus loops between posterior margin of ventral sucker and posterior end of body. Eggs small, oval, operculated. Excretory bladder Y-shaped, excretory pore terminal.

#### *Lecithaster salmonis* Yamaguti, 1934

*Hosts.* *Osmerus mordax* and *Hypomesus japonicus*.

*Locality.* Akkeshi Gulf, Hokkaido Japan (43°02'N, 144°85'E).

*Intensity of infection.* 7 specimens per fish.

*Site.* Intestine.

#### *Description* (based on 4 specimens; fig. 1; table 3)

Body elongated oval, smooth. Pre-oral lobe present. Oral sucker subterminal, prepharynx absent, pharynx spherical, oesophagus short. Intestinal bifurcation 0.119–0.193 mm from ventral sucker. Caeca reach vitellarium level. Ventral sucker in middle of anterior half of body. Testes round, symmetrical, close to posterior margin of ventral sucker. Seminal vesicle elongated, at level of ventral sucker. Pars prostatica elongated, lined with vesicular cells and surrounded by numerous prostatic cells. Sinus-like sac oval, immediately posterior to intestinal bifurcation, penetrated by hermaphroditic duct. Genital pore median, immediately posterior to or at the level of intestinal bifurcation. Ovary consists of four round or oval lobes, at median line immediately posterior to testes. Seminal receptacle oval, dorsal to ovary. Vitellarium consists of seven drop-shaped lobes, located in posterior third of body, post-ovarian. Uterus loops between middle of ventral sucker and posterior end of body. Eggs oval, operculated. Excretory bladder Y-shaped, excretory pore terminal.

#### *Hysterolecithoides epinepheli* Yamaguti, 1934

*Host.* *Siganus fuscescens* (Houttuyn, 1782).

*Locality.* Coastal water off Cat Ba Island, Halong Bay, northern Vietnam (20°84'N, 106°59'E).

*Site.* Intestine.

*Intensity of infection.* 17 specimens per fish.

#### *Description* (based on 9 specimens; fig. 1; table 3)

Body fusiform, smooth. Pre-oral lobe present. Oral sucker subterminal, prepharynx absent, pharynx spherical, oesophagus short. Intestinal bifurcation located 0.246–0.674 mm from ventral sucker. Caeca reach close to posterior edge of body; terminations asymmetrical in post-uterine region. Ventral sucker large, on border of anterior and posterior half of body. Testes round or transversely oval, symmetrical, close to posterior margin of ventral sucker. Seminal vesicle tubular, sinuous, at middle of ventral sucker level. Pars prostatica sinuous, sigmoid, lined with vesicular cells and surrounded by numerous prostatic cells, apex loops dorsally over distal part of sinus sac to level of posterior end or middle of sinus sac. Sinus sac oval, pre-acetabular, penetrated by hermaphroditic duct. Genital pore median. Pit connected with thin-walled sac located on median line anterior to genital pore. Gland cells close to pit. Genital pore and pit open separately into genital atrium. Ovary transversely oval, between posterior border of testis and anterior margin of vitellarium. Juel's organ large and oval or elongated, located left of vitellarium. Vitellarium consists of 3–4 drop-shaped lobes (one specimen

was three-lobed), immediately posterior to ovary. Uterus reaches close to posterior extremity. Eggs small, oval, operculated. Excretory bladder Y-shaped, subterminal. Excretory pore opens ventrally.

#### *Molecular analysis*

Three phylogenetic trees for Lecithasteridae were reconstructed with Bayesian analysis based on a 28S rDNA fragment (872 bp), an ITS2 rDNA fragment (448 bp) and a mitochondrial COI gene fragment (686 bp), respectively. The 28S-rDNA-based phylogenetic tree was subdivided into four clades. Clade I represents the monophyletic genus *Lecithaster* (fig. 2). Within this clade, there are two subclades. The first consists of *L. confusus*, *L. sayori*, *L. mugilis*, *L. sudzuhensis* and *Lecithaster* sp.; *L. confusus* and *L. sayori* are closely related to each other and *Lecithaster* sp. is basal to these two species. *Lecithaster mugilis* and *L. sudzuhensis* are closely related to each other. The second subclade includes *L. salmonis* from Japan and *L. gibbosus* from the United Kingdom. Clade II consists of representatives of the genus *Aponurus* and *Lecithophyllum botryophorum*, which are closely related to each other with high statistical support. Clade III comprises all specimens of *H. epinepheli* from *S. fuscescens*. Clade IV includes species of four genera: *Quadrifoliovarium*, *Bilacinia*, *Unilacinia* and *Machidatrema*. Within this clade, *Quadrifoliovarium* and *Bilacinia* are closely related to each other, and *Unilacinia assymetrica* appears as a sister taxon to these two genera. *Machidatrema chilostoma* is basal for clade IV.

Lecithasterid species were subdivided into three main clades and form a polytomy in the ITS-rDNA-based phylogenetic tree (fig. 3). Clade I represents the genus *Lecithaster*. Within this clade, there are three subclades. The first subclade consists of the evidently closely related *L. confusus* + *L. sayori* as well as closely related *L. stellatus* + *Lecithaster* sp. The second subclade comprises *L. sudzuhensis* + *L. mugilis*, and the third subclade comprises only Japanese *L. salmonis*. *Aponurus laguncula* forms clade II. Clade III contains representatives of the genera *Quadrifoliovarium*, *Monorchimacradena*, *Bilacinia*, *Unilacinia* and *Hysterolecithoides*. Within this clade, *Hysterolecithoides epinepheli* from our study is identical to *H. guangdongensis*. The genus *Hysterolecithoides* appears as a sister taxon relative to other species within clade III.

The phylogenetic tree based on nucleotide sequences from a COI gene fragment (fig. 4) includes only species of *Lecithaster* and *Hysterolecithoides* from this study. Separate lineages correspond to these two genera. The genus *Lecithaster* has a polytomy with four clades that represent *L. confusus* (clade I), *Lecithaster* sp. and *L. salmonis* (clade II), *L. sayori* (clade III) and *L. mugilis* + *L. sudzuhensis* (clade IV), respectively. *Hysterolecithoides epinepheli* appears as a sister taxon to *Lecithaster*.

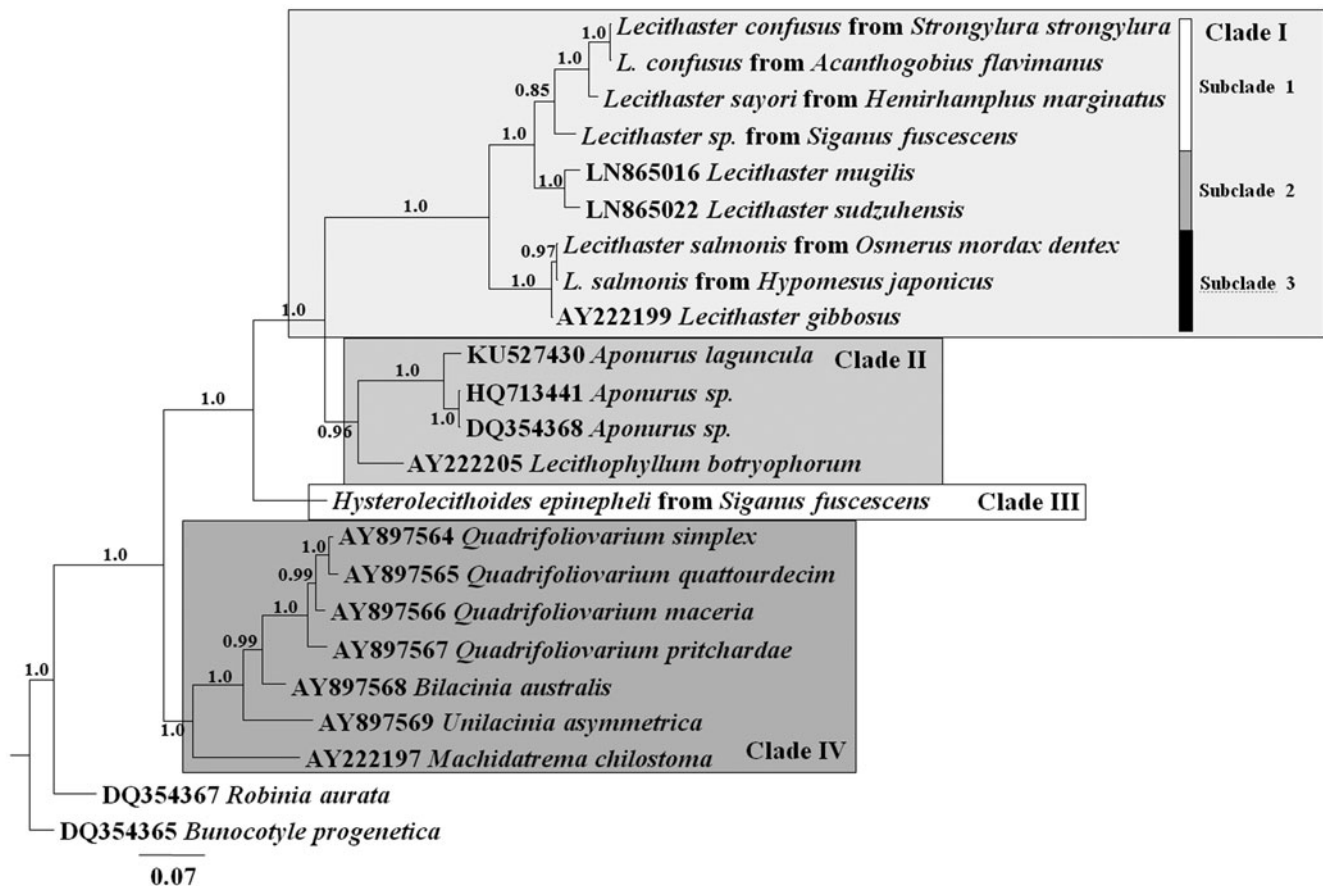
Ribosomal 18S rDNA gene V4 region sequences (291 bp) of *H. epinepheli*, *H. frontilatum* and *H. guangdongensis* were highly similar to each other. Only one variable site (no. 108) was detected (fig. 5).

#### *Discussion*

##### *Taxonomic conclusion with respect to Lecithaster and Hysterolecithoides species*

Representatives of the genus *Lecithaster*, like most worms that belong to the same genus, can resemble each other closely,





**Fig. 2.** Phylogenetic relationships of the family Lecithasteridae obtained with the Bayesian algorithm, based on partial 28S rRNA gene sequences. Nodal numbers are posterior probabilities that indicate statistical support of phylogenetic relationships.

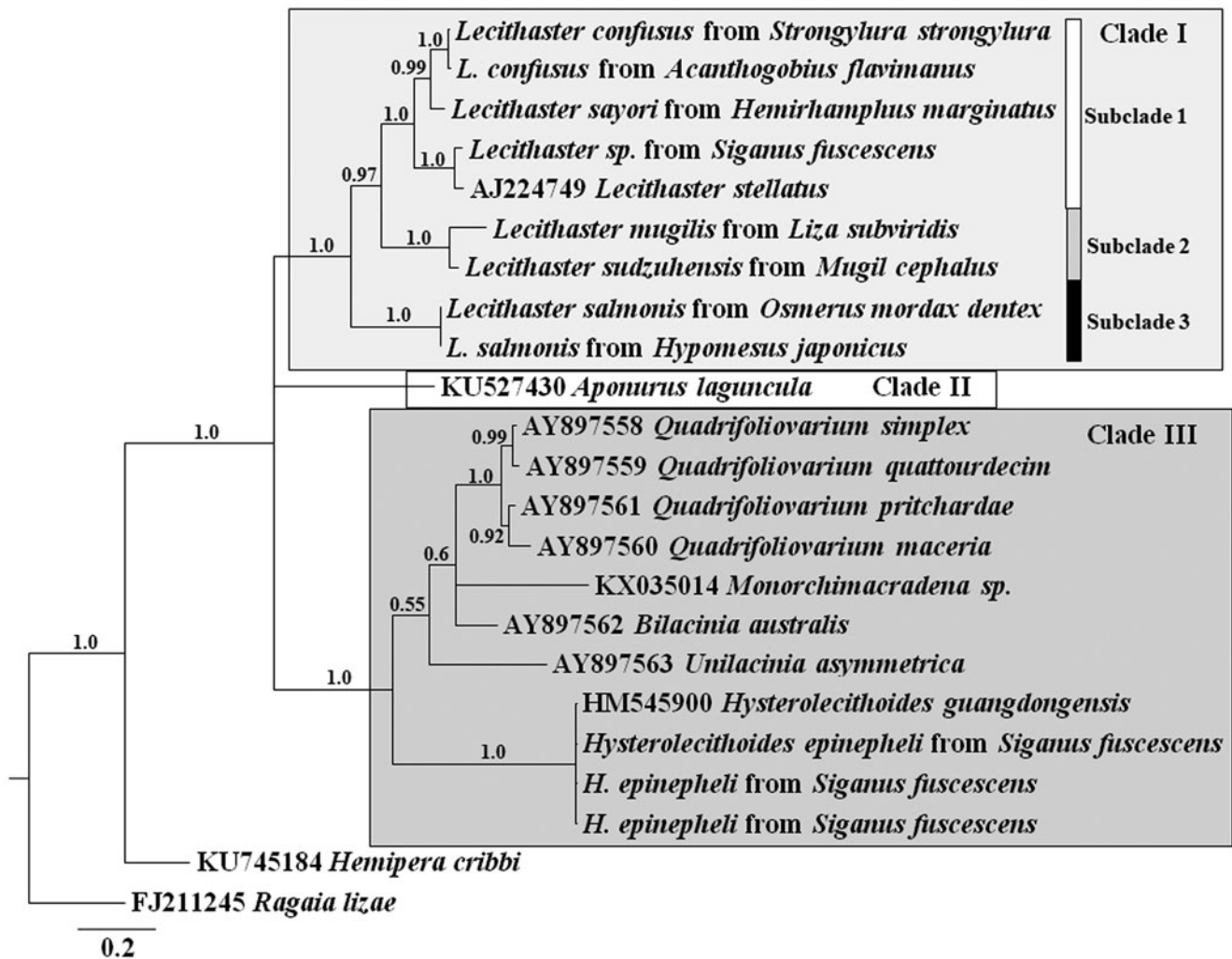
making species identification based solely on morphometric analysis difficult. Trematodes from Vietnamese *S. strongylura* resemble *L. confusus* and *L. stellatus*, based on metric index values (table 3). Morphologically, and by organ arrangements, particularly the vitellarium lobe form and position, these trematodes resemble *L. confusus* and *L. stellatus* from the study of Yamaguti (1953), in which the lobes were flask-like and the vitellarium was postero-ventrally to the ovary. Vietnamese worms, however, were different from *L. stellatus* specimens with respect to these two parameters, as reported by Looss (1908, cited in Skrjabin, 1954) and Bray et al. (1993). These authors indicated that the vitellarium with elongated lobes was partially (Looss, 1908, cited in Skrjabin, 1954) or completely (Bray et al., 1993) at the level of the ovary.

*Lecithaster confusus* has been described in *Alosa finta* (Clupeiformes) from Egypt by Odhner, 1905 (cited in Skrjabin, 1954). Subsequently, this trematode species was detected in representatives of six fish orders from northern European waters, the Atlantic coast of North America, and the Mediterranean and Black seas (Skrjabin, 1954; Pérez-del-Olmo et al., 2006) and in East Asia off the Chinese coast (Pan, 1984). *Lecithaster stellatus* was originally detected from the intestines of perciform fishes from the coastal waters of Trieste, Mediterranean Sea (Skrjabin, 1954). Later, it was found in beloniform species from coastal waters of Japan (Yamaguti, 1934), Australia (Bray et al., 1993) and China (Liu et al., 2010). Thus, both *L. confusus* and *L. stellatus* are reported to exhibit a wide host range and overlapping distributions. At the same time, data from different authors reveal

discrepancies in morphological characteristics of these species. Results from studies of *L. confusus* collected from *Micropogon undulatus* Linnaeus, 1766 and *Alosa chrysochloris* Rafinesque, 1820 from the Gulf of Mexico (Overstreet, 1973) and *Boops boops* Linnaeus, 1758 from Malpica, Spain (Perez-del-Olmo et al., 2006) confirm considerable variation in morphometric indices of these worms (table 3) that may be caused by trematode infection of fish with different taxonomic affiliations. On the other hand, morphometric differences possibly indicate that these specimens belong to separate species. Molecular data are necessary to resolve this ambiguity. There is a single nucleotide sequence for *L. stellatus* from in or near Moreton Bay, eastern Australia (Anderson and Barker, 1998). Comparative analysis among ITS rDNA nucleotide sequences from *L. stellatus* from GenBank and *Lecithaster* from Vietnamese *S. strongylura* in our study indicates that these trematodes belong to different species (fig. 3). Based on these data and morphometric analysis, we conclude that trematodes from Vietnamese *S. strongylura* belong to *L. confusus*.

*Lecithaster sayori* Yamaguti, 1938 has been found in *Hyporhamphus sajori* Temminck and Schlegel, 1846 in Hamana Lake, Japan, which is connected to the sea (Yamaguti, 1938). Manter and Pritchard (1960) recognized *L. sayori* as a synonym of *L. stellatus* based on morphometric similarity. A unique feature of *L. sayori*, however, is the arrangement of the medial vitellarium at the same level as the ovary and elongated follicles of the vitellarium. Metrical (table 3) and morphological data, including





**Fig. 3.** Phylogenetic relationships of the family Lecithasteridae obtained with the Bayesian algorithm, based on ITS2 rDNA gene sequences. Nodal numbers are posterior probabilities that indicate statistical support of phylogenetic relationships.

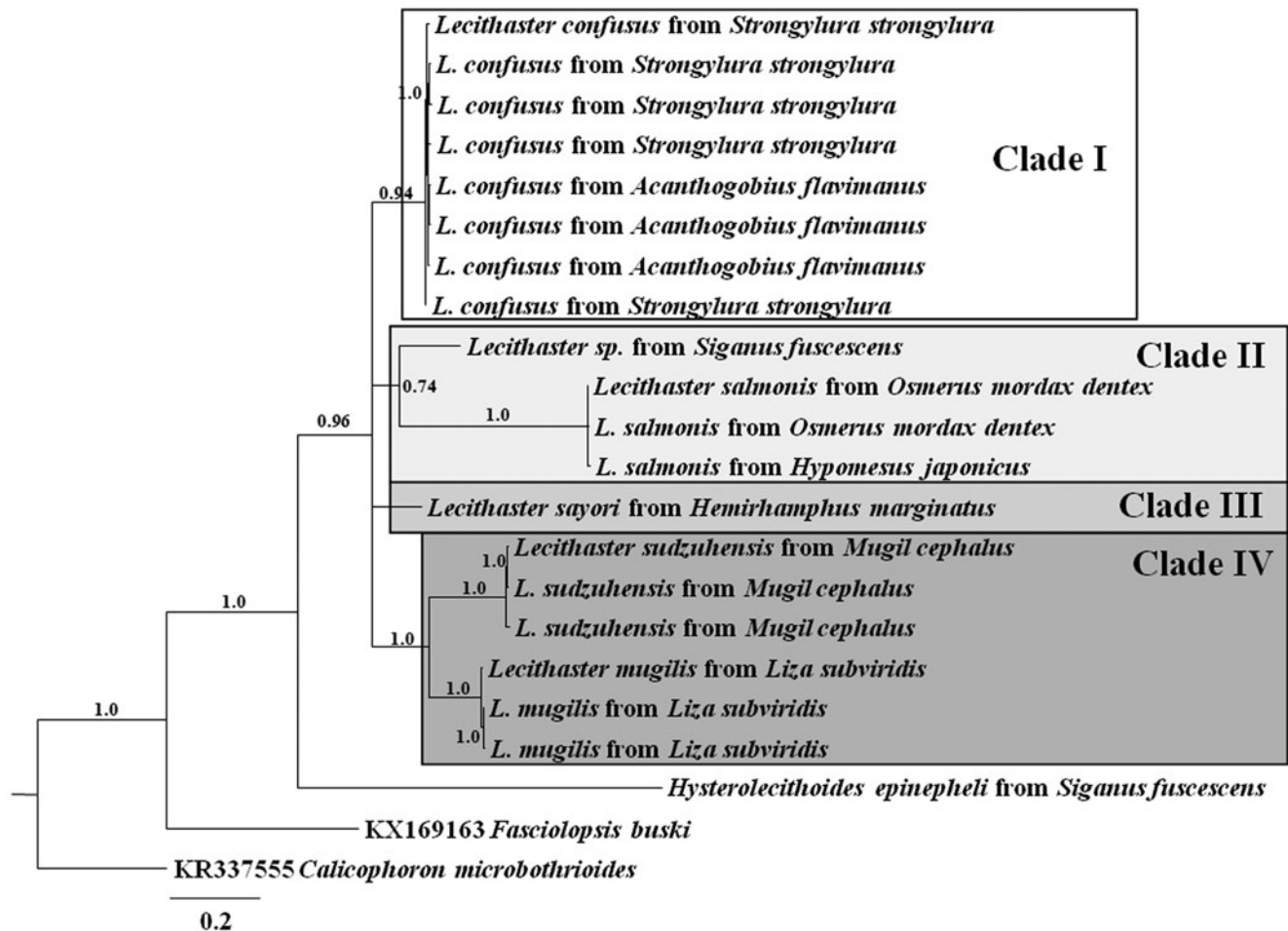
vitellarium arrangement and vitellarium lobe form, for trematodes from Vietnamese *H. marginatus* from our study indicated that these worms were identical to *L. sayori* described by Yamaguti and *L. stellatus* reported by Bray *et al.* (1993). Phylogenetic analysis showed that Vietnamese trematodes differed from *L. stellatus*, deposited in GenBank, in their ITS rDNA nucleotide sequences. This finding confirmed the validity of Vietnamese trematodes (fig. 3). Thus, based on the morphological similarity of Vietnamese trematodes to *L. sayori*, and molecular data, *L. sayori* and *L. stellatus* were apparently synonymized unreasonably.

Wang (1999) described a new *Lecithaster* species, namely *L. fusiformis* from Chinese *S. fuscescens*. Morphometrically, these worms resemble both *L. stellatus* reported in Bray *et al.* (1993) and *L. sayori*. However, as there are no molecular data for *L. stellatus* from Bray *et al.* (1993) or for *L. fusiformis* it is not possible to clarify the taxonomic status of these worms.

*Lecithaster salmonis*, as well as other *Lecithaster* species, are often low-specificity fish parasites that have been detected in fish orders Salmoniformes, Siluriformes and Perciformes from Japan (Yamaguti, 1934, 1940). In this study, this species was found in Japanese osmerid fishes. These worms were identical

to specimens morphologically (fig. 1) and metrically (table 3) described by Yamaguti (1934). Molecular data confirmed the validity of *L. salmonis* from this study. In our opinion, synonymization of this species with *L. gibbosus* is unreasonable.

*Hysterolecithoides epinepheli* was first described in *Epinephelus akaara* from Japan and also detected in the fish genera *Caranx* Lacépède, 1801 and *Siganus* Forsskål, 1775 (Yamaguti, 1934, 1953; Bray and Cribb, 2000). Adult *H. frontilatus* (Manter, 1969) that were morphologically and metrically (table 3) similar to *H. epinepheli* have been described from *Siganus* from New Caledonia (Manter, 1969; Bray and Cribb, 2000). Based on morphological analysis of *H. epinepheli* and *H. frontilatus* specimens from different areas and host species, Bray and Cribb (2000) concluded that these species are valid. They showed that these worms were identical with respect to most metric and morphological characters, including 3–6 vitellarium lobes and a pit anterior to the genital pore. A single difference between these worms was the location of the distal part of the pars prostatica. For *H. epinepheli* it was always posterior to the sinus sac, whereas for *H. frontilatus* it loops dorsally to the sinus sac. Among the worms collected from Vietnamese *Siganus*, we found specimens that had either the first or second variant of the distal part of the



**Fig. 4.** Phylogenetic relationships of the genus *Lecithaster* obtained with the Bayesian algorithm, based on partial mitochondrial COI gene sequences. Nodal numbers are posterior probabilities that indicate statistical support of phylogenetic relationships.

pars prostatica position. Herewith, the first variant of the position of the distal part of the pars prostatica is usually at the lateral arrangement of worms, while the second is at the dorsoventral level. Moreover, the distal part of the pars prostatica is usually posterior from the sinus sac for young, small specimens with a poorly developed uterus. According to Yamaguti (1953), the pars prostatica of *H. epinepheli* is sigmoid (S-shaped curve), similar to other Vietnamese worms. A sigmoidal pars prostatica occurs when the worm is in a lateral position. When the worm is in a ventral position, the distal pars prostatica is loop-like. Shared areas, the same definitive host species and morphometric similarity indicate that *H. epinepheli* and *H. frontilatus* belong to the same species. Molecular data confirm the conspecificity of these worms, indicating high identity of ribosomal 18S rDNA gene V4 region sequences of these species (fig. 5). Based on these results, we believe that worms collected from Vietnamese *Siganus* in this study belong to *H. epinepheli*, and *H. frontilatus* is the junior synonym of *H. epinepheli*. In addition, *H. guangdongensis* (Wu, 2000) from Chinese *Siganus oramin* (Bloch & Schneider) is identical to *H. epinepheli* in morphometric indices. Molecular data support that these worms belong to the same species (figs 3 and 5). Based on these data we conclude that *H. guangdongensis* is a synonym of *H. epinepheli*.

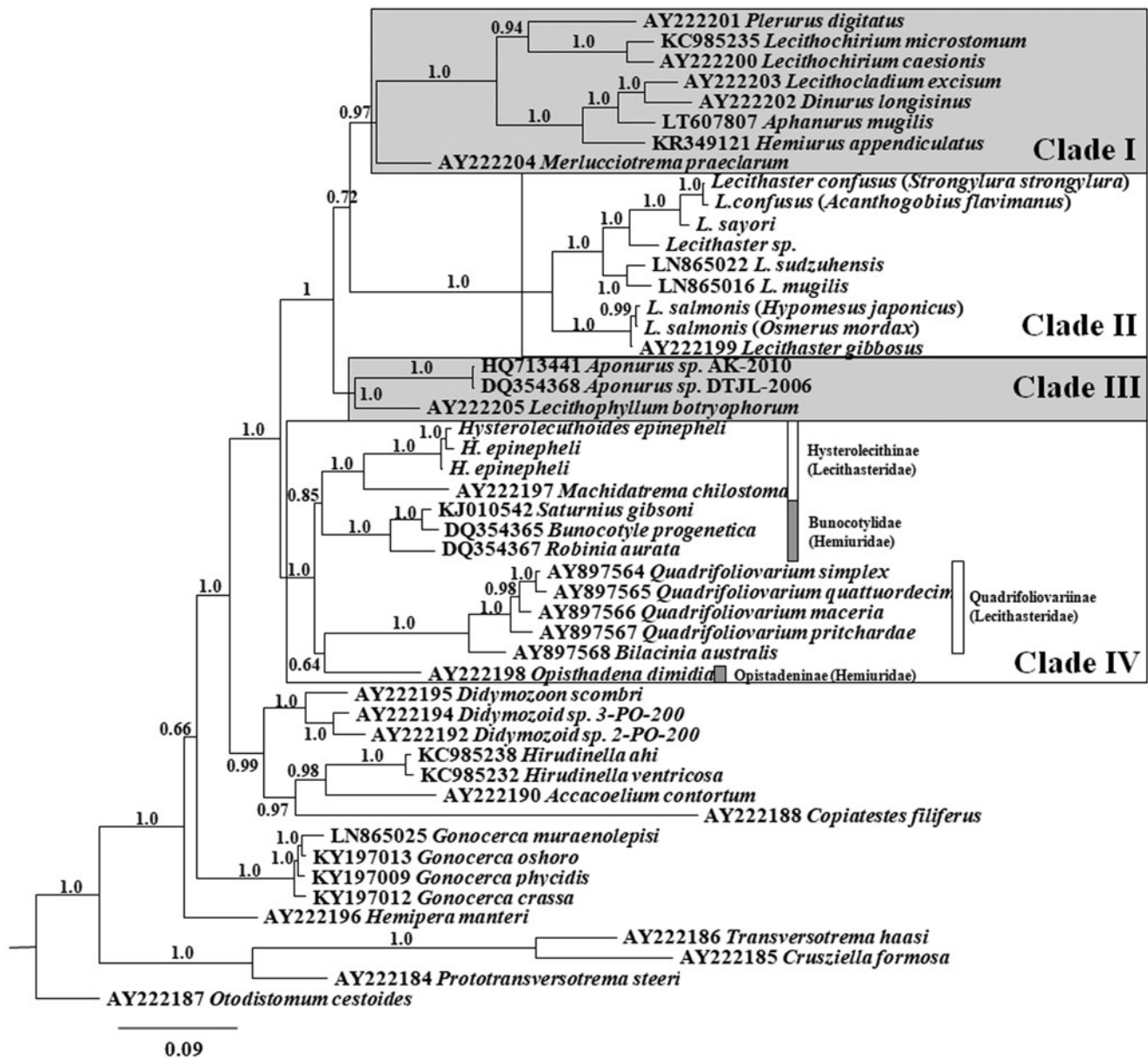
#### Interrelationships of Lecithasteridae

All phylogenetic trees showed a distinct position of the genus *Lecithaster* within the Hemiuroidea. In the 28S-rDNA-based phylogenetic reconstruction, this genus formed a clade with internal differentiation into three subclades: *L. confusus*, *L. sayori* + *Lecithaster sp.* within the first, *L. mugilis* + *L. sudzuhensis* within the second and *L. salmonis* + *L. gibbosus* within the third (fig. 6).

Phylogenetic studies of hemiurid and lecithasterid trematodes demonstrate an association between molecular differentiation and definitive host-specificity within different trematode groups and provide an explanation from the point of view of host-switching processes (León-Règagnon, 1998; León-Règagnon et al., 1998). Numerous data show that a number of representatives of *Lecithaster* infect a wide range of definitive host species. However, molecular-based phylogenetic reconstructions (figs 2 and 3) indicate that members of the subclades recognized here generally display a distinct host preference. For example, *L. mugilis* + *L. sudzuhensis* have been reported only from mugilid fish species (Besprozvannykh et al., 2017), and *L. salmonis* and *L. gibbosus* infect mainly salmonids, but also species of Beloniformes, Clupeiformes, Gadiformes, Perciformes and other orders (Skrjabin, 1954). *Lecithaster confusus* from the first subclade has







**Fig. 6.** Phylogenetic relationships of Hemiuroidea obtained with the Bayesian algorithm based on partial 28S rRNA gene sequences. Nodal numbers are posterior probabilities that indicate statistical support of phylogenetic relationships.

Nevertheless, this clade included the hemiurid subfamily Opisthadeninae, which was thought to have a controversial taxonomic status (León-Régagnon *et al.*, 1998; Pankov *et al.*, 2006), and restored family Bunocotyliidae, which includes the genera *Bunocotyle* Odhner, 1928, *Robinia* Pankov, Webster, Blasco-Costa, Gibson, Littlewood, Balbuena & Kostadinova, 2006 and *Saturnius* Manter, 1969. Later studies have accumulated evidence that the family Bunocotyliidae Dollfus, 1950 is valid (Atopkin *et al.*, 2017; Faltýnková *et al.*, 2017; Sokolov *et al.*, 2018b). However, we conclude that trematodes of Quadrifoliovariinae and Hysteroleclithinae, along with Opisthadeninae, belong to the family Bunocotyliidae, which differs considerably from both Hemiuridae and Lecithasteridae based on molecular data.

**Author ORCIDs.** D.M. Atopkin 0000-0001-8417-3424.

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**Conflict of interest.** None.

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