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FISH PARASITOLOGY - ORIGINAL PAPER

Morphometric and molecular analyses of Carassotrema koreanum Park 1938 and Elonginurus mugilus Lu 1995 (Digenea: Haploporidae) Srivastava, 1937 from the Russian Far East and Vietnam

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Abstract
Adult worms that belong to *Carassotrema* Park 1938 and *Elonginurus* Lu, 1955 were found in the intestine of *Carassius gibelio* Bloch, 1782 from the southern Russian Far East and *Mugil cephalus* Linnaeus, 1758 from northern Vietnam, respectively. Morphometric parameters, geographic location and host species composition of these worms correspond to *Carassotrema koreanum* Park 1938, which is a known parasite of cyprinid fish in Korea, Japan and China, and *Elonginurus mugilus* Lu, 1955, first described from *M. cephalus* in China. The validity of *Carassotrema ginezinskajae* Kulakova, Ha Ky, 1976, a synonym of *C. koreanum*, first described from *Spinibarbichthys denticulatus* Oshima, 1926 in Vietnam, supported the morphometric data. Phylogenetic analysis based on combined ITS2 ribosomal DNA (rDNA) and 28S rRNA indicated that *C. koreanum* and *E. mugilus* belong to the subfamily Waretrematinae and are closely related to the genera *Skrjabinolecithum* and *Parasaccocoelium*, respectively. Species similarity, revealed through molecular analysis, agreed with the generic diagnoses for *Parasaccocoelium* and *Elonginurus*, as well as for *Carassotrema* and *Skrjabinolecithum*.

Keywords Haploporidae · *Carassotrema* · *Elonginurus* · 28S · *Mugil*

Introduction

Carassotrema koreanum Park, 1938 is a parasite of cyprinids in eastern Asia. Park (1938) described *C. koreanum* as representative of a new genus from *Carassius auratus* (Linnaeus, 1758) in Seoul, Korea. Later, trematodes reported as *C. koreanum* were found in fish species of Japan, China, the Russian Far East and Vietnam (Yamaguti 1942; Tang and Lin 1979; Bykhovskaya-Pavlovskaya and Kulakova 1987; Moravec and Sey 1989; Shimazu 2005). Shimazu (2013) provided a detailed description of *C. koreanum* specimens based on material from different Japanese fish species and revealed a number of morphological differences among worms reported as *C. koreanum* from various territories of the species' natural habitat (as described by different authors). *Elonginurus mugilus* Lu 1995 is the single member of its genus that has been described as a parasite of *Mugil cephalus* Linnaeus, 1758 from the South China Sea (Lu 1995). The species *Phanurus oligoovus* (Liu and Yang 2002), which was detected in *Liza haematocheila* (Temminck and Schlegel, 1845) from the South China Sea (Liu and Yang 2002), was synonymised with *E. mugilus* (Overstreet and Curran 2005). *Carassotrema koreanum* and *E. mugilus* (Waretrematinae Srivastava, 1937) are species of respective genera for which only morphometric data are available; molecular data are absent. These circumstances create some difficulties for species identification of potential representatives of these two genera, especially for those that have been found outside of their type localities. Moreover, this data absence does not allow identification of phylogenetic relationships at intergeneric or higher levels.

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In the present study, we detected trematodes of *C. koreanum* in *Carassius gibelio* (Bloch, 1782) in Lebedinoe Lake of the southern Russian Far East and trematodes of *E. mugilus* trematodes in *M. cephalus* from coastal waters of Cat Ba Island, Halong Bay, Vietnam. We present morphometric and molecular data for these worms in consideration of their taxonomic identification and phylogenetic relationship within Waretrematinae.

Materials and methods

Specimen collection

Seventy-five (75) adult *C. koreanum* worms were found in the intestine of *C. gibelio* in Lebedinoe Lake, Chasansky district, Primorsky Region, Russia. Twenty-nine (29) adult worms of *E. mugilus* were found in *M. cephalus* in coastal waters of Cat Ba Island, Halong Bay, Vietnam. Worms 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 for descriptive purposes were prepared by staining the specimens with alum carmine, dehydrating the worms in a graded ethanol series and clearing in clove oil and then mounting in Canada balsam under a coverslip on a slide. All measurements are provided in micrometer. 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 Russian Academy of Sciences, Vladivostok, Russia; e-mail: petrova@ibss.dvo.ru).

DNA extraction, amplification and sequencing

Adult *C. koreanum* and *E. mugilus* worms were fixed in 96% ethanol. Three *C. koreanum* and five *E. mugilus* adult specimens were used for molecular analysis (Table 1). Total DNA was extracted from flukes using a “hot shot” technique (Truett 2006).

28S ribosomal DNA (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′) (Tkach et al. 2003) with an annealing temperature of 55 °C. 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 primer pairs were included.

Polymerase chain reaction (PCR) products were directly sequenced using an ABI Big Dye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, USA), following the manufacturer’s recommendations, with the internal sequencing primers described by Tkach et al. (2003) for 28S rDNA and Luton et al. (1992) for ITS. PCR product sequences were analysed using an ABI 3130 genetic analyser at the Federal Scientific Center of the East Asia Terrestrial Biodiversity FEB RAS. Sequences were submitted to the GenBank database (NCBI).

Alignments and phylogenetic analysis

Ribosomal DNA sequences were assembled with SeqScape v.2.6 software, provided by Applied Biosystems. Alignments and estimations of the number of variable sites and sequence differences were performed using MEGA 7.0 software (Kumar et al. 2016). The values of genetic p-distances were calculated for the 28S rDNA fragment. Phylogenetic relationships were obtained using a concatenated data set of partial 28S rRNA and ITS2 rDNA. Phylogenetic analysis was performed for concatenated ITS2 and 28S rDNA using the Bayesian algorithm with MrBayes v.3.2.6 software (Huelsenbeck et al. 2001). The best nucleotide substitution model, the general time reversible (Tavare 1986) with estimates of invariant sites and gamma-distributed among-site variation (GTR + I + G), was 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 with the standard deviation of split frequencies at 0.0099. Summary parameters and the phylogenetic tree were calculated with a burn-in of 250,000 generations. The significance of the phylogenetic relationships was estimated using posterior probabilities (Huelsenbeck et al. 2001). Accession numbers, authority and supporting information about rDNA sequences from Gene Bank, used for phylogenetic analyses, were provided in Table 1 (Blasco-Costa et al. 2009; Pulis et al. 2013; Pulis and Overstreet 2013; Andres et al. 2014, 2015, 2018; Atopkin et al. 2015; Besprozvannykh et al. 2015b; Briscoe et al. 2016; Besprozvannykh et al. 2017; Curran et al. 2018; Huston et al. 2018; Atopkin et al. 2019). *Brachyphallus goliath* was used as an outgroup (authors of these data and accession numbers are given in the Table 1).

Results

Worms description

***Elonginurus mugilus* Lu, 1995**

Host. Mugil cephalus Linnaeus, 1758

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

Site. Intestine

Intensity of infection. 29 worms

Description (based on 7 specimens; Fig. 1a, b; Table 2). Body is elongate-oval, with retractable posterior end, spined from the anterior end to posterior third. Eye-spots’ pigment is diffuse in the anterior third of body. Oral sucker is

Table 1 List of taxa used for molecular analysis

Species	N (28S/ ITS)	Definitive host	Authors	Accession number in the NCBI	
				28S	ITS1-5.8S-ITS2
Haploporoidae					
Waretrematinae					
Elonginurus mugilus	2/2	*Mugil cephalus*	Present study	MH763761 - MH76362	MH763766 - MH763767
Carassotrema koreanum	3/3	*Carassius gibelio*	Present study	MH763758 - MH763760	MH763763 - MH763765
S. pyriforme	1/1	*Liza haematocheila*	Besprozvannykh et al. 2017a	HE806359	LN864990
S. spasskii	1/1	*Liza haematocheila*	Besprozvannykh et al. 2015b; Atopkin et al. 2015	LN614538	LK022754
S. spinosum	1/1	*Mugil cephalus*	Besprozvannykh et al. 2017b	MF176829	MF176832
Parasaccocoelium mugili	1/1	*Liza haematocheila*	Atopkin et al. 2019	MG098882	MG098884
Spiritestis herveyensis	1/1	*Moolgarda seheli*	Pulis and Overstreet 2013	KC206500	FJ211263-FJ211264
Capitimitta costata	1/1	*Selenotoca multifasciata*	Pulis and Overstreet 2013	KC206497	FJ211267
Capitimitta darwinensis	1/1	*Selenotoca multifasciata*	Pulis and Overstreet 2013	KC206498	FJ211248
Haploporinae					
Unisaccus tonkini	1/1	*Moolgarda cunnesius*	Besprozvannykh et al. 2017b	MF176840	MF176835
Saccocoelium brayi	1/1	*Liza saliens*	Blasco-Costa et al. 2009	FJ211234	FJ211244
Lecithobotrys putrescen	1/1	*Liza saliens*	Blasco-Costa et al. 2009	FJ211236	FJ211246
Litosaccus_ brisbanensis	1/1	*Mugil cephalus*	Andres et al. 2014	KM253765	
Haploporus benedeni	1/1	*Liza ramado*	Blasco-Costa et al. 2009	FJ211237	FJ211247
Ragaia lizae	1/1	*Liza aurata*	Blasco-Costa et al. 2009	FJ211235	FJ211245
Forticulcitinae					
Forticulcita gibsoni	1/1	*Mugil cephalus*	Blasco-Costa et al. 2009	FJ211239	FJ211249
F. apiensis	1/1	*Mugil cephalus*	Andres et al. 2015	KP761087	
F. platana	1/1	*Mugil liza*	Andres et al. 2015	KP761086	
Xiha fastigata	1/1	*Mugil cephalus*	Andres et al. 2015	KP761088	
Chalcinotrematinae					
Saccocoelioides beauforti	1/1	*Mugil cephalus*	Curran et al. 2018	MG925104	MG925103
Saccocoelioides elongatus	1/1	*Prochilodus lineatus*	Curran et al. 2018	MG925108	MG925107
Saccocoelioides magnus	1/1	*Cyphocharax voga*	Curran et al. 2018	MG925112	MG925111
Saccocoelioides nanii	1/1	*Prochilodus lineatus*	Curran et al. 2018	MG925114	MG925113
Saccocoelioides orosiensis	1/1	*Poecilia gillii*	Curran et al. 2018	MG925118	MG925117
Saccocoelioides tkachi	1/1	*Astyanax aeneus*	Curran et al. 2018	MG925122	MG925121
Intromugil mugilicolus	1/1	*Mugil cephalus*	Pulis et al. 2013	KC430096	
Intromugil alachuaensis	1/1	*Mugil cephalus*	Pulis et al. 2013	KC430095	
Hapladeninae					
Hapladena acanthuri	1/1	*Acanthurus chirurgus*	Andres et al. 2018	MH244119	
Hapladena cf. *varia*	1/1	*Acanthurus chirurgus*	Andres et al. 2018	MH244120	
Megasoleninae					
Megasolena hysterospina	1/1	*Archosargus rhomboidalis*	Andres et al. 2018	MH244121	

Table 1 (continued)

Species	N (28S/ITS)	Definitive host	Authors	Accession number in the NCBI	
				28S	ITS1-5.8S-ITS2
Megasolena sp. m MA-2018	1/1	*Holacanthus ciliaris*	Andres et al. 2018	MH244122	
Cadenatelinae					
Cadenatella americana	1/1	*Kyphosus sectatrix*	Andres et al. 2018	MH244117	
Cadenatella floridae	1/1	*Kyphosus incisor*	Andres et al. 2018	MH244118	
Atractotrematidae					
Isorchis anomalus	1/1	*Chanos chanos*	Huston et al. 2018	KU873018	
Isorchis currani	1/1	*Selenotoca multifasciata*	Huston et al. 2018	KU873017	
Isorchis megas	1/1	*Selenotoca multifasciata*	Huston et al. 2018	KU873015	
Plagiorchiidae					
Brachycladium goliath	1/1	*Balaenoptera acutorostrata*	Briscoe et al. 2016	KR703279	
Monorchiidae					
Hyrleytrematoides chaetodoni	1/1	*Chaetodon striatus*	Andres et al. 2018	MH244116	

n, number of sequences

subterminal, transversally oval. Prepharynx is short. Pharynx is round or oval. Oesophagus is longer; intestinal bifurcation occurs at the level of the posterior half-ventral sucker. Caeca is short, saccular. Ventral sucker is equal or slightly larger or less than the oral sucker at the border of the anterior and middle third of the body. Testis is single, round or transversally oval, in the posterior part of the body. External seminal vesicle is saccular or elongated and curved, between the middle of the ventral sucker and ovary. Metraterm is short. Hermaphroditic sac is sacciform, median and overlaps the ventral sucker; it

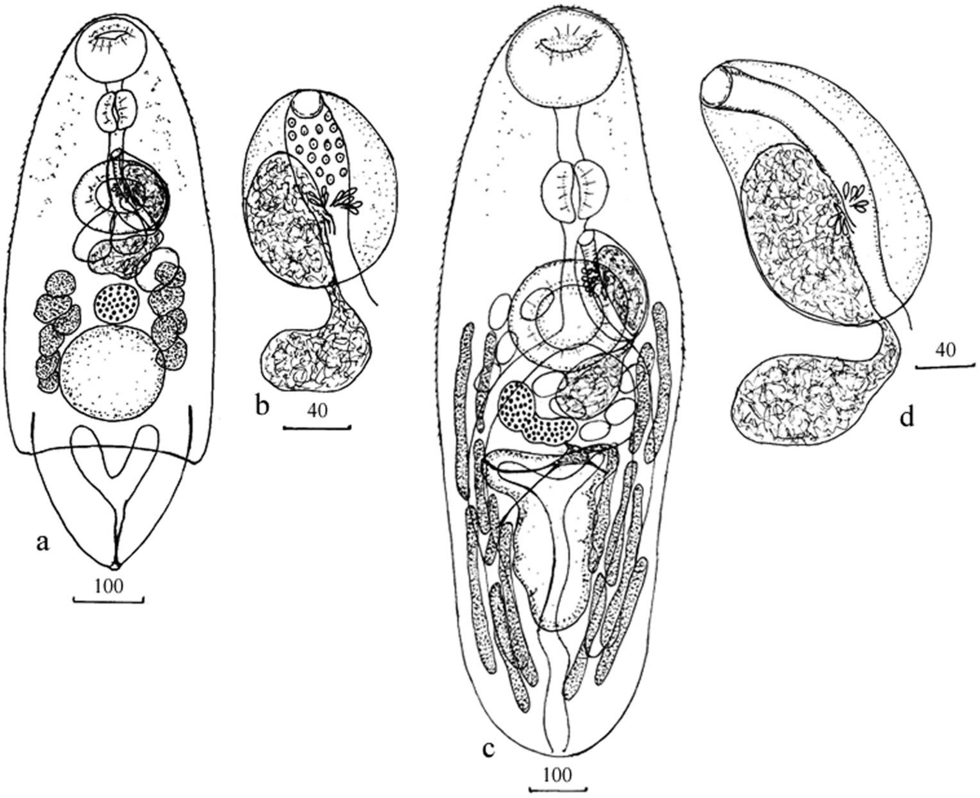

Fig. 1 *Elonginurus mugilus* Lu, 1995: **a** adult worms, **b** hermaphroditic sac; *Carassotrema koreanum* Park, 1938 **c** adult worms, **d** hermaphroditic sac. Scale bar is micrometer

Table 2 Sizes (μm) adult worms *Elonginurus mugilus* Lu 1995 and *Carassotrema koreanum* Park 1938

	E. mugilus			*C. koreanum*			*C. ginezinskajae*
	Our data	(Lu 1995)	(Liu et Yang, 2002)	Our data	(Park 1938)	(Shimazu 2013)	Kulakova, Ha Ky, (1976)
Body length	662–1032	472.5–945	604–784	1278–1602	684–1915	1520–2260	1000–1500
Body width	246–416	180–367.5	288–360	431–601	325–599	600–850	500–700
Oral sucker length	85–108	52.5–112.5	84–110	158–182	86–188	170–230	160–240
Oral sucker width	96–146	525–137.5	112–130	189–239	88–214	190–240	170–220
Forebody	185–293	–	–	420–566	290–350	510–720	
Ventral sucker length	96–135	60–107.5	92–120	162–262	109–205	210–260	170–270
Ventral sucker width	96–146	625–132.5	108–128	163–250	133–239	250–300	180–240
Prepharynx length	15–46	10–32.5	6–40	50–154	61	50–120	55
Pharynx length	54–92	52.5–100	52–72	119–146	80–139	140–190	150–220
Pharynx width	50–81	50–97.5	68–86	104–131	72–125	140–170	140–200
Oesophagus length	116–189	62.5–150	154–204	146–258	103–333	160–290	
Ovary length	50–62	45–117.5	64–90	58–80	88–154	150–320	76–108
Ovary width	50–62	37.5–100	30–74	135–189	103–257	80–160	55–76
Testis length	112–154	70–187.5	90–168	308–400	280–496	390–620	270–360
Testis width	96–154	67.5–175	142–238	154–250	205–302	260–550	250–360
Hermaphroditic sac length	108–146	85–162.5	120–150	193–212	–	230–390	–
Hermaphroditic sac width	65–119	62.5–150	90–132	116–135	–	150–220	–
Posterior end	135–154	–	216–304	–	–	–	–
Eggs length	69	52.5–72.5	56–64	54–58	57–64	56–62	57–72
Eggs width	54–58	42.5–55	40–57	35–39	24–35	34–42	31–38
Body/forebody length ratio %	19.1–33.4%	–	–	29.0–41.8%	–	32–38%	–
Ventral/oral sucker length ratio	1:1.13–1.54	–	–	1: 0.90–1.44	–	–	–
Ventral/oral sucker width ratio	1:0.82–1.1	–	–	1: 0.86–1.2	–	1: 1.2–1.3	–

contains an internal seminal vesicle, prostatic complex, metraterm and hermaphroditic duct. Pars prostatica is small, surrounded by few prostatic cells. Hermaphroditic duct is longer than the metraterm with pads. Genital pore is median, between the pharynx and ventral sucker. Ovary is round, submedian to median, pretesticular. Uterus is short. Vitellarium is composed of oval or irregular follicles in lateral fields between the levels of half-ventral sucker and anterior testis half. Eggs are large with 1–2 in the uterus, operculated and unembryonated. Excretory bladder is Y-shaped.

Molecular data

For two *E. mugilus* specimens, there were 1221 and 1342 alignable characters available for analysis in the 28S rRNA gene and the ITS1-5.8S-ITS2 rDNA fragment datasets, respectively. Nucleotide sequences of the *E. mugilus* 28S rRNA gene fragment contained no variable sites, while the ITS1-5.8S-ITS2 rDNA fragments had 9 variable sites. The sequences were submitted to the NCBI database with accession numbers MH763761 - MH76362 and MH763766 - MH763767.

***Carassotrema koreanum* Park, 1938**

Host. Carassius gibelio (Bloch, 1782)

Locality. Lebedinoe Lake, Chasansky district, Primorsky Region, southern Russian Far East, 42° 34' N, 130° 41' E

Site. Intestine

Intensity of infection. 75 worms

Description (based on 5 specimens; Fig. 1c, d; Table 2). Body is elongate-oval, spined from the anterior end to posterior third. Eye-spots' pigment is diffuse in the anterior third of body. Numerous large gland cells present in the forebody. Oral sucker is subterminal; inner wall bears small spines. Prepharynx is shorter than the oesophagus. Pharynx is round or oval. Oesophagus is long and reaches the middle level of the ventral sucker. Caeca ends blindly at the posterior margin of testis or slightly farther. Ventral sucker is round on anterior half of body, and inner wall bears small spines. Testis is single, T-shaped, in the middle hindbody. External seminal vesicle is saccular, between the middle ventral sucker and ovary.

Hermaphroditic sac is sacciform, sinistral to the ventral sucker and partly covering it, and contains an internal seminal vesicle, prostatic complex, metraterm and hermaphroditic duct. Pars prostatica is small, surrounded by few prostatic cells. Metraterm is shorter than the hermaphroditic duct. Hermaphroditic duct is long, muscular and surrounded by small gland cells. Genital pore is between the pharynx and ventral sucker. Ovary consists of two lobes transversally reniform or of an irregular form, submedian and pretesticular. Seminal receptacle is uterine. Uterus is between the hermaphroditic sac and anterior testis border. Vitellarium forms elongated follicles, between the middle of the ventral sucker and posterior extremity of the body. Vitelline fields are ventral and dorsal to intestines and partly cover the ventral ovary and testis. Eggs are few, operculated and unembryonated. Excretory bladder is Y-shaped; stem is dorsal to testis and bifurcates dorsally to the anterior testis.

Molecular data

For three *C. koreanum* specimens, there were 1127 and 1232 alignable characters available for analysis in the 28S rRNA gene and ITS1-5.8S-ITS2 rDNA fragment datasets, respectively. Both the *C. koreanum* 28S rRNA gene fragment and the ITS1-5.8S-ITS2 rDNA fragment were identical. The sequences were submitted to the NCBI database with accession numbers MH763758 - MH763760; MH763763 - MH76375.

Discussion

Morphological analysis

Adult worms from *C. gibelio* of the southern Russian Far East corresponded to the genus *Carassotrema* and were identical to *C. koreanum* morphologically (Fig. 1; Table 2). The natural area of these worms is restricted to eastern Asia, where this species has been detected in freshwater and anadromous Cyprinidae. Using results of morphological data analysis for *C. koreanum* from Japan and other areas of its natural habitat, Shimazu (2013) indicated that specimens from publications of different authors are characterised by the presence (Kulakova and Ky 1976; Tang and Lin 1979; Shimazu 2013) or absence (Park 1938; Yamaguti 1942; Wang and Pan 1984; Shimazu 2005) of spines on sucker walls. There are two possible interpretations for this phenomenon: these trematodes belong to different species or to the same species if spines were overlooked for a number of specimens. Moreover, *Carassotrema ginezinskajae* Kulakova, Ha Ky, 1976 from Vietnam was synonymised with *C. koreanum* (Moravec and Sey 1989), an action that was questionable from the viewpoint of Shimazu (2013); we agree with him. *Carassotrema ginezinskajae* differs from *C. koreanum* described by Park (1938) based on the ovary form, oval or irregular versus two lobes transversally reniform or of an irregular form, and its size (Table 2), as well as the vitellaria arrangement: vitellaria fields extends from the posterior boundary of the ventral sucker to the end of caeca versus from the anterior boundary of the median part of ventral sucker to the posterior end of the body. However, to fully resolve the *C. ginezinskajae* species status, additional molecular data are needed for worms from the type locality (Vietnam). Definitive hosts for *C. koreanum* are chiefly freshwater Cyprinidae, whose habitats are stagnant or flowing water reservoirs in Japan or continental reservoirs in Korea, south of the Russian Far East, China and Vietnam, which are geographically isolated from Japanese water bodies. Freshwater cyprinids within continental areas are also somewhat isolated from each other, although Korea, China and Vietnam have shared watercourses. The *C. koreanum* locality in the present study is Lebedinoe Lake in the southern Russian Far East. This lake belongs to the bottomland of the Karasik River, which is adjacent to the Tumannaya River that flows along the border of North Korea, China and the southern Russian Far East. This feature creates the possibility for exchange of freshwater ichthyofauna between the Tumannaya and Karasik Rivers. Such exchange is possible during great floods or as a result of animal introductions from one reservoir to another. At the same time, anadromous fish species of the genus *Tribolodon* were noted as definitive hosts for *C. koreanum*. Migrations of these fish species are restricted to coastal waters near its swapping and wintering areas, namely rivers. Thus, species of the genus *Tribolodon*, as definitive hosts for trematodes, could play a key role for maintaining connections between *C. koreanum* populations in the Tumannaya and Karasik Rivers. At the same time, the absence of long-distance marine migrations of *Tribolodon* species indicates complete isolation of *C. koreanum* Japanese island and continental Asian populations. Isolation factors could result in deep divergence of trematode populations marked as *C. koreanum*. The consequences of this process may not be reflected in trematode species morphology; i.e. morphologically similar worms may belong to different species based on molecular analysis. Trematodes *C. koreanum* from the Japanese island and continental East Asian populations have no significant morphological differences. Molecular data were obtained only for southern Russian Far East specimens. Thus, the question of species status for Japanese trematodes remains unresolved until additional molecular data become available.

Worms that were found in Vietnamese mullet fish are identical to *E. mugilus* morphometrically (Fig. 1, Table 2). The set of characteristics for these worms, including their morphometrical similarity, use of mullet fish as definitive hosts and their detection in the South China Sea basin, all suggest that these worms belong to the same species.

Molecular analysis

The concatenated ITS1-5.8S-ITS2 + 28S rDNA-based phylogenetic tree showed monophyly of haploporids, including Waretrematinae, Haploporinae, Chalcinotrematinae and Forticulticinae, which infect of mullet fish (Fig. 2). The initial *E. mugilus* and *C. koreanum* molecular data support their membership in the subfamily Waretrematinae. *Carassotrema koreanum* from Russian Far East *C. auratus* was closely related molecularly to Chinese *Carassotrema* sp. from *C. auratus*, with 99.73% similarity in the 28S rDNA 1112 base pairs (bp). *Carassotrema* was most closely related to *Skrjabinolecithum* trematodes in the terminal part of the tree. *E. mugilus* was closest to *Parasaccocoelium mugili*. These four genera formed a highly supported group with a sister clade that contained *Capitimitta* species and represented the monophyletic Waretrematinae subfamily. The phylogenetic position of *C. koreanum* indicates that there are considerable amounts of molecular characteristics for this species identical to *Skrjabinolecithum* species; for 28S rDNA, 1089–1090 bp (96.6–96.7%) of 1127 bp were conserved and for the ITS1-5.8S-ITS2 rDNA region, 1070–1156 bp (86.8–93.8%) of the 1232 bp were the same. Minimal values were obtained for the species pair *C. koreanum*/*S. spasskii*. The molecular identity of 28S rDNA and ITS1-5.8S-ITS2 rDNA for *E. mugilus* and *P. mugili* was also relatively high; they comprised 1106 (90.5%) of 1221 bp and 1062 (79.1%) of 1342 bp, respectively. These values are similar to interspecific and, in some examples, to intergeneric divergence level of the family Haploporidae (Blasco-Costa et al. 2009; Andres et al. 2015; Besprozvannykh et al. 2017a), and this finding supports the taxonomical status of separate genera for *C. koreanum* and *E. mugilus*. Molecular similarity between *Elonginurus* and *Parasaccocoelium* and between *Carassotrema* and

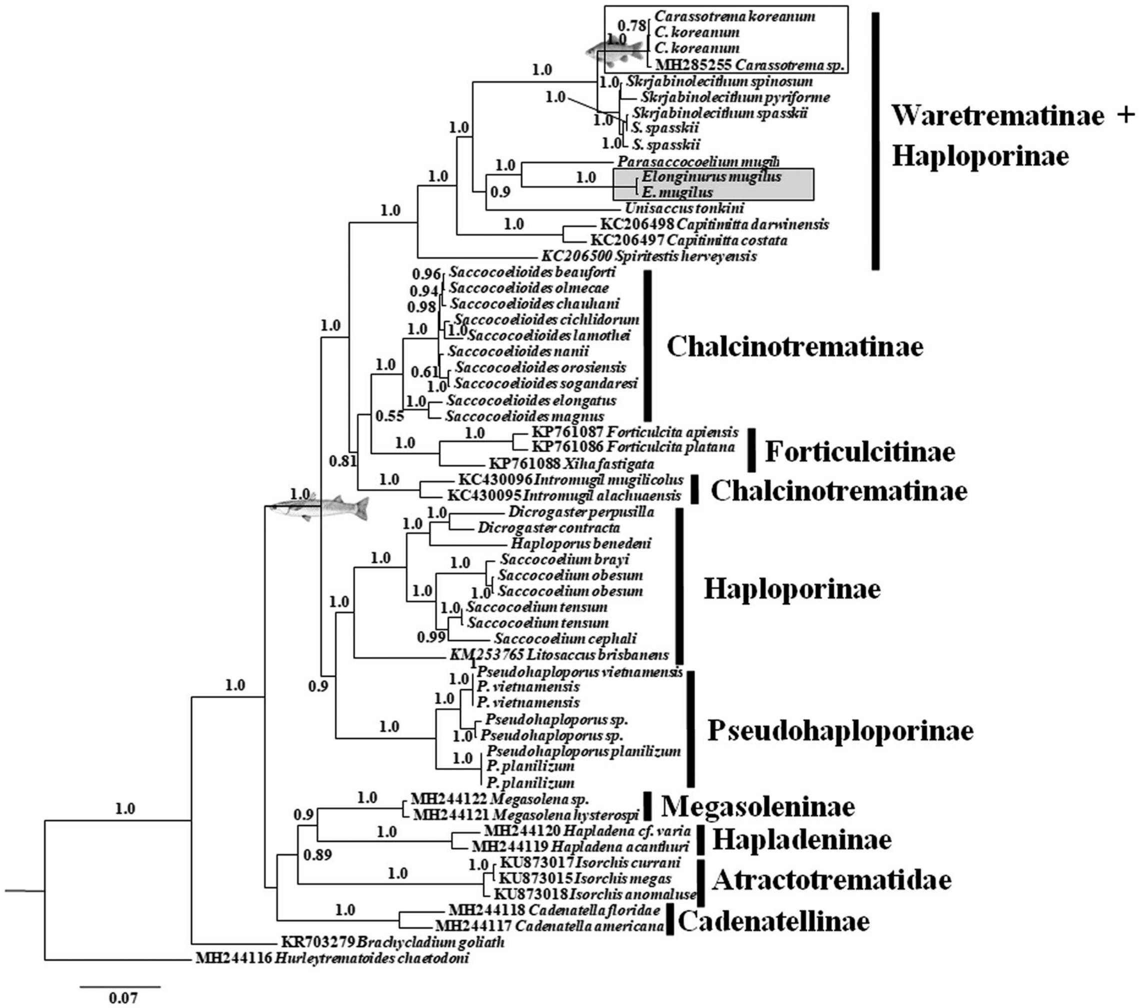

Fig. 2 Phylogenetic relationships of the family Haploporidae obtained with Bayesian algorithm analysis based on concatenated nucleotide sequence data set of ITS1-5.8S-ITS2 rDNA and partial 28S rRNA gene (overall 1925 bp). Nodal numbers— posterior probabilities that indicate statistical support of phylogenetic relationships; only significant values (0.9–1.0) are shown

Skrjabinolecithum agrees with the morphological similarities of representatives of the respective genera (Overstreet and Curran 2005; Besprozvannykh et al. 2015a, 2015b). The type species of *Elonginurus* (*E. mugilus*) and *Parasaccocoelium* (*P. mugili*) are similar with regard to organ morphology and arrangement. Moreover, these worms possess a retractable posterior body end, and unlike other haploporids, they possess a short uterus that contains minimal eggs with an undeveloped embryo (Lu 1995; Overstreet and Curran 2005; Besprozvannykh et al. 2015b). Concerning *Carassotrema* and *Skrjabinolecithum*, vitellaria morphology is different in the representatives of these genera. Vitellarium of species in *Carassotrema* consists of elongated, ellipse-shaped or tubular follicles, while vitellarium of species in*Skrjabinolecithum* thread-like and consists of small, round follicles. Further, species of *Elonginurus*, *Parasaccocoelium* and *Skrjabinolecithum* were detected only for mugilids and *Carassotrema*—for Cyprinidae and Mugilidae. The morphological and molecular similarities for *Elonginurus* and *Parasaccocoelium*, and shared definitive host species in east Asia, possibly indicate these species originated from a common ancestor. Differentiation of specimens of these genera is potentially related with mugilid phylogeny. For example, macro-populations of *Mugil cephalus* and *Planiliza haematochelia* possess genotype structure polymorphism and are split into mesopopulations due to Pleistocene glaciations (Jamandre et al. 2009; Han et al. 2013). These events could result in speciation and differentiation of Haploporidae faunas of the north-western Pacific and Southeastern Asia. Apparently, there are also close phylogenetic relationships between *Carassotrema* and *Skrjabinolecithum*. Andres et al. (2018) proposed, on the basis of concatenated 28S and ITS2 nucleotide sequence data, that marine eupercarian fish as hosts represents the basal state of the superfamily Haploporoidea. The authors support the "ecological bridge" hypothesis of Manter (1957), which proposes that mugilids (*Mugil cephalus* in particularly) is a key group of definitive hosts for haploporids divergence in estuarine and freshwater fish species. This hypothesis is useful for explaining the phylogenetic closeness of *C. koreanum* and *Skrjabinolecithum* spp. However, for complete resolution of this question, molecular data for *Carassotrema* from mugilid species are needed.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Andres MJ, Pulis EE, Cribb TH, Overstreet RM (2014) Erection of the haploporid genus *Litosaccus* n.g. and its phylogenetic relationship within the Haploporidae Nicoll, 1914. Syst Parasitol 89:185–194. https://doi.org/10.1007/s11230-014-9521-4

Andres MJ, Curran SS, Fayton TJ, Pulis EE, Overstreet RM (2015) An additional genus and two additional species of Forticulcitinae (Digenea: Haploporidae). Folia Parasitol 62(025). https://doi.org/10.14411/fp.2015.025

Andres MJ, Pulis EP, Curran SS, Overstreet RM (2018) On the systematic of some marine haploporids (Trematoda) with the description of a new species of *Megasolena* Linton, 1910. Parasitol Int 67:805–815. https://doi.org/10.1016/j.parint.2018.08.002

Atopkin DM, Beloded AY, Ngo HD (2015) Molecular genetic characterization of the far eastern trematode *Skrjabinolecithum spasskii* Belous, 1954, (Digenea, Haploporidae), a parasite of mullets. Russ J Mol Biol 49:373–379. https://doi.org/10.7868/S0026898415030027

Atopkin DM, Besprozvannykh VV, Ha DN, Nguyen VH, Nguyen VT, Chalenko KP (2019) A new subfamily, Pseudohaploporinae subfam. n. (Digenea: Haploporidae), with morphometric and molecular analyses of two new species: *Pseudohaploporus vietnamensis* n. g., sp. n. and *Pseudohaploporus planilizum* n. g., sp. n. from Vietnamese mullet. Par Int 69:17–24. https://doi.org/10.1016/j.parint.2018.11.001

Besprozvannykh VV, Atopkin DM, Ermolenko AV, Nikitenko AY (2015a) Restoration of the genus *Parasaccocoelium* Zhukov, 1971 (Digenea: Haploporidae) and a description of two new species from mugilid fish in the Far East of Russia. J Helminthol 89:565–576. https://doi.org/10.1017/S0022149X14000443

Besprozvannykh VV, Atopkin DM, Ngo HD, Beloded AY, Ermolenko AV, Ha NV, Tang NV (2015b) *Skrjabinolecithum spasskii* Belous, 1954 (Digenea: Haploporidae), parasite of mullets (Mugilidae) from Primorsky region (Far East of Russia) and Tonkin Bay (North Vietnam): morphology and molecular data. Russ J Mar Biol 41: 267–275. https://doi.org/10.1134/S1063074015040021

Besprozvannykh VV, Atopkin DM, Ermolenko AV Beloded AY (2017a) Morphometric and molecular analyses for a new species *Skrjabinolecithum pyriforme* n. sp. (Digenea: Haploporidae) in mullet fish from the Primorsky Region, Russia. J Helminthol 91:625–632. https://doi.org/10.1017/S0022149X16000626

Besprozvannykh VV, Atopkin DM, Ngo HD, Ha NV, Tang NV, Beloded AY (2017b) Morphometric and molecular analyses of two digenean species from the mullet: *Skrjabinolecithum spinosum* n. sp. from the Russian southern Far East and *Unisaccus tonkini* n. sp. from Vietnam. J Helminthol 92:713–724. https://doi.org/10.1017/S0022149X17000943

Blasco-Costa I, Balbuena JA, Kostadinova AA, Olson PD (2009) Interrelationships of the Haploporinae (Digenea: Haploporidae): a molecular test of the taxonomic framework based on morphology. Parasitol Int 58:263–269. https://doi.org/10.1016/j.parint.2009.03.006

Briscoe AG, Bray RA, Brabec J, Littlewood DT (2016) The mitochondrial genome and ribosomal operon of *Brachycladium goliath* (Digenea: Brachycladiidae) recovered from a stranded minke whale. Parasitol Int 65:271–275. https://doi.org/10.1016/j.parint.2016.02.004

Bykhovskaya-Pavlovskaya IE, Kulakova AP (1987) Class TREMATODA — Trematoda Rudolphi, 1808. In: Bauer ON (ed) Parasitic Metazoa, Part 2. Nauka, Leningrad, pp. 77–198. (In Russian)

Curran SS, Pulis EE, Andres MJ, Overstreet RM (2018) Two new species of *Saccocoelioides* (Digenea: Haploporidae) with phylogenetic analysis of the family, including species of *Saccocoelioides* from North, Middle and south America. J Parasitol 104:221–239. https://doi.org/10.1645/17-189

Darriba D, Taboada GL, Doallo R, Posada D (2012) jModeltest2: more models, new heuristics and parallel computing. Nat Meth 9:772. https://doi.org/10.1038/nmeth.2109

Han Z-Q, Han G, Gao T-X, Wang Z-Y, Shui B-N (2013) Genetic population structure of *Liza haematocheilus* in north-western Pacific detected by amplified fragment length polymorphism markers. J Mar Biol Assoc UK 93:373–379. https://doi.org/10.1017/S0025315412000872

Huelsenbeck JP, Ronquist F, Nielsen R, Bollback JP (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. Science 294:2310–2314. https://doi.org/10.1126/science.1065889

Huston DC, Cutmore SC, Cribb TH (2018) *Isorchis cannoni* n. sp. (Digenea: Atractotrematidae) from great barrier reef rabbitfishes and the molecular elucidation of its life cycle. J Helmointhol 92: 604–611. https://doi.org/10.1017/S0022149X17000906

Jamandre BW, Durand JD, Tzeng WN (2009) Phylogeography of the flathead mullet Mugil cephalus in the north-West Pacific as inferred from the mtDNA control region. J Fish Biol 75:393–407

Kulakova AP, Ky H (1976) *Carassotrema koreanum* Park, 1938 (Trematoda, Waretrematidae) and a new species of this genus from freshwater fishes of North Viet-Nam. Parazitologiya 10:460–462 **(In Russian with English abstract)**

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0. Mol Biol Evol 33:1870–1874. https://doi.org/10.1093/molbev/msw054

Liu SF, Yang TB (2002) *Phanurus oligoovus* gen. et sp. nov. (Digenea: Waretrematidae) from *Liza carinatus* from the South China sea. J Parasitol 88:362–364. https://doi.org/10.1645/0022-3395(2002)088[0362:POGESN]2.0.CO;2

Lu JU (1995) Notes of digenetic trematodes parasitic in fishes near shallow sea in Guangdong province, China-III. Three new species, two new genera and one new subfamily of Digenea. Acta Zootaxon Sin 20:141–152

Luton K, Walker D, Blair D (1992) Comparisons of ribosomal internal transcribed spacers from two congeneric species of flukes (Platyhelminthes: Trematoda: Digenea). Mol Biochem Parasitol 56:323–327. https://doi.org/10.1016/0166-6851(92)90181-I

Manter HW (1957) Host specificity and other host relationships among the digenetic trematodes of marine fishes. In: Proceedings of First Symposium on Host Specificity Among Parasites of Vertebrates, Institut de Zoologie, Universite de Neuchâtel, Neuchatel, Switzerland, pp. 185–198

Moravec F, Sey O (1989) Some trematodes of freshwater fishes from North Vietnam with a list of recorded endohelminths by fish hosts. Folia Parasitol 36:243–262

Overstreet R, Curran S (2005) Family Haploporidae Nicoll, 1914. In: Gibson DI, Jones A, Bray RA (eds) Keys to the Trematoda, vol 2. CAB International, Wallingford, pp 127–166

Park JT (1938) A new fish trematode with single testis from Korea. Keijo J Med 9:290–298

Pulis E, Overstreet R (2013) Review of haploporid (Trematoda) genera with ornate muscularisation in the region of the oral sucker, including four new species and a new genus. Syst Parasitol 84:167–191. https://doi.org/10.1007/s11230-012-9401-8

Pulis E, Fayton T, Curran S, Overstreet R (2013) A new species of *Intromugil* (Digenea: Haploporidae) and redescription of *Intromugil mugilicolus*. J Parasitol 99:501–508. https://doi.org/10.1645/12-106.1

Shimazu T (2005) Digeneans found in fresh- and brackish-water fishes of Lake Ogawara in Aomori Prefecture, Japan. Bull Nat Sci Mus, Tokyo Ser A Zool 31:137–150

Shimazu T (2013) Digeneans parasitic in freshwater fishes (Osteichthyes) of Japan. I. Aporocotylidae, Bivesiculidae and Haploporidae. Bull Nat Sci Mus Tokyo Ser A Zool 39:167–184

Tang Z-Z, Lin H-M (1979) Studies on *Carassotrema* Park, 1938. Life-histories and distribution. Xiamen Daxue Bao 1:81–98. (In Chinese with English abstract)

Tavare S (1986) Some probablistic and statistical problems on the analysis of DNA sequences. Lect Math Life Sci 17:57–86

Tkach VV, Littlewood DTJ, Olson PD, Kinsella JM, Swiderski Z (2003) Molecular phylogenetic analysis of the Microphalloidea Ward, 1901 (Trematoda: Digenea). Syst Parasitol 56:1–15

Truett GE (2006) Preparation of genomic DNA from animal tissues. In: Kieleczawa J (ed) DNA sequencing II: optimizing preparation and cleanup. Jones & Bartlett, Sudbury, MA, pp 33–46

Wang W-J, Pan J-P (1984) Studies on the *Carassotrema* Park, 1938 (Digenea: Haploporidae Nicoll, 1914) in China. In: Institute of Hydrobiology Academia Sinica (ed) Parasitic Organisms of Freshwater Fish of China. Agricultural Publishing House, Beijing, pp 139–148 **(In Chinese)**

Yamaguti S (1942) Studies on the helminth fauna of Japan. Part 39. Trematodes of fishes mainly from Naha. Trans Biogeogr Soc Jap 3:329–398