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New trematode species *Lecithostaphylus halongi* n. sp. (Zoogonidae, Microphalloidea) and *Gymnotergestia strongyluri* n. sp. (Fellodistomidae, Gymnophalloidea) from beloniform fishes in Vietnam

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Research Paper

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Abstract

In this study we described two new trematode species, *Lecithostaphylus halongi* n. sp. (Zoogonidae, Lecithostaphylinae) and *Gymnotergestia strongyluri* n. sp. (Fellodistomidae, Tergestiinae), on the basis of morphological and molecular data. Adult worms of these two species were collected from, respectively, *Hemiramphus* spp. (Hemiramphidae) and *Strongylura strongylura* (Belonidae) caught in the coastal waters of Vietnam. Adult worms of *L. halongi* n. sp. are morphologically close to *Lecithostaphylus gibsoni* Cribb, Bray & Barker, 1992 ex *Abudefduf whitleyi* from Heron Island and *Lecithostaphylus depauperati* Yamaguti, 1970 ex *Hemiramphus depauperatus* from Hawaii, but differ from these species in having a larger cirrus sac and a different arrangement of vitelline fields. They also differ from *Lecithostaphylus brayi* Cabañas-Granillo, Solórzano-García, Mendoza-Garfias & Pérez-Ponce de León, 2020 in the 28S ribosomal DNA (rDNA) sequence data at the interspecific level. Adult worms of *G. strongyluri* n. sp. ex *S. strongylura* are morphologically similar to *Gymnotergestia chaetodipteri*, the only previously known species of this genus, described from *Chaetodipterus faber* in Jamaica. The new species differs from *G. chaetodipteri* in body shape, testicular arrangement and the size of the pharynx and eggs. The 28S rDNA-based phylogenetic analysis indicates that *G. strongyluri* n. sp. is closely related to *Tergestia* spp., rendering *Tergestia* paraphyletic. Genetic divergence values between *G. strongyluri* n. sp. and *Tergestia* spp. are similar to those among species in the genera *Tergestia*, *Steringophorus* and *Proctoeces*. Our molecular results indicate that *G. strongyluri* n. sp. and *Tergestia* spp. may belong the same genus, but additional molecular data are needed for the final conclusion.

Introduction

The genus *Lecithostaphylus* currently comprises 11 species of trematodes parasitizing the intestines of marine fish from several orders. The genus is cosmopolitan, and the range of its definitive hosts is fairly broad (Zhang *et al.*, 1986; Cribb *et al.*, 1992; Toman, 1992; Ramadan *et al.*, 2003; Châari *et al.*, 2013; Cabañas-Granillo *et al.*, 2020). The East Asian territory is the type region for a single species of this genus, *Lecithostaphylus fugus* Zhang, Qiu & Li, 1986, detected from tetraodontids in China (Zhang *et al.*, 1986). The nearest localities from which *Lecithostaphylus* spp. have been reported are the Indian Ocean for *Lecithostaphylus pomacentri* Toman, 1992, Pacific waters near Australia for *Lecithostaphylus gibsoni* Cribb, Bray, & Barker, 1992 and the Hawaiian Islands for *Lecithostaphylus depauperati* Yamaguti, 1970.

Bray (2008) considered *Lecithostaphylus* as a valid genus of the subfamily Lepidophyllinae Stossich, 1904. Later, several complex studies indicated that *Lecithostaphylus* was phylogenetically distant from the genus *Lepidophyllum* Odhner, 1902 and could be separated into a distinct subfamily Lecithostaphylinae Odhner, 1911 (Cabañas-Granillo *et al.*, 2020; Sokolov *et al.*, 2021a; 2021b). On the basis of molecular-based species clustering, Sokolov *et al.* (2021b) resurrected the subfamily Lecithostaphylinae and provided a diagnosis of Lecithostaphylinae *sensu lato*, including the genera *Lecithostaphylus* (as the type genus), *Deretrema* Linton, 1910, *Proctophantases* Odhner, 1911 and *Steganoderma* Stafford, 1904.

The genus *Gymnotergestia* Nahhas & Cable, 1964 (Fellodistomidae Nicoll, 1909) contains a single species, *Gymnotergestia chaetodipteri* Nahhas & Cable, 1964, described from the perciform fish *Chaetodipterus faber* (Broussonet, 1782) from the Caribbean Sea (Nahhas & Cable,

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1964). This species was also detected from *Serranus scriba* (Linnaeus, 1758) in the Mediterranean Sea off Libya (Al-Bassel, 1999). There is a report of *Gymnotergestia* sp. in beloniform fish *Arrhamphus sclerolepis* Günther, 1866 from Australian coastal waters, and a drawing of this trematode is provided in the Keys to the Trematoda (Bray, 2002, p. 291). However, morphological description and metric data for these worms are absent.

At present, the structure of the families Zoogonidae and Fellodistomatidae and the species differentiation within *Lecithostaphylus* and *Gymnotergestia* are mainly based on morphological data. Though there are several molecular studies of trematodes from each of these two families (Bray *et al.*, 1999; Hall *et al.*, 1999; Olson *et al.*, 2003; Cribb *et al.*, 2014, 2015; Cutmore *et al.*, 2014; Antar & Gargouri, 2015; Sokolov *et al.*, 2016; Wee *et al.*, 2017; Cutmore *et al.*, 2018; Pérez-Ponce de León *et al.*, 2018; Cabañas-Granillo *et al.*, 2020; Krupenko *et al.*, 2020; Sokolov *et al.*, 2021a, b), most of them do not provide morphological validation of the species under study. In many cases, this means that no sound taxonomical conclusions can be made.

During our survey of the trematode fauna of fish in the coastal waters of northern Vietnam, we found mature trematode specimens morphologically similar to *Lecithostaphylus* Odhner, 1911 (Zoogonidae Odhner, 1902: Lepidophyllinae Stossich, 1903) and *Gymnotergestia* Nahhas & Cable, 1964 (Fellodistomidae Nicoll, 1909: Tergestiinae Skrjabin & Koval, 1957) in the intestines of *Hemiramphus* Cuvier, 1816 and *Strongylura* van Hasselt, 1824, respectively. In this study, we describe two new trematode species based on the results of morphological and molecular examination of these worms.

Materials and methods

Collection of trematodes

Specimens of trematodes from the families Zoogonidae and Fellodistomidae were sampled from four individuals of *Hemiramphus far* (Forsskål, 1775), from one out of two individuals of *Hemiramphus marginatus* (Forsskål, 1775), and from ten out of 18 individuals of *Strongylura strongylura* (van Hasselt, 1823) in the coastal waters of Cat Ba Island, northern Vietnam (20°84′N, 106°59′E). The worms were briefly rinsed in distilled water and killed in hot distilled water. Several specimens were preserved in 70% ethanol for preparation of slides, while others were fixed in 96% ethanol for DNA extraction. Whole-mounts for the descriptions of adult worms were made by staining them with alum carmine, dehydrating them in a graded ethanol series, clearing in xylene and mounting in Canada balsam under a coverslip on a slide. All measurements are given in micrometres.

DNA extraction, amplification and sequencing

Adult specimens of *Lecithostaphylus halongi* n. sp. ($n = 2$) and *Gymnotergestia strongyluri* n. sp. ($n = 2$) were used for molecular analysis (table 1). Total DNA was extracted from the worms fixed in 96% ethanol using a 'hot shot' technique (Truett, 2006).

28S ribosomal DNA (rDNA) was amplified using 28S-A forward primer (5′-GCACCCGCTGAAYTTAAG-3′) (Matejusova & Cunningham, 2004) and 1500R (5′-GCTATCCTGAGGG AAACTTCG-3′) (Tkach *et al.*, 2003). Initial polymerase chain reaction (PCR) reaction was performed in a total volume of 25 µl containing 0.25 mM of each primer pair, 25 ng of total DNA in water and 12.5 µl of Promega GoTaq Green Master mix (Madison, Wisconsin, USA). Amplification of a 1200-bp fragment of 28S ribosomal RNA (rRNA) gene was performed in a GeneAmp 9700 (Applied Biosystems, Waltham, Massachusetts, USA), with a 5-min denaturation at 96°C, 35 cycles of 1 min at 96°C, 20 s at 55°C and 2 min 30 s at 72°C, and a 10-min extension at 72°C. Negative and positive controls were made with the use of both primers.

PCR products were directly sequenced using an ABI Big Dye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, Massachusetts, USA), as recommended by the manufacturer, with the internal sequencing primers described by Tkach *et al.* (2003) for 28S rDNA. PCR products were analysed

Table 1. List of taxa incorporated in the molecular analysis of the superfamily Microphalloidea with the number of 28S rDNA sequences given in parentheses.

Species	Author	Accession numbers
Microphalloidea		
Zoogonidae		
Cephaloporinae		
Zoogonoides viviparus ($n = 1$)	Olson *et al.*, 2003	AY222271
Plectognathotrema kamegaii ($n = 1$)	Cutmore *et al.*, 2014	KM505035
Lecithostaphylinae		
Deretrema nahaense ($n = 1$)	Olson *et al.*, 2003	AY222273
Lecithostaphylus brayi ($n = 3$)	Cabañas-Granillo *et al.*, 2020	MT704137–MT704139
Lecithostaphylus halongi n. sp. ($n = 2$)	This study	OK636406-OK636407
Proctophantastes gillissi ($n = 2$)	Sokolov *et al.*, 2016	KU163452–KU163453
Steganoderma eamiqtrema (Lecithostaphylinae *s.l. sensu* Sokolov *et al.*, 2021b, $n = 1$)	Sokolov *et al.*, 2021b	MW264135
Lepidophyllinae		
Lepidophyllum cameroni ($n = 2$)	Sogrina *et al.*, 2019 (unpublished)	MN217107–MN217108
Lepidophyllum steenstrupi ($n = 1$)	Lockyer *et al.*, 2003	AY157175
Faustulidae		
Antorchis pomacanthi ($n = 2$)	Olson *et al.*, 2003; Cribb *et al.*, 2015	AY222268, KR149729
Bacciger lesteri ($n = 1$)	Olson *et al.*, 2003	AY222269
Trigonocryptus conus ($n = 1$)	Olson *et al.*, 2003	AY222270
Eucotylidae		
Paratanaisia bragai ($n = 2$)	Unwin *et al.*, 2013	JX231098–JX231099
Tamerlania zarudnyi ($n = 2$)	Tkach *et al.*, 2001; Suleman *et al.*, 2021	AF184248, MW131090
Tanaisia fedtschenkoi ($n = 1$)	Olson *et al.*, 2003	AY116870
Tanaisia valida ($n = 3$)	Soares *et al.*, 2016 (unpublished)	KX913712–KX913714

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using an ABI 3500 genetic analyser at the Federal Scientific Center of the East Asia Terrestrial Biodiversity FEB RAS. The sequences were submitted to the National Center for Biotechnology Information (NCBI) database with accession number: (OK636406 - OK636409).

Alignment and phylogenetic analysis

rDNA sequences were assembled with SeqScape v.2.6 software (provided by Applied Biosystems, Waltham, Massachusetts, USA). Alignments and estimation of the number of variable sites and sequence differences were performed using MEGA 7.1 (Kumar *et al.*, 2016).

Phylogenetic analysis of nucleotide sequences was undertaken using Maximum Likelihood (ML) and Bayesian Inference (BI) methods. Prior to the analysis, the nucleotide substitution model was estimated using Akaike's information criterion for ML (Akaike, 1974) and Bayesian information criterion (BIC) for BI (Huelsenbeck *et al.*, 2001) using jModeltest v.3.07 software (Darriba *et al.*, 2012). The model TVM + I + G (Posada, 2003) was estimated as best fitting the 28S rDNA sequence data of Zoogonidae for both ML and BI analyses. The models GTR + I +G and TPM3uf + G (Posada, 2003) were estimated as optimal for 28S rDNA of the Fellodistomidae dataset for ML and BI algorithms, respectively. Phylogenetic trees were reconstructed with PhyML 3.1 (Guindon & Gascuel, 2003) and MrBayes v.3.1.2 software (Huelsenbeck *et al.*, 2001). A Bayesian algorithm was performed using the Markov Chain Monte Carlo option with ngen = 10,000,000, nruns = 2, nchains = 4, temp = 0.5 and samplefreq = 100. Burn-in values for 'sump' and 'sumt' options made up 25% of the number of generations (ngen). Phylogenetic relationship significance was estimated using posterior probabilities for both ML and BI analyses (Huelsenbeck *et al.*, 2001).

Phylogenetic relationships were inferred from our data and the nucleotide sequences of 28S rDNA from other trematode specimens from the superfamilies Microphalloidea and Gymnophalloidea obtained from the NCBI GenBank database (tables 1 and 2).

Results

Lecithostaphylus halongi n. sp.

Taxonomic summary

Type host. *Hemiramphus far* (Forsskål, 1775).
Other host. *Hemiramphus marginatus* (Forsskål, 1775).
Site. Intestine.
Intensity of infection. 1–5 specimens.
Type locality. Coastal water of Cat Ba Island, Tonkin Bay, northern Vietnam (20°84′N, 106°59′E).
Type deposition. Holotype no. 194–Tr, paratypes nos. 195–198–Tr.
Materials deposited. Materials are deposited in the parasitological collection of the Zoological Museum (deposited 20 November 2020, Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia; e-mail: petrova@biosoil.ru).

Etymology. Species name refers to Halong Bay, Vietnam, where the fish infected with this parasite were caught.

Description

Adult. Based on five specimens (table 3 and fig. 1a). Body broadly fusiform. Tegumental spines lacking. Oral sucker globular,

Table 2. List of taxa incorporated in the molecular analysis of the family Fellodistomidae with the number of 28S rDNA sequences given in parentheses.

Species	Author	Accession numbers
Gymnophalloidea		
Fellodistomidae		
Coomera brayi (*n* = 1)	Cribb *et al.*, 2014	KJ425462
Fellodistomum agnotum (*n* = 2)	Krupenko *et al.*, 2020	MT216757–MT216758
Fellodistomum fellis (*n* = 3)	Krupenko *et al.*, 2020	MT216752, MT216755–MT216756
Gymnotergestia strongiluri n. sp. (*n* = 2)	This study	OK636408-OK636409
Lintonium crowcrofti (*n* = 1)	Cribb *et al.*, 2021	MZ687079
Lintonium currani (*n* = 1)	Cribb *et al.*, 2021	MZ687080
Lintonium droneni (*n* = 1)	Cribb *et al.*, 2021	MZ687081
Lintonium kostadinovae (*n* = 1)	Cribb *et al.*, 2021	MZ687082
Lintonium madvaviae (*n* = 1)	Cribb *et al.*, 2021	MZ687083
Lintonium pulchrum (*n* = 3)	Cribb *et al.*, 2021	MZ687084–MZ687086
Oceroma praecox (*n* = 1)	Cribb *et al.*, 2014	KJ425464
Olssonium turneri (*n* = 1)	Olson *et al.*, 2003	AY222283
Proctoeces choerodoni (*n* = 1)	Wee *et al.*, 2017	KX671299
Proctoeces insolitus (*n* = 1)	Wee *et al.*, 2017	KX671300
Proctoeces maculates (*n* = 3)	Olson *et al.*, 2003; Antar & Gargouri, 2015	AY222284, KU052940–KU052941
Proctoeces major (*n* = 3)	Wee *et al.*, 2017	KX671307–KX671309
Steringophorus blackeri (*n* = 1)	Bray *et al.*, 1999	AJ405296
Steringophorus dorsolineatum (*n* = 1)	Bray *et al.*, 1999	AJ405291
Steringophorus furciger (*n* = 2)	Krupenko *et al.*, 2020	MT216753–MT216754
Steringophorus haedrichi (*n* = 1)	Bray *et al.*, 1999	AJ405293
Steringophorus liparidis (*n* = 1)	Sokolov *et al.*, 2021a	MT872201
Steringophorus margolisi (*n* = 1)	Olson *et al.*, 2003	AY222281
Steringophorus merretti (*n* = 1)	Bray *et al.*, 1999	AJ405299
Steringophorus occidentalipacificus (*n* = 1)	Sokolov *et al.*, 2021a	MT872202
Steringophorus pritchardae (*n* = 1)	Bray *et al.*, 1999	AJ405295
Steringophorus thulini (*n* = 2)	Bray *et al.*, 1999	AJ405297–AJ405298
Steringotrema robertpoulini (*n* = 1)	Pérez-Ponce de León *et al.*, 2018	MG696894
Symmetrovesicula chaetodontis (*n* = 1)	Cribb *et al.*, 2021	MZ687087

(*Continued*)

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Table 2. (*Continued.*)

Species	Author	Accession numbers
Symmetrovesicula gracilis (*n* = 1)	Cribb *et al.*, 2021	MZ687088
Tergestia clonacantha (*n* = 1)	Wee *et al.*, 2017	MF155627
Tergestia henryi (*n* = 1)	Wee *et al.*, 2017	MF155628
Tergestia maryae (*n* = 1)	Wee *et al.*, 2017	MF155626
Tergestia sp. (*n* = 1)	Cribb *et al.*, 2014	KJ425467
Tandanicolidae		
Prosogonarium angelae (*n* = 1)	Olson *et al.*, 2003	AY222285
Outgroup		
Rhipidocotyle fennica (*n* = 1)	Stunžėnas *et al.*, 2014	KM068119

subterminal. Ventral sucker round, larger than oral sucker, pre-equatorial. Prepharynx short. Pharynx round. Oesophagus extremely short, bifurcates immediately anteriorly to ventral sucker. Caeca extend to posterior third of hindbody. Testes round to oval, symmetric, separated by uterus, located just anteriorly of border between anterior and posterior part of hindbody. Cirrus sac elongate, curved in posterior part, dorsally to ventral sucker, dextral to mid-body line, extending from posterior end of ventral sucker to genital pore. Genital pore sinistral, lateral at level of posterior half of pharynx. Seminal vesicle elongate, occupies posterior third of cirrus sac. Pars prostatica tubular, surrounded by few prostatic cells. Ejaculatory duct long. Ovary submedian, transversally oval, partially overlapping ventral sucker. Seminal receptacle median, in space between ovary and testes. Vitellarium in two clusters of 9–10 round follicles, extending from level of posterior border of ventral sucker or from level of ovary to middle or posterior third of testes. Uterus mainly in post-testicular region. Metraterm long, extending from mid-level of ventral sucker to genital pore. Eggs numerous, operculate. Excretory vesicle not observed, excretory pore terminal.

Molecular data

Partial sequences of 28S rRNA gene 1164 bp in length of two specimens of *L. halongi* n. sp. (OK636406-OK636407) were aligned with all available ribosomal large subunit sequences of the Zoogonidae and the Faustulidae and trimmed to the most optimal alignment length (1105 bp) for the available dataset; missing data were considered for the new species when building the alignment. The two 28S rDNA sequences of *L. halongi* n. sp. obtained in our study were identical.

Gymnotergestia strongyluri *n. sp.*

Taxonomic summary

Type host. *Strongylura strongylura* (van Hasselt, 1823).
Site. Intestine.
Intensity of infection. 1–26 specimens.
Type locality. Coastal water of Cat Ba Island, Tonkin Bay, northern Vietnam (20°84′N, 106°59′E).
Type deposition. Holotype no. 199–Tr, paratypes nos. 200–203–Tr.
Materials deposited. Materials are deposited in the parasitological collection of the Zoological Museum (deposited 20 November 2018, Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia; e-mail: petrova@biosoil.ru).
Etymology. The species name refers to the definitive host, *S. strongylura*.

Description

Adult. Based on five specimens (table 3 and fig. 1b, c). Body elongate oval. Tegumental spines lacking. Oral sucker globular, subterminal. Ventral sucker round, larger than oral sucker, pre-equatorial. Prepharynx not observed. Pharynx large, elongate, conical, expanding anteriorly and tapering posteriorly. Oesophagus extremely short, bifurcates at midway between oral and ventral sucker. Caeca slightly not reaching posterior end of body. Testes round to oval, tandem, adjacent to each other, located in posterior third of body. Cirrus sac pyriform with expanding anterior part located immediately anteriorly to ventral sucker and elongate posterior part at level of anterior half of ventral sucker. Genital pore with muscular sphincter, sinistral, submedian between intestinal bifurcation and ventral sucker, opening into genital atrium. Genital atrium oval, surrounded by numerous small glandular cells. Seminal vesicle elongate, occupies elongate part of cirrus sac and partly penetrates expanding part of cirrus sac. Pars prostatica thick-walled, surrounded by numerous prostatic cells. Ejaculatory duct curved. Ovary round or oval, submedian, immediately before testes. Vitellarium in two lateral fields of irregular follicles. Vitelline fields extending from level of middle of distance between ovary and ventral sucker to anterior half of posterior testis. Uterus occupying much of hindbody and part of lateral region, sinistral to testes and ovary. Metraterm thin-walled, long, curved, extending from posterior end of ventral sucker to genital pore. Eggs numerous, operculate. Excretory vesicle Y-shaped, pore terminal.

Molecular data

Partial sequences of 28S rRNA gene 1270 bp in length of two specimens of *G. strongyluri* n. sp. (OK636408-OK636409) were aligned with all available ribosomal large subunit sequences of the Fellodistomidae, resulting in a 908 bp alignment dataset. The two 28S rDNA sequences of *G. strongyluri* n. sp. differed by four variable sites, which represents 0.46 ± 0.21% of divergence.

Remarks

The worms found in *Hemiramphus* spp. from Vietnam apparently belong to the genus *Lecithostaphylus* and are morphologically similar to *L. gibsoni* Cribb, Bray & Barker, 1992 ex *Abudefduf whitleyi* Allen & Robertson, 1974 (Pomacentridae) of Heron Island (Cribb *et al.*, 1992) and to *L. depauperati* Yamaguti, 1970 ex *Hemiramphus depauperatus* Lay & Bennett, 1839 off Hawaii (Yamaguti, 1970). There are no marked differences between the two trematodes described in this study and the two known species just mentioned in the metric parameters of the body and the eggs and the size of most organs, except the length of the cirrus sac (table 3). The cirrus sac of trematodes from our study is longer than that of *L. gibsoni* and *L. depauperati*. The worms collected in Vietnam also differ from *L. gibsoni* and *L. depauperati* by organ topology. In trematodes ex *Hemiramphus* spp., vitelline fields extend from the level of the posterior border of the ventral

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Table 3. Measurements (μm) of adult worms Zoogonidae (Microphalloidea) and Fellodistomidae (Gymnophalloidea).

	Lecithostaphylus halongi n. sp.		*Lecithostaphylus depauperati* (Yamaguti, 1970)		*Lecithostaphylus gibsoni* (Cribb Bray & Barker, 1992)	*Gymnotergestia strongyluri* n. sp.		*Gymnotergestia chaetodipteri* (Nahhas & Cable, 1964)	
	Holotype	Range	Mean	Range	Range	Holotype	Range	Mean	Range
Body length	1448	1278–1663	1445	900–1250	1026–1658	2064	1294–2295	1.723	1660–4280
Body width	493	462–601	508	250–400	399–615	616	462–616	527	380–567
Bw/Bl (%)	34	28.7–42.0	35.2	–		29.8	22.6–45.2	30.6	–
Forebody length	385	354–447	397	–	279–424	616	447–724	578	–
Fo/Bl (%)	26.6	26.6–27.9	27.5		19.3–27.1	29.8	28.8–38.1	33.5	–
Oral sucker length	154	154–193	170	70–120	140–164	223	185–231	207	140–187
Oral sucker width	181	162–196	176	90–190	159–171	243	185–246	216	200–280
Ventral sucker length	289	250–289	267	170–270	216–247	281	223–308	257	320–440
Ventral sucker width	285	243–296	276	–	183–196	289	243–308	269	247–340
Suckers length ratio	1.88	1:1.30–1.88	1:1.57	–	–	1:1.26	1:1.11–1.33	1:1.24	1:1.67
Suckers width ratio	1.57	1:1.46–1.73	1:1.57	–	1:1.09–1.22	1:1.20	1:1.19–1.25	1:1.25	–
Pharynx length	123	104–135	121	–	107–120	204	154–204	182	300–413
Pharynx width	123	123–154	136	–	114–127	181	142–181	157	123–173
Ovary length	96	96–108	102	80–100	101–152	154	123–189	162	126–186
Ovary width	119	104–127	115	70–100	120–167	116	104–231	159	106–133
Testis left length	189	189–316	228	–	161–222	204	142–227	178	173–286
Testis left width	193	135–193	151	–	107–196	270	154–277	233	140–173
Testis right length	200	196–300	232	–	164–253	243	142–262	202	–
Testis right width	196	112–196	154	–	139–222	270	169–285	242	–
Cirrus sac length	504	354–504	429	200–280	284–334	308	227–339	307	–
Cirrus sac width	89	77–100	89	40–60	114–120	166	139–189	167	–
Oval part cirrus sac length	–	–	–	–	–	212	135–212	169	–
Elongated part cirrus sac length	–	–	–	–	–	96	96–262	159	–
Elongated part cirrus sac width	–	–	–	–	–	62	58–92	69	–
Post-testicular field length	447	419–493	452	–	–	270	116–381	217	–
Eggs length	42–46	–	–	33–42	34–43	23–27	23–27	–	30–36
Eggs width	23–27	–	–	18–23	18–26	12–15	12–15	–	20–25

Bw, Body width; Bl, Body length; Fo, Forebody.

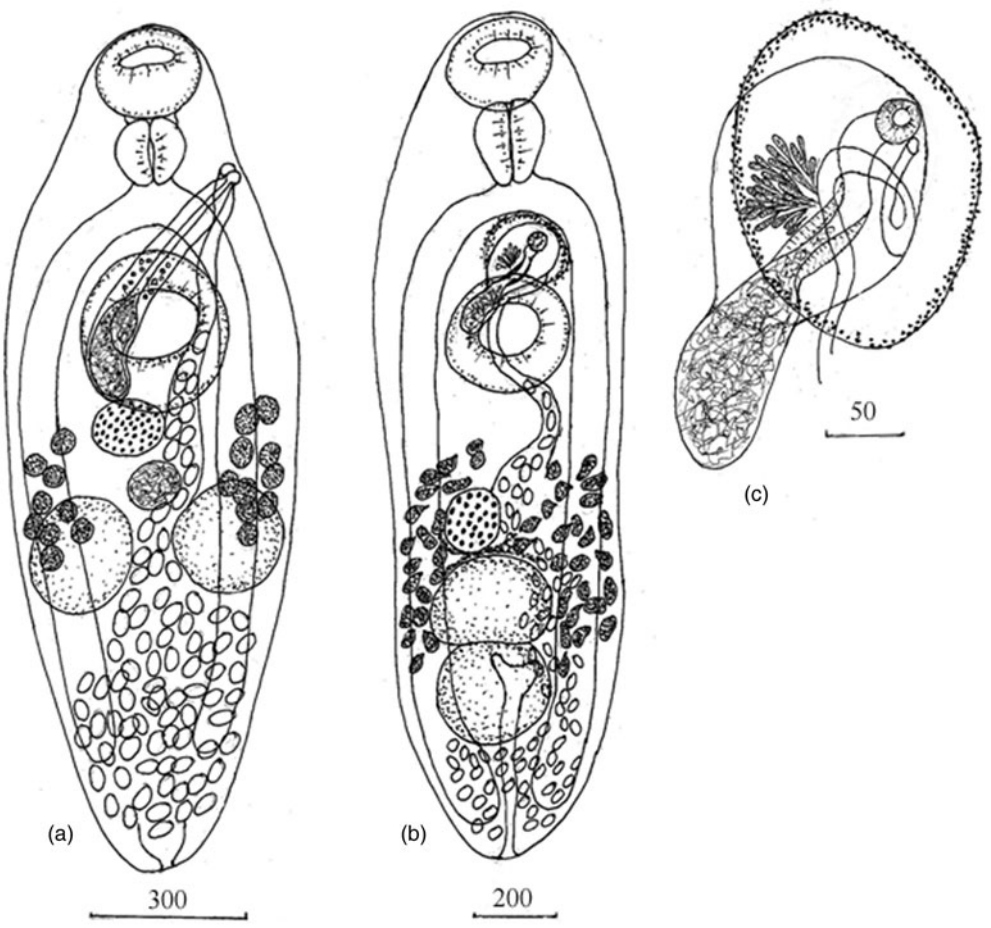

Fig. 1. Adult worms: (a) *Lecithostaphylus halongi* n. sp., ventral view; (b, c) *Gymnotergestia strongyluri* n. sp., ventral view. Measurements are given in μm.

sucker or ovary to the middle or posterior third of the testes, while in *L. gibsoni*, vitelline fields are predominantly located anteriorly to the testes at the level of the ventral sucker, and in *L. depauperati*, vitelline fields reach the anterior border of the ventral sucker. On the basis of these data, we recognize the worms ex *Hemiramphus* spp. from Vietnam as a new species and name it *L. halongi* n. sp.

To check the validity of the new species, ML and BI phylogenetic analyses based on 28S rDNA partial sequences were performed for the Zoogonidae. These two algorithms generated phylogenetic trees with an identical topology (fig. 2). We omitted the gymnophalloid clade of the Zoogonidae because its species always clustered with the Gymnophalloidea in previous molecular phylogenetic analyses (Sun *et al.*, 2014; Cutmore *et al.*, 2018; Diaz, 2018; Pérez-Ponce de León & Hernández-Mena, 2019; Sokolov *et al.*, 2021a).

The results of the phylogenetic analysis indicate that *L. halongi* n. sp. is closely related to *Lecithostaphylus brayi* Cabañas-Granillo, Solórzano-García, Mendoza-Garfias & Pérez-Ponce de León, 2020 with a divergence value of 7.14 ± 0.91%, generating a monophyletic highly supported clade. *Deretrema* spp. and *Proctophantastes* spp. formed a distinct clade, a sister to *Lecithostaphylus,* with a high statistical support and a genetic divergence of 17.08 ± 0.98%. For comparison, the interspecific genetic divergence value for the monophyletic genus *Lepidophyllum* in our study was 14.76 ± 1.12%, which is similar to the divergence values between the different zoogonid genera on the phylogenetic tree. These results unambiguously indicate that the new species is a member of the genus *Lecithostaphylus* within the subfamily Lecithostaphylinae, which also includes *Deretrema nahaense* Yamaguti, 1942 and *Proctophantastes gillissi* (Overstreet & Pritchard, 1977) Bray & Gibson, 1986 from the present dataset.

We disagree with the opinion of Sokolov *et al.* (2021a) about the existence of Lecithostaphylinae *sensu lato*, which includes the species mentioned above and *Steganoderma eamiqtrema* Blend & Racz, 2020. *Steganoderma eamiqtrema* has high *p*-distance values, 20.44 ± 1.21%–23.68 ± 1.23%, as compared with species of Lecithostaphylinae *sensu stricto* by 28S rDNA sequence data, and the phylogenetic relationships of this species are poorly supported in the study by Sokolov *et al.* (2021b). Moreover, in our study, *S. eamiqtrema* (Zoogonidae) was sister to [Lecithostapyhllinae + Zoogoninae + Faustulidae (*Antorchis pomacanthi*, *Trigonocryptus conus* and *Bacciger lesteri*)] clade on both ML and BI trees with a high support (figs 2 and 3). In our opinion, the molecular data indicate that *S. eamiqtrema* belongs to a distinct subfamily. Thus, we suggest that *Lecithostaphylus* should be removed from the Lepidophyllinae and that the subfamily Lecithostaphylinae Odhner, 1911 should be recognized, including at least three genera: *Lecithostaphylus*, *Proctophantastes* and *Deretrema*.

The diagnostic characters of trematode specimens found in *S. strongylura* from Vietnam correspond to those of *Gymnotergestia*. This genus contains a single species, *G.*

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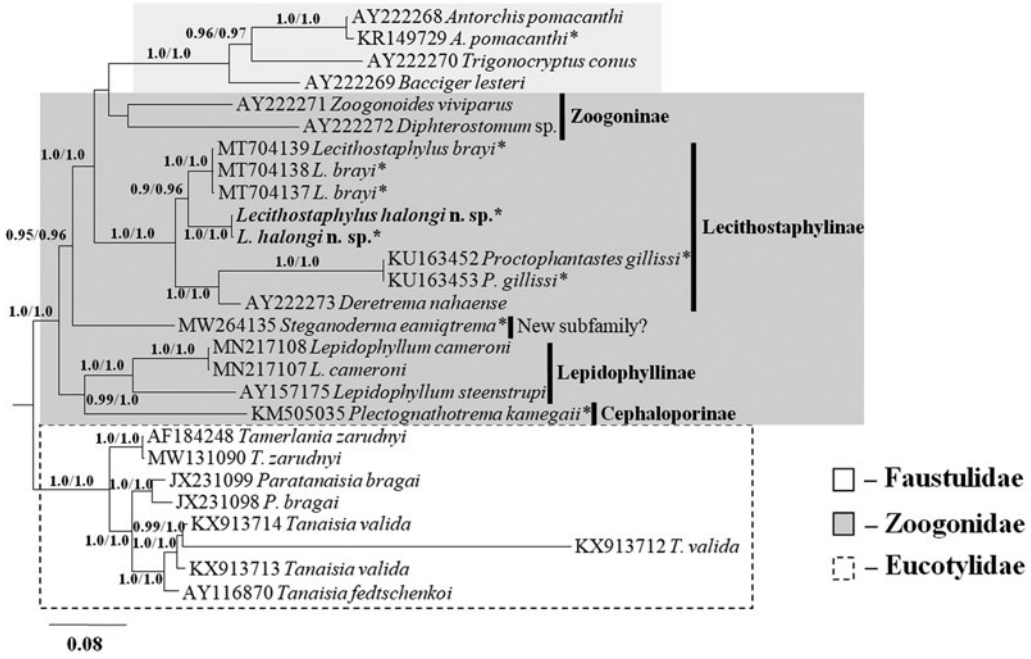

Fig. 2. Bayesian phylogenetic tree of Zoogonidae based on the analysis of partial 28S rRNA gene sequences; nodal numbers indicate posterior probabilities. Sequences from the present study are in bold. *Morphologically validated species. Scale bar shows the number of substitutions per site.

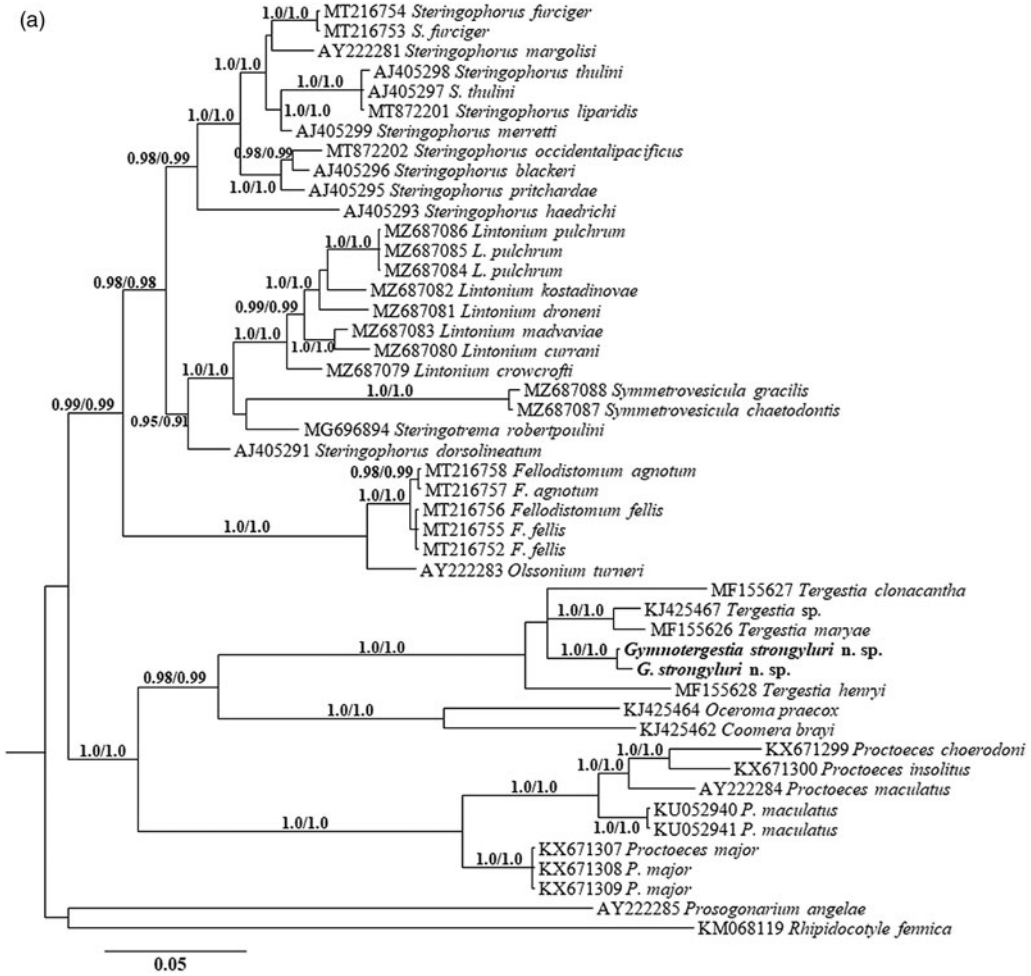

Fig. 3. (a) Phylogenetic tree of the superfamily Gymnophalloidea based on the analysis of partial 28S rRNA gene sequences; nodal numbers indicate posterior probabilities for ML/BI algorithms. Sequences from the present study are in bold. (b) Fragment of ML phylogenetic tree topology exhibited relationships of *Gymnotergestia strongyluri* n. sp. Sequences from the present study are in bold. Scale bar shows the number of substitutions per site.

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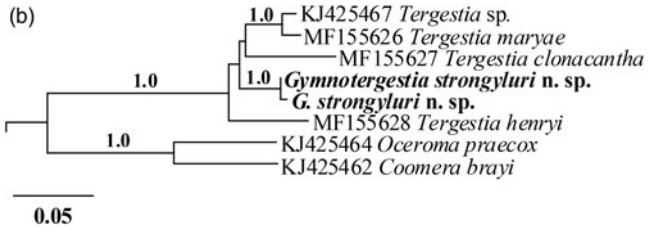

Fig. 3. Continued.

chaetodipteri Nahhas & Cable, 1964, ex *C. faber* from Jamaica (Nahhas & Cable, 1964). The worms from our material are similar to *G. chaetodipteri* in having a conical pharynx, tandem testes, a pre-testicular ovary, vitelline follicles in lateral fields posterior to the ventral sucker and in not having external seminal vesicle and seminal receptacle. At the same time, the worms ex *S. strongylura*, unlike *G. chaetodipteri*, possess an elongate to oval body vs. elongate slender body, and testes adjacent to each other vs. testes separated by uterine coils. Besides, there are considerable differences in the pharynx length, egg size and the suckers ratios between these two species (table 3).

Taken together, these data justify the establishment of a new species, *G. strongyluri* n. sp., for the worms described in our study. Morphologically, they are most similar to the specimens of *Gymnotergestia* sp. ex *A. sclerolepis* from Australia in the shape of the cirrus sac and the relative arrangement of testes and ovary, on the basis of the figure provided in Keys to the Trematoda (Bray, 2002 p. 291). However, these worms differ from each other by the cirrus sac and vitellarium arrangement relative to other organs. Unfortunately, there are no published morphometric data for Australian worms, but judging from the figure in Bray (2002), they may belong to a new species of *Gymnotergestia*.

It is also impossible to perform a meaningful comparative analysis of the worms from our material and those from the study of Al-Bassel (1999), who found *G. chaetodipteri* in *S. scriba* from the Mediterranean Sea. The illustration provided in Al-Bassel (1999) is poorly informative for taxonomical interpretations, and molecular data are needed for any conclusion.

Phylogenetic trees of the Fellodistomatidae based on 28S rDNA have identical topologies in ML and BI analyses, except relationships within the clade containing *G. strongyluri* n. sp. and the genus *Tergestia* Stossich, 1899 (fig. 3a, b). These relationships appear as a poorly supported polytomy of *G. strongyluri* n. sp. and *Tergestia* spp. (except *Tergestia henryi* Wee, Cutmore, Yong & Cribb, 2017) on the Bayesian tree (fig. 3a) and as a poorly supported dichotomy on the ML tree (fig. 3b), indicating paraphyly of *Tergestia*. Genetic divergence by 28S rDNA sequence data of *G. strongyluri* n. sp. and *Tergestia* spp. ranged from 4.61 ± 0.68% to 7.48 ± 0.81%, which was comparable with that for interspecific divergence values within *Tergestia* (1.85 ± 0.44 to 9.09 ± 0.94%) and for two other fellodistomid genera: *Steringophorus* Odhner, 1905 (1.61 ± 0.39 to 7.59 ± 0.86%) and *Proctoeces* Odhner, 1911 (4.26 ± 0.71 to 8.9 ± 0.98%). For the latter genus, a minimal value of 4.26 ± 0.71% was observed for different specimens of *Proctoeces maculatus* (Looss, 1901) Odhner, 1911 ex *Archosargus probatocephalus* (Walbaum, 1792), Gulf of Mexico, Mississippi, USA (Olson *et al.*, 2003) and ex *Sabella pavonina* Savigny, 1822, Bizerte Lagoon, Tunisia (Antar & Gargouri, 2015). Antar & Gargouri (2015) provided conclusive morphological and molecular evidence that the specimen of *P. maculatus* ex *A. probatocephalus* belongs to a different species of *Proctoeces*. Thus, we can use this value as reliable criterion for the minimal broad interspecific genetic divergence between *Proctoeces* species.

In our study, *G. strongyluri* n. sp. and *Tergestia* spp. clustered together on the phylogenetic tree, differing from each other by the *p*-distance values at the interspecific level. This indicates that these trematodes may belong to the same genus. However, additional molecular data for most of the species of these two genera, especially the type species, are needed for a final taxonomical conclusion.

In our analysis, we did not consider the genetic divergence between *Olssonium turneri* Bray & Gibson, 1980 and *Fellodistomum* spp., which ranged from 2.41 ± 0.5% to 2.52 ± 0.52% by 28S rDNA sequences. These values correspond to the interspecific divergence level. However, this fact was ignored by all the authors of the phylogenetic studies of the Fellodistomidae employing a molecular approach, even though relatively representative data were available (Bray *et al.*, 1999; Cribb *et al.*, 2014; Antar & Gargouri, 2015; Wee *et al.*, 2017; Pérez-Ponce de León *et al.*, 2018; Krupenko *et al.*, 2020). In our opinion, it should be checked in further complex studies whether *O. turneri* in fact belongs to the genus *Fellodistomum*.

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Conflicts of interests. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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