

Morphometric and molecular analyses of *Skrjabinolecithum pyriforme* n. sp. (Digenea: Haploporidae) in mullet fish from the Primorsky Region, Russia

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Abstract

A new species of *Skrjabinolecithum* (Digenea: Waretrematinae), *Skrjabinolecithum pyriforme* n. sp., has been found in the intestines of *Liza haematocheila* and *Mugil cephalus* from the Primorsky Region, Russia. These worms differ from *S. vitellosum* and *S. loboolecithum* by the presence of two caeca, as do *S. spasskii*, *S. indicum* and *S. bengalensis*. These species differ morphologically from *S. pyriforme* n. sp. by a number of features, including body width, oral sucker, pharynx, eggs and ratio of length and width of the body. The most similar species to *S. pyriforme* n. sp. is *Platydidymus flecterotestis* (Zhukov, 1971) with some differences in maximal body length, testis and egg sizes. The results of molecular analysis confirmed that this new species belongs to *Skrjabinolecithum* on the basis of close relationships with *S. spasskii*-type species.

Introduction

The genus *Skrjabinolecithum* (Waretrematinae, Srivastava, 1937) was first proposed by Belous (1954) based on the type species *S. spasskii* from the intestine of redlip mullet *Liza haematocheila* (Temminck et Schlegel, 1845) (old name: *Mugil soiiuy*) from Razdolnaya River (Suifun), Primorsky Region, Russia. Overstreet & Curran (2005) revised the family Haploporidae and included in *Skrjabinolecithum indicum* (Zhukov, 1972), *S. vitellosum* (Martin, 1973), *S. loboolecithum* (Martin, 1973) and *S. bengalensis* (Madhavi, 1979), which infect marine and estuarine fishes in the eastern hemisphere. Besprozvannykh *et al.* (2015b) published new morphological and molecular data on *S. spasskii* collected from *L. haematocheila* and *Mugil cephalus* Linnaeus, 1758 from the Primorsky Region, Russia, and from Vietnamese *M. cephalus*. A number of diagnostic features for *S. spasskii* were revised, and intraspecific molecular variation of this species

was detected. Here, we provide morphometric and molecular data for a new species of *Skrjabinolecithum* from the intestines of *L. haematocheila* and *M. cephalus* from the Primorsky Region, Russia.

Materials and methods

Collection of trematodes

Trematodes were collected during parasitological investigations of *L. haematocheila* and *M. cephalus* fish in estuaries of the Kievka River (42°52'N, 133°39'E) and the Razdolnaya River (43°20'N, 131°47'E) in the Primorsky Region, Russia. Following removal from the intestine, worms from each fish species were rinsed in distilled water, killed in hot distilled water and preserved in 70% ethanol. Following fixation, flukes were transferred to 96% ethanol and whole mounts were stained in alum carmine, dehydrated in an ethanol series, cleared in xylene and mounted in Canada balsam. All sizes are given in millimetres (mm).

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Table 1. List of taxa used for molecular analysis.

Species	n	Definitive host	Authors	Accession number in the European Nucleotide Archive	
				28S	ITS1–5.8S–ITS2
Haploporoidea					
Waretrematinae					
<i>S. pyriforme</i> n. sp., Kievka River, Primorsky Region		<i>Liza haematocheila</i>	Present study	HE806359–HE806362	LN864990–LN864993
<i>S. spasskii</i> , Razdolnaya River, Primorsky Region	7	<i>Liza haematocheila</i>	Atopkin et al., 2015	HE806371, HE806376, LK022754–LK022755	HG530210, HG530211, LK022759–LK022760
<i>S. spasskii</i> , Cat Ba Island, Vietnam		<i>Mugil cephalus</i>	Atopkin et al., 2015	HG530228–HG530230	HG530207–HG530209
<i>Parasaccocoelium mugili</i> , Primorsky Region*		<i>Liza haematocheila</i>	Besprozvannykh et al., 2015a	HF548473, HF548468	–
<i>Intromugil mugilicolus</i>	1	<i>Mugil cephalus</i>	Pulis & Overstreet, 2013		KC430096
<i>Intromugil alachuaensis</i>	1	<i>Mugil cephalus</i>	Pulis & Overstreet, 2013		KC430095
<i>Spiritestis herveyensis</i>	1	<i>Moolgarda seheli</i>	Pulis et al., 2013		KC206500
<i>Capitimitta costata</i>	1	<i>Selenotoca multifasciata</i>	Pulis et al., 2013		KC206497
<i>Capitimitta darwinensis</i>	1	<i>Selenotoca multifasciata</i>	Pulis et al., 2013		KC206498
Haploporinae					
<i>Saccocoelium brayi</i>	1	<i>Liza saliens</i>	Blasco-Costa et al., 2009	FJ211234	FJ211244
<i>S. cephalii</i>	1	<i>Mugil cephalus</i>	Blasco-Costa et al., 2009	FJ211233	FJ211243
<i>S. obesum</i>	2	<i>Liza ramada</i>	Blasco-Costa et al., 2009	FJ211259	FJ211265
<i>S. tensum</i>	2	<i>Liza ramada</i>	Blasco-Costa et al., 2009	FJ211257	FJ211263
<i>Dicrogaster contracta</i>	2	<i>Liza aurata</i>	Blasco-Costa et al., 2009	FJ211261	FJ211267
<i>D. perpusilla</i>	1	<i>Liza ramada</i>	Blasco-Costa et al., 2009	FJ211238	FJ211248
<i>Lecithobotrys putrescens</i>	1	<i>Liza saliens</i>	Blasco-Costa et al., 2009	FJ211236	FJ211246
<i>Litosaccus brisbanensis</i>	1	<i>Mugil cephalus</i>	Andres et al., 2014		KM253765
Forticulcitinae					
<i>Forticulcita gibsoni</i>	1	<i>Mugil cephalus</i>	Blasco-Costa et al., 2009	FJ211239	FJ211249
<i>F. apiensis</i>		<i>Mugil cephalus</i>	Andres et al., 2015		KP761087
<i>F. platana</i>		<i>Mugil liza</i>	Andres et al., 2015		KP761086
<i>Xiha fastigata</i>		<i>Mugil cephalus</i>	Andres et al., 2015		KP761088
Chalcinotrematinae					
<i>Saccocoelioides</i> sp.*		Unidentified molly (Poecilidae)	Curran et al., 2006	EF032696	–
Paragonimidae					
<i>Paragonimus westermani</i>	1	Unknown	Narain et al., 2006, direct submission	DQ836244	DQ836243

*Nucleotide sequences used only for p-distance calculation.

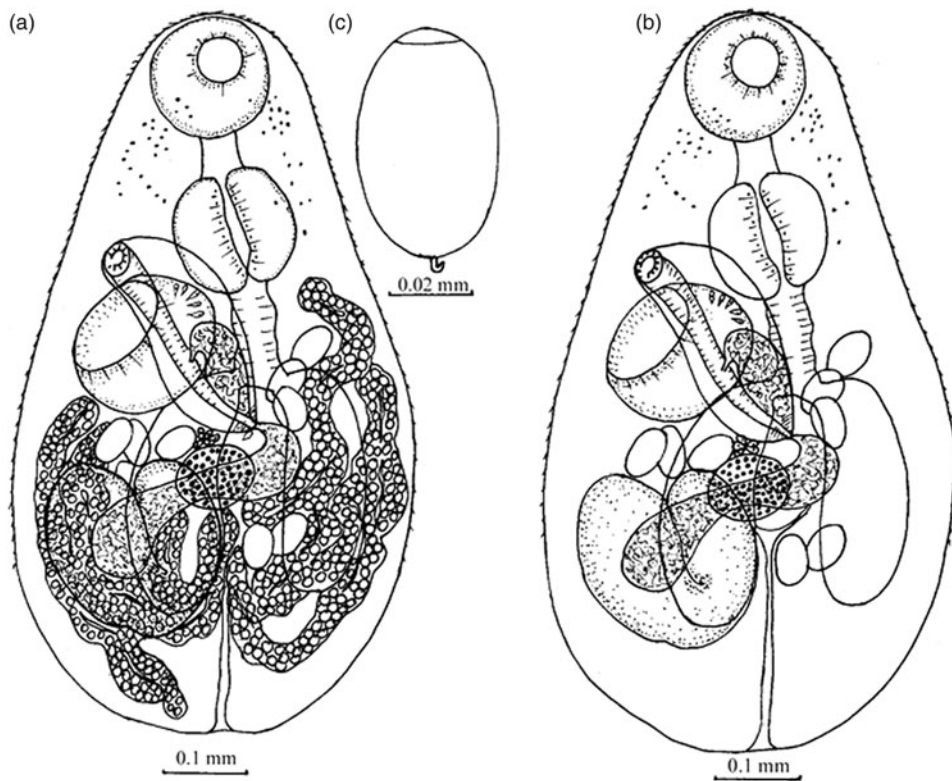


Fig. 1. *Skrjabinolecithum pyriforme* n. sp.: (a) ventral view of holotype, (b) ventral view of holotype without vitellarium, (c) egg.

DNA extraction, amplification and sequencing

Four adult specimens of *S. pyriforme* n. sp. from *L. haematocheila* collected from the Kievka River of the Primorsky Region 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 following primers: DIG12 (5'-AAG CAT ATC ACT AAG CCG-3') and 1500R (5'-GCT ATC CTG AGG GAA ACT TCG-3') (Tkach *et al.*, 2003) at 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 CCG 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, Waltham, Massachusetts, USA), as recommended by the manufacturer, with the internal sequencing primers described by Tkach *et al.* (2003) for 28S rDNA and by Luton *et al.* (1992) for ITS. Products of sequencing were analysed using an ABI 3130 genetic analyser at the Institute of Biology and Soil Sciences, Far Eastern Branch of Russian Academy of Sciences (FEB RAS). Sequences

were submitted to the European Nucleotide Archive (ENA).

Alignments and the 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 the MEGA 6.0 software (Tamura *et al.*, 2013). The values of genetic p-distances were calculated for two ribosomal DNA fragments separately. Phylogenetic relationships were obtained using a concatenated dataset of partial sequences of the 28S rRNA gene and ITS2 rDNA. Phylogenetic analysis was performed using the Bayesian algorithm with the MrBayes v. 3.1.2 software (Huelsenbeck *et al.*, 2001). The best nucleotide substitution model, the general time reversible model (Tavare, 1986) with estimates of invariant sites and gamma-distributed among-site variation (GTR + I + G), was estimated using 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 burn-in of 2,500,000 generations. The significance of the phylogenetic relationships was estimated using posterior probabilities (Huelsenbeck *et al.*, 2001).

Table 2. Morphometric comparison of *Skrjabinolecithum pyriforme* n. sp. with similar species.

Features	<i>Skrjabinolecithum pyriforme</i> n. sp. Holotype	<i>Skrjabinolecithum pyriforme</i> n. sp. Range	<i>Skrjabinolecithum pyriforme</i> n. sp. Mean	<i>Skrjabinolecithum spasskii</i> (Besprozvannykh <i>et al.</i> , 2015)	<i>Skrjabinolecithum indicum</i> (Zhukov, 1972)	<i>Platydidymus flecterotestis</i> (Zhukov, 1971)	<i>Carassotrema flecterotestis</i> (Yiu, Peng & Liu, 2005)
Body length	0.878	0.878–1.090	0.979	0.939–1.510	1.00–1.20	0.95–2.10	0.10–1.70
Body width	0.493	0.493–0.755	0.579	0.292–0.430	0.28–0.37	0.52–0.93	0.49–0.70
Oral sucker length	0.142	0.142–0.158	0.149	0.065–0.131	0.083–0.120	0.13–0.17	0.094–0.160
Oral sucker width	0.142	0.142–0.166	0.149	0.080–0.146	0.110–0.120	0.12–0.23	0.150–0.230
Forebody	0.362	0.270–0.362	0.334	0.270–0.447	–	–	0.334–0.536
Ventral sucker length	0.135	0.135–0.185	0.151	0.104–0.177	0.120–0.150	0.17–0.27	0.148–0.240
Ventral sucker width	0.185	0.166–0.185	0.175	0.108–0.177	0.140–0.170	0.18–0.27	0.176–0.278
Prepharynx length	0.046	0.035–0.046	0.041	0.035–0.058	0.120–0.170	–	0.020–0.05
Pharynx length	0.154	0.154–0.173	0.158	0.039–0.096	0.110–0.140	0.13–0.18	0.120–0.180
Pharynx width	0.146	0.146–0.177	0.155	0.042–0.096	0.080–0.110	0.13–0.21	0.122–0.190
Oesophagus length	0.092	0.092–0.173	0.114	0.050–0.244	–	–	0.272–0.440
Caeca length	0.335	0.308–0.443	–	0.539–0.801	–	0.41–0.66	0.200–0.536
Ovary length	0.081	0.069–0.096	0.085	0.054–0.100	0.062–0.083	0.050–0.092	0.080–0.172
Ovary width	0.096	0.096–0.123	0.108	0.054–0.085	0.062–0.083	0.041–0.104	0.046–0.072
Testis length	0.239	0.192–0.239	0.206	0.112–0.231	0.21–0.30	0.37–0.91	0.750–1.660
Testis width	0.223	0.162–0.223	0.195	0.092–0.239	0.12–0.14	0.066–0.20	0.088–0.184
Hermaphroditic sac length	0.262	0.227–0.277	0.257	0.154–0.285	–	0.35–0.46	0.176–0.384
Hermaphroditic sac width	0.127	0.116–0.142	0.148	0.100–0.177	–	0.14–0.20	0.096–0.192
From posterior end of body to testis	0.119	0.119–0.262	0.204	0.270–0.500	–	–	–
Eggs, length	0.054–0.058	0.054–0.060	–	0.050–0.065	0.071–0.079	0.071–0.083	0.072–0.088
Eggs, width	0.033–0.035	0.033–0.035	–	0.031–0.042	0.039–0.043	0.033–0.048	0.038–0.050
Length/width ratio	56%	48–76%	59.1%	27.8–33.3%	–	–	40.7–54.5%
Forebody/body length ratio	41%	27.4–41%	34.1%	27–30.8%	–	–	27.7–38.2%
Suckers, ratio	1:1.24	1:1.02–1.27	1:1.19	1:1.31–2.03	–	–	1:1.08–1.39

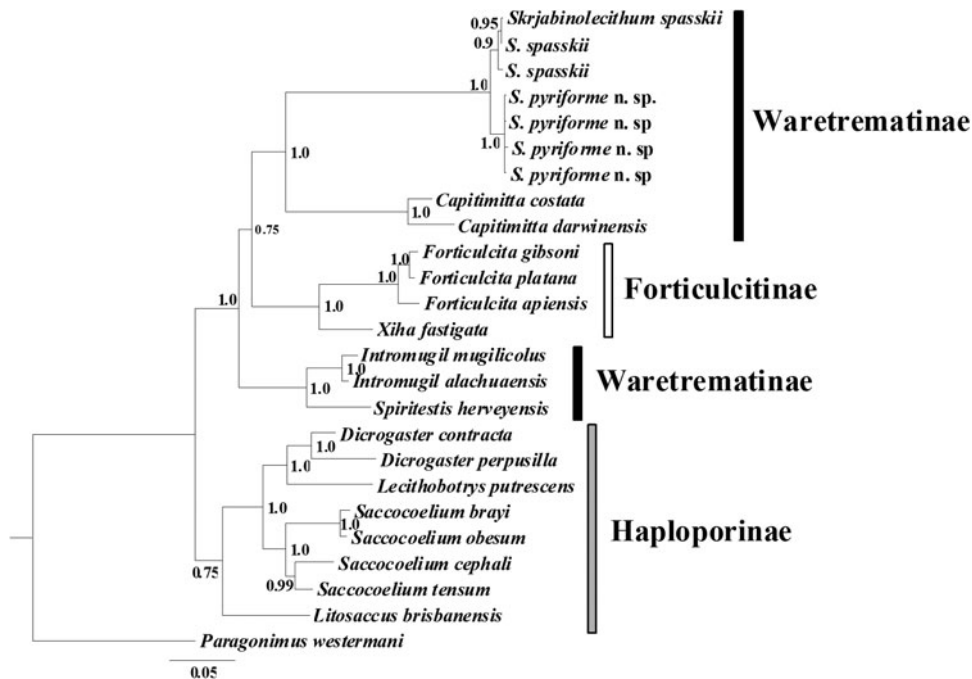


Fig. 2. Phylogenetic relationships of the family Haploporidae obtained with the Bayesian algorithm based on a concatenated nucleotide sequence dataset of ITS2 rDNA and partial 28S rRNA gene. Nodal numbers: posterior probabilities that indicate statistical support of phylogenetic relationships.

Results

Skrjabinolectithum pyriforme n. sp.

Taxonomic summary

Type host. *Liza haematocheila* (Temminck et Schlegel).

Other host. *Mugil cephalus* L.

Type locality. Kievka River (43°52'N, 133°39'E), Primorsky Region (southern Far East, Russia).

Other locality. Razdolnaya River (43°20'N, 131°47'E), Primorsky Region.

Site. Intestine.

Intensity of infection. *Liza haematocheila*: 4–27 worms per fish; *M. cephalus*: 3–6 worms per fish.

Material examined. Fifty specimens. Worms were found in the intestines of 9 of the 27 specimens of *L. haematocheila* and in 2 of the 7 specimens of *M. cephalus*.

Type deposited. Holotype No. Tr-68, paratypes No. Tr-69–73. This material is held at the parasitological collection of the Zoological Museum (Institute of Biology and Soil Science, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia), e-mail: petrova@ibss.dvo.ru. Deposited: 29 July 2010.

Etymology. The name of the species refers to the pyriform shape of the body.

Description

Based on 15 specimens (fig. 1, table 2).

Adult worms. Body pyriform, spined from anterior end to posterior third. Eye-spots in anterior third of body, diffuse. Oral sucker subterminal; prepharynx short; pharynx round, equal to size of oral sucker. Oesophagus longer than prepharynx; caeca wide, reach posterior margin of testis. Ventral sucker equal or slightly larger than oral sucker. Testis single, V-shaped, far posterior to ventral sucker. External seminal vesicle well-developed, may reach level of testis. Hermaphroditic sac sacciform, dorsal to ventral sucker and partly covered by it, with internal seminal vesicle and few prostatic cells. Posterior margin of hermaphroditic sac at level of posterior margin of ventral sucker. Genital pore dextral to median line of body, anterior to ventral sucker, with muscle sphincters at the posterior end of the hermaphroditic sac. Ovary round or irregular form, adjacent to anterior margin of testis. Mehlis' gland composed of large cells. Vitellarium, thread-like, consists of small, round follicles, in close contact with each other. Vitelline fields from ventral sucker to posterior end of body, merge at median line of body and cover ovary, testis and caeca. Uterus short, between hermaphroditic sac and anterior border of testis, with 7–22 eggs. Eggs light-yellow, operculated, with knob, unembryonated. Excretory bladder I-shaped.

Miracidium. The development of miracidia takes place only in water and finishes (at a temperature of 21–22°C) with hatching on days 8–9. Miracidium with eye-spot, span 11 h.

Molecular data

For *S. pyriforme* n. sp., totals of 1243 and 1294 alignable characters were available for analysis in the 28S rRNA gene and ITS1–5.8S–ITS2 rDNA fragment datasets, respectively. Three variable sites were detected within the ITS2 fragment. The 28S rRNA gene fragment was conservative. Alignment of the concatenated nucleotide sequence dataset was trimmed by adding ribosomal DNA sequences for Haploporidae species obtained from GenBank for phylogenetic analysis. Thus, for Bayesian analysis 1525 bp (608-bp and 917 bp fragments of the 28S rRNA gene and ITS1–5.8S–ITS2 rDNA, respectively) were available (fig. 2).

Remarks

Specimens of *S. pyriforme* n. sp. resemble representatives of the type species, *S. spasskii* (in the description of Besprozvannykh *et al.*, 2015b) in a number of morphological characteristics: thread-like vitelline fields, V-shaped testis and unembryonated eggs in the uterus. *Skrjabinolectithum pyriforme* n. sp. differs from *S. vitellosum* and *S. lobolecithum* by the presence of two caeca. *Skrjabinolectithum vitellosum* and *S. lobolecithum* have a single caecum. Besides that, *S. pyriforme* n. sp. differs from *S. spasskii* and *S. indicum* species in body width, sizes of oral sucker, pharynx and eggs, and ratio of length and width of the body (table 2). The new species differs from *S. bengalensis* in having a threefold smaller body size, V-shaped testis and thread-like vitelline (Madhavi, 1979). Specimens of *S. pyriforme* n. sp. were similar in most morphological and metric features to *Platydidymus flecterotestis*, which was first described by Zhukov (1971) in *Leiocassis brashnikovi* (Berg, 1907) and *L. haematocheila* from the Liao River. Overstreet & Curran (2005) noted that the generic name ‘*Haplotrema*’ is *nomen praecoccupatum*, studied a number of specimens of worms collected by Zhukov and included them in a new genus, *Platydidymus*, as type species *P. flecterotestis* (Waretrematinae). Yiu *et al.* (2005), on the basis of a study of the morphology of worms found in *Liza carinatus* from Taiwan, transferred *Haplotrema flecterotestis* to *Carassotrema* as *C. flecterotestis* (Waretrematinae). *Skrjabinolectithum pyriforme* n. sp. is most similar morphologically to the worms presented in the publication of Zhukov (1971). In both cases, these worms have a thread-like vitellarium and V-shaped testis. However, specimens of *S. pyriforme* n. sp. differ from the worms described by Zhukov in having lower maximal sizes of body length, and lower sizes of testis and eggs (table 2). Specimens of *S. pyriforme* n. sp. differ from *C. flecterotestis*, as described by Yiu *et al.* (2005), by the same metric features (table 2). In descriptions of *C. flecterotestis* and *P. flecterotestis*, the vitellarium consists of separate elongated follicles (Overstreet & Curran, 2005; Yiu *et al.*, 2005). Besides that, species of these two genera differ from *S. pyriforme* n. sp. by the presence of miracidia in eggs in the uterus (Overstreet & Curran, 2005). Trematodes identified in mullet fish from the Primorsky Region and described in the present paper, therefore, cannot be attributed to any of the species described previously by Zhukov (1971), Yiu *et al.* (2005) or Overstreet & Curran (2005) on the basis of morphological features.

Sequence differentiation, as estimated through p-distance calculation (tables 3 and 4) of *S. pyriforme* n. sp.

Table 3. Genetic differentiation (below the diagonal, %) and standard error (above the diagonal, %) of species of the family Haploporidae based on nucleotide sequences of ITS2 rDNA.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
<i>Skrjabinolectithum pyriforme</i> n. sp.	0.67	1.96	2.07	1.91	2.06	2.15	2.53	2.45	2.56	2.48	2.34	2.33	2.42	2.48	2.44	2.44	2.49	2.49
<i>S. spasskii</i>	2.14	1.95	1.97	1.89	2.05	2.15	2.49	2.36	2.53	2.48	2.36	2.33	2.5	2.5	2.44	2.44	2.62	2.45
<i>Capitrimitia costata</i>	16.1	16.3	0.93	1.81	1.52	1.61	1.94	2.02	2	1.98	2.08	2.08	1.94	1.94	1.86	1.96	2.12	2.02
<i>C. darwiniensis</i>	16.9	15.6	4.07	1.79	1.57	1.67	2.02	2.07	2.1	2.08	2.07	2.19	2.02	2.01	2	1.99	2.24	2
<i>Spiritesis herveyensis</i>	15.6	15.7	14	13.7	1.41	1.52	1.87	1.96	1.93	2.23	1.97	1.95	1.93	2.05	2	2.03	2.18	1.95
<i>Intronuigil alachuensis</i>	17.3	17.4	10.6	10.8	8.84	0.8	1.71	1.83	1.77	2.2	1.85	1.85	1.64	1.88	1.83	1.86	1.92	1.75
<i>Intronuigil mugiliculus</i>	18.1	18.3	11.6	12.4	9.86	2.9	1.83	1.99	1.9	2.16	1.93	1.91	1.72	1.99	1.89	1.97	1.9	1.78
<i>Forticulcita platana</i>	22.7	22.4	15.3	16.4	13.5	11.9	13.8	0.78	0.47	2.34	2.24	2.41	2.24	2.33	2.25	2.4	2.48	1.62
<i>F. apensis</i>	21.6	20.7	16.2	17.1	15.2	13.9	15.8	3.15	0.88	2.35	2.31	2.47	2.33	2.46	2.3	2.47	2.6	1.64
<i>F. gibsoni</i>	23.3	22.9	16.1	17.3	14.5	13	14.8	1.1	3.83	2.41	2.16	2.36	2.21	2.33	2.25	2.35	2.4	1.64
<i>Litosacus brisbanensis</i>	21.5	21.3	16.4	17.6	18.7	17.9	20.7	20.8	21.6	15	1.98	1.79	1.87	2.09	1.89	2.15	1.93	2.24
<i>Dicrogaster contracta</i>	20.8	20.9	16.9	17	15.6	13.2	14	18.7	19.4	17.6	15	1.12	1.2	1.52	1.29	1.4	1.45	2
<i>D. perpusilla</i>	21.4	21.4	17.9	19	15.9	14.1	14.7	21.2	22.5	20.6	13.4	5.72	1.35	1.64	1.39	1.57	1.49	2.13
<i>Saccocoelium tensum</i>	21.9	22.1	15.8	16.7	15.3	11.6	12.4	19	20.3	18.4	14.2	6.64	8.33	1.39	0.91	1.3	1.46	2.23
<i>S. obesum</i>	22.8	22.8	16.4	17.5	17	13.7	14.8	20.3	22.2	20	16.7	9.6	10.4	1.34	1.34	0.57	1.47	2.38
<i>S. cephalii</i>	22.2	22.3	15	16.9	16.4	13.4	13.7	19.8	20.8	19.5	14.2	7.6	8.12	8.32	1.38	1.38	1.44	2.25
<i>S. brayi</i>	22.5	22.1	16.4	17.2	16.4	13.4	14.5	20.5	21.8	20	17.3	8.83	7.35	1.55	8.33	1.49	1.49	2.32
<i>Lecithobotrys putrescens</i>	23.4	24.3	18.3	19.5	18.9	15	15.4	22.3	23.6	21	14.3	9.33	9.58	10.1	9.34	10.4	10.4	2.21
<i>Xitha fastigata</i>	22.7	22.4	15.3	15.1	14.3	12.3	13	10.9	11.2	11.4	19.5	17.3	18.2	21.1	19.8	20.5	19.8	19.8

Table 4. Genetic differentiation (below the diagonal, %) and standard error (above the diagonal, %) of species of the family Haploporidae based on nucleotide sequences of partial 28S rRNA gene.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1 <i>Skrjabinolectithum pyriforme</i> n. sp.		0.27	1.03	1.05	0.98	0.99	0.97	0.94	1.02	1.01	1.01	1.03	1.04	1.08	0.94	0.98	1	1.01	1.02	1.01	1.03	0.99	0.98	1.06	1.1
2 <i>S. spasskii</i>	0.93		1.06	1.04	1.02	0.99	0.97	0.97	1.02	1.01	1.01	1.05	1.05	1.1	0.93	0.98	1.02	1.02	1.03	1.02	1.05	1.01	0.99	1.09	1.11
3 <i>Capitimitta costata</i>	13.1	13.2		0.55	0.96	1.02	1.05	0.92	1.05	1.06	1.05	1.03	1.03	1.06	1.1	1.07	1	1.03	1.02	1.02	1.03	1.04	0.96	1.1	1.14
4 <i>C. darwinensis</i>	13.7	13.5	3.6		1	1.08	1.12	0.91	1.09	1.1	1.09	1	1.04	1.07	1.09	1.03	1.01	1.05	1.03	1	1.03	1	0.98	1.13	1.13
5 <i>Spiritestis herveyensis</i>	12.3	12.5	13.2	13.4		0.95	0.96	1.02	0.99	0.96	0.96	0.98	0.93	0.96	0.98	0.98	0.85	0.9	0.88	0.9	0.9	0.93	0.89	1.04	0.99
6 <i>Introugil alachuensis</i>	12.4	12.8	11.6	13	11.6		0.55	0.96	0.91	0.92	0.92	0.84	0.84	0.97	0.93	0.97	0.9	0.92	0.86	0.9	0.86	0.84	0.87	0.98	1.05
7 <i>I. mugilicolus</i>	11.8	11.9	12.4	13.7	11.6	3.51		1.01	0.95	0.94	0.95	0.9	0.85	0.98	0.93	0.96	0.88	0.92	0.85	0.91	0.86	0.86	0.89	1	1.02
8 <i>Parasaccoelium mugili</i>	11.1	11.4	11.3	11	11.5	12.6	12.8		1.04	1.05	1.03	0.99	1.02	1.02	1	0.98	1.01	0.99	0.99	0.97	0.98	0.98	0.95	1.06	1.15
9 <i>Forticulcita apiensis</i>	12.1	12.1	12.6	12.8	11.2	10.4	10.5	11.8		0.38	0.37	0.9	0.94	0.99	0.99	0.99	0.89	0.95	1.01	0.91	0.98	0.94	0.73	1.01	1.03
10 <i>F. platana</i>	12.1	12.1	12.2	12.6	10.8	9.93	10	12	1.5		0.21	0.92	0.96	1.01	1	1	0.89	0.96	0.96	0.89	0.95	0.93	0.74	1	1.03
11 <i>F. gibsoni</i>	12	12	12.1	12.5	10.9	10	10.1	11.7	1.4	0.5		0.91	0.96	1.01	1	0.99	0.89	0.95	0.96	0.89	0.94	0.93	0.73	1.01	1.02
12 <i>Saccoceolioides</i> sp.	12	12.3	12	12.1	10.9	8.33	8.53	11.3	9.32	9.42	9.32		0.91	0.98	0.96	0.93	0.9	0.95	0.95	0.93	0.95	0.93	0.86	1.06	1.06
13 <i>Dicrogaster contracta</i>	13.1	13.4	12.4	12.5	11.5	9.53	9.32	12	10.4	10.6	10.5	9.52		0.65	0.76	0.73	0.78	0.63	0.72	0.74	0.69	0.67	0.86	1.04	1.07
14 <i>D. perpusilla</i>	14.3	14.9	14	13.8	12	12.1	11.9	12.3	11.5	12	11.9	10.9	4.5		0.85	0.84	0.86	0.79	0.82	0.83	0.8	0.79	0.9	1.04	1.1
15 <i>Haploporus benedeni</i>	12.9	12.8	14.1	13.7	12.4	10.7	10.7	11.8	10.4	10.7	10.6	11.2	6.41	8.01		0.62	0.86	0.77	0.9	0.87	0.89	0.83	0.88	1.04	1.05
16 <i>Lecithobotrys putrescens</i>	13	13.1	13.5	12.9	11.6	10.8	10.5	11.5	10.8	10.8	10.7	10.5	6.01	7.61	4.4		0.81	0.7	0.85	0.79	0.83	0.77	0.84	1.05	1.06
17 <i>Litosaccus brisbanensis</i>	12.5	12.8	11.5	12.6	10.3	8.65	8.84	12.1	10.1	10	10	8.24	7.43	8.94	8.23	8.33		0.73	0.82	0.76	0.8	0.7	0.81	0.96	1.06
18 <i>Ragaia lizae</i>	13.3	13.6	13.3	13.3	10.9	9.73	9.72	12.2	10.8	11	10.9	9.82	4.9	6.81	6.71	6.01	6.63		0.72	0.72	0.69	0.71	0.83	1.01	1.01
19 <i>Saccoceolium brayi</i>	13.2	13.6	13	13.1	11.2	9.73	9.72	11.8	11.2	11.1	11	10.3	6.41	8.31	8.61	7.51	8.13	6.41		0.68	0.31	0.65	0.84	0.99	1.02
20 <i>S. cephalii</i>	13.2	13.6	12.9	12.6	11	10	10.3	11.9	10.4	10.6	10.5	9.82	6.31	7.91	8.21	7.01	7.03	6.21	4.8		0.67	0.46	0.81	0.97	1.06
21 <i>S. obesum</i>	13.1	13.5	12.8	12.9	11	9.73	9.72	11.6	10.8	10.7	10.6	10	6.01	7.91	8.21	7.21	7.43	5.81	0.9	4.6		0.62	0.84	0.97	1.03
22 <i>S. tensum</i>	12.9	13.3	12.4	12	10.7	9.33	9.52	11.4	10.4	10.6	10.5	9.42	5.41	7.21	7.41	6.61	6.33	5.61	4.1	2.4	3.7		0.81	0.94	1.07
23 <i>Xiha fastigata</i>	11.5	11.7	11.3	11.6	9.35	8.84	9.04	11.1	5.82	5.92	5.82	7.63	9.33	10.2	9.53	9.53	8.55	9.13	9.23	8.63	8.93	8.53		0.94	0.98
24 <i>Pseudomegasolena ishigakiense</i>	15.1	15.6	15.4	15.7	14.6	13.7	13.8	14.3	13.4	13.1	13.2	13.6	13.9	14.7	13.9	14.2	12.7	13.3	13.8	13.2	13.3	12.6	12.3		1.03
25 <i>Hapladena nasonis</i>	16	16.3	16.9	16.7	14.6	13.7	14.2	14.3	13.8	13.8	13.7	13.4	13.4	14.8	13.1	13.9	13.4	13.6	12.7	13.1	12.5	12.7	12.3	13.9	

Skrjabinolectithum pyriforme n. sp. in mullet fish from Russia

and *S. spasskii*, was $0.93 \pm 0.27\%$ and $2.14 \pm 0.67\%$ according to 28S rDNA and ITS rDNA sequence data, respectively. These values correspond with the interspecific level of genetic differentiation estimated by ribosomal DNA fragments, obtained earlier for other representatives of Haploporidae (Blasco-Costa *et al.*, 2009; Pulis *et al.*, 2013). For example, genetic differentiation within *Saccocoelium* ranged from $0.9 \pm 0.31\%$ to $4.8 \pm 0.68\%$ by 28S rDNA and from $1.55 \pm 0.57\%$ to $8.33 \pm 1.39\%$ by ITS rDNA sequence data, respectively (tables 3 and 4). Within Waretrematinae, genetic differentiation of *S. pyriforme* n. sp. ranged from $11.1 \pm 0.94\%$ (*Parasaccocoelium mugili*) to $13.7 \pm 1.05\%$ (*Capitimitta darwinensis*) by 28S rDNA, and from $15.6 \pm 1.91\%$ (*Spiritestis herveyensis*) to $18.1 \pm 2.15\%$ (*Intrromugil mugilicolus*) by ITS rDNA sequence data. The same values of genetic differentiation were obtained after comparative analysis of *S. spasskii* relative to other Waretrematinae species (tables 3 and 4).

Phylogenetic tree topology (fig. 2) showed close relationships of *S. pyriforme* n. sp. to *S. spasskii*. These species formed a clade closely related to the genus *Capitimitta* of the subfamily Waretrematinae. However, *I. mugilicolus*, *I. alachuaensis* and *S. herveyensis*, which are present in the subfamily Waretrematinae (Pulis *et al.*, 2013) are highly differentiated from the [*Skrjabinoecithum*/*Capitimitta*] clade, suggesting polyphyly of the subfamily Waretrematinae.

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Conflict of interest

None.

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