

Research Paper

Cite this article: Besprozvannykh VV, Atopkin DM, Ngo HD, Ha NV, Tang NV, Beloded AYU (2018). 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. *Journal of Helminthology* **92**, 713–724. <https://doi.org/10.1017/S0022149X17000943>

Received: 2 June 2017

Accepted: 6 September 2017

First published online: 17 October 2017

Author for correspondence:

D.M. Atopkin, Fax: +7 4232310193

E-mail: atop82@gmail.com

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

V.V. Besprozvannykh¹, D.M. Atopkin^{1,2}, H.D. Ngo³, N.V. Ha³, N.V. Tang³ and A.Yu. Beloded¹

¹Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of the Russian Academy of Sciences; ²Department of Cell Biology and Genetics, Far Eastern Federal University, Vladivostok, Russia and ³Institute of Ecology and Biodiversity, Vietnamese Academy of Sciences and Technology, Hanoi, Vietnam

Abstract

Adults of *Skrjabinolecithum spinosum* n. sp. were discovered in *Mugil cephalus* from the Gulf of Peter the Great in southern Far-East Russia. Additionally, adults of *Unisaccus tonkini* n. sp. were found in the intestine of *Moolgarda cunnesius* and *Moolgarda seheli* from the coastal waters of Cat Ba Island, Tonkin Bay, northern Vietnam. *Skrjabinolecithum spinosum* n. sp. possesses a larger body, and ventral and oral sucker size in comparison with *Skrjabinolecithum vitellosum*, a smaller pharynx size and body length/width rate ratio in comparison to *Skrjabinolecithum pyriforme*, a smaller body length and prepharynx size in comparison to *Skrjabinolecithum lobolectum* and a smaller pharynx length and egg size in comparison to *Skrjabinolecithum indicum* and *S. lobolectum*. The new species also differs from *S. indicum*, *S. lobolectum* and *S. vitellosum* by the form of the testis, and from the last two species by the presence of a two-branched intestine. The morphometric parameters of *S. spinosum* n. sp. are similar to those of *Skrjabinolecithum spasskii*. However, *S. spinosum* n. sp., unlike *S. spasskii*, has an armed hermaphroditic duct. *Unisaccus tonkini* n. sp. is similar to *Unisaccus spinosus* (Martin, 1973), *Unisaccus brisbanensis* (Martin, 1973) and *Unisaccus overstreeti* (Ahmad, 1987) in body size but differs in oral sucker, pharynx and hermaphroditic sac size from *U. spinosus*, and in ventral sucker and ovary size from *U. brisbanensis* and *U. overstreeti*. Bayesian phylogenetic analysis, based on combined data of internal transcribed spacer 2 (ITS2) and partial 28S rRNA gene sequences, confirmed the validity of *S. spinosum* n. sp. and *U. tonkini* n. sp. Analysis of interrelationships of the family Haploporidae, including molecular data on new species, showed that the Waretrematinae subfamily is more heterogeneous in comparison with Haploporinae and Forticulcitinae, and includes *U. tonkini* n. sp.

Introduction

At the present time, six species are recognized within the genus *Skrjabinolecithum* Belous, 1954 (Waretrematinae, Srivastava, 1937): *Skrjabinolecithum indicum* (Zhukov, 1972), *Skrjabinolecithum bengalensis* (Madhavi, 1979), *Skrjabinolecithum vitellosum* (Martin, 1973), *Skrjabinolecithum lobolectum* (Martin, 1973), *Skrjabinolecithum spasskii* (Belous, 1954) and *Skrjabinolecithum pyriforme* (Besprozvannykh *et al.*, 2016). *Skrjabinolecithum indicum* and *S. bengalensis* were detected in cichlid fish from the Arabian Sea, and mugilid fish from the Bay of Bengal, respectively (Zhukov, 1972; Madhavi, 1979). Other species were detected in mullet fish from the western coastal waters of the Pacific Ocean: *S. vitellosum* and *S. lobolectum* from Queensland, Australia, *S. spasskii* and *S. pyriforme* from southern Russian Far East and northern coastal waters of Vietnam (Belous, 1954; Martin, 1973a; Besprozvannykh *et al.*, 2015, 2017). The validity of the species *S. spasskii* and *S. pyriforme* have been confirmed by morphological and molecular analyses. Other species of the genus *Skrjabinolecithum* have been validated with morphological data only. The representatives of *Unissacus* (Haploporinae, Nicol, 1973) are known from Australian mullet fish: *Unissacus sprengi* (Martin, 1973), *Unissacus spinosus* (Martin, 1973) and *Unissacus brisbanensis* (Martin, 1973); and Indian mullet: *Unissacus mugilis* (Rekharani & Madhavi, 1985), *Unissacus overstreeti* (Ahmad, 1987) and *Unissacus martini* Ahmad, 1986 (Martin, 1973b, c; Rekharani & Madhavi, 1985; Ahmad 1986, 1987; Blasco-Costa *et al.*, 2009). Identification of these species was based on general morphology. In the present study, morphometric and molecular data are presented of two digeneans: *Skrjabinolecithum spinosum* n. sp. from *Mugil cephalus*

(Linnaeus, 1758) of the southern Russian Far East and *Unisaccus tonkini* n. sp. from *Moolgarda cunnesius* (Valenciennes, 1836) and *Moolgarda seheli* (Forsskål) of Vietnam.

Materials and methods

Collection of trematodes

Adult *Skrjabinolecithum* were found in the intestine of *M. cephalus* from estuaries of the Kievka River (42°52'N, 133°39'E) in the Primorsky Region, Russia. Specimens of adult *Unisaccus* were found in the intestine of *M. cunnesius* and *M. seheli* from coastal waters of Cat Ba Island, northern Vietnam (20°84'N, 106°59'E). Worms were rinsed in distilled water for a very short time, killed in hot distilled water and preserved in 70% ethanol for morphological study with light microscopy. Whole-mounts for adult descriptions were made by staining the specimens with aluminium carmine, dehydrating the worms in a graded ethanol series and clearing in xylene, followed by mounting in Canada balsam under a coverslip on a slide. All measurements are given in millimetres (mm).

DNA extraction, amplification and sequencing

Adult worms of both *S. spinosum* n. sp. and *U. tonkini* n. sp. were fixed in 96% ethanol. Three adult specimens of *S. spinosum* n. sp. and five specimens of *U. tonkini* n. sp. 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, Waltham, Massachusetts, USA), as recommended by the manufacturer, with the internal sequencing primers described by Tkach *et al.* (2003) for 28S rDNA and Luton *et al.* (1992) for internal transcribed spacers (ITS). PCR product sequences were analysed using an ABI 3130 genetic analyser (Applied Biosystems) at the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences. Sequences were submitted to the GenBank database (National Center for Biotechnology Information; 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 6.0 software (Tamura *et al.*, 2013). The values of genetic p-distances were calculated for the 28S ribosomal DNA fragment. 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 (Tavare, 1986) with estimates of invariant sites and gamma-distributed among-site variation (GTR + I + G) were estimated with jModeltest v. 2.1.5 software (Darriba *et al.*, 2012). Bayesian analysis was performed using 10,000,000 generations with two independent runs. Summary parameters and the phylogenetic tree were calculated with a burn-in of 3,000,000 generations. The significance of the phylogenetic relationships was estimated using posterior probabilities (Huelsenbeck *et al.*, 2001). Combined molecular data for phylogenetic reconstructions contained only ITS2 rDNA sequences, which allowed us to use the maximal amount of species. *Paragonimus westermani* was used as outgroup, authors of these data and accession numbers are given in table 1.

Results

Skrjabinolecithum spinosum n. sp.

Taxonomic summary

Host. *Mugil cephalus* L.

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

Site. Intestine.

Type deposition. Type No. 84-Tr, paratype No. 85-93-Tr. This material is held in the collection of the Zoological Museum (Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia); e-mail: petrova@ibss.dvo.ru. Deposited 25 October 2016.

Etymology. The specific name refers to the spines on the hermaphroditic duct.

Material examined. Ten specimens.

Description

Adult worm. Shown in fig. 1 and described in table 2. Body elongate, spined from the anterior end to back third. Eye-spot diffuse, placed at the anterior third of the body. Oral sucker sub-terminal. Prepharynx short, pharynx transversely oval. Oesophagus longer in comparison with prepharynx. Caeca are wide, reach posterior margin of the testis. Ventral sucker size equal to the oral sucker, placed on the border of the anterior and middle third of the body. Testis single, V-shaped, usually at the end of the middle third of the body. External seminal vesicle is sac-shaped or another form, depending on the quality of sperm stored. Most specimens possess a vesicle that reaches the level of the ovary or anterior margin of the testis. Hermaphroditic sac oval or sac-shaped, located dorsally from the ventral sucker. Posterior end of hermaphroditic sac crosses the border of the posterior margin of the ventral sucker. Internal seminal vesicle size is smaller than the external seminal vesicle. Internal seminal vesicle sizes depend on the quality of sperm stored. Internal seminal vesicle duct is placed in the posterior third of the hermaphroditic duct. Prostatic cells are located around the middle part of the hermaphroditic duct. The hermaphroditic duct is muscular with six spirally arranged rows of pads, with 6–8 pads per row. Each pad has two spines on a reticular sclerotized base. Distal part of the hermaphroditic duct is eversible. Genital opening is on the midline of the body immediately before the ventral sucker. Metraterm is short and thin-walled. Vitellarium extends from the middle level of the ventral sucker or middle part of the body length up to the posterior end of the body, has the form

Table 1. List of taxa used for molecular analysis.

| Species | n | Definitive host | Authors | Accession number in the NCBI | |
|---|---|----------------------------------|--|------------------------------|-------------------|
| | | | | 28S | ITS1–5.8S–ITS2 |
| Waretrematinae | | | | | |
| <i>Unisaccus tonkini</i> n. sp. | 5 | <i>Moolgarda cunnesius</i> | Present study | MF176840–MF176844 | MF176835–MF176839 |
| <i>Skrjabinolecithum spinosum</i> n. sp. | 3 | <i>Mugil cephalus</i> | Present study | MF176829–MF176831 | MF176832–MF176834 |
| <i>S. pyriforme</i> , Kievka River, Primorsky Region | 1 | <i>Liza haematocheila</i> | Besprozvannykh <i>et al.</i> , 2017 | HE806359 | LN864990 |
| <i>S. spasskii</i> , Razdolnaya River, Primorsky Region | 7 | <i>Liza haematocheila</i> | Atopkin <i>et al.</i> , 2015 | LN614538 | LK022754 |
| <i>Parasaccocoelium mugili</i> , Primorsky Region | 1 | <i>Liza haematocheila</i> | Besprozvannykh <i>et al.</i> , 2015 | HF548468 | – |
| <i>P. haematochelium</i> | 1 | <i>Liza haematocheila</i> | Besprozvannykh <i>et al.</i> , 2015 | HF548462 | – |
| <i>P. polyovum</i> | 1 | <i>Liza haematocheila</i> | Besprozvannykh <i>et al.</i> , 2015 | HF548474 | – |
| <i>Intromugil mugilicolus</i> | 1 | <i>Mugil cephalus</i> | Pulis <i>et al.</i> , 2013 | | KC430096 |
| <i>Intromugil alachuaensis</i> | 1 | <i>Mugil cephalus</i> | Pulis <i>et al.</i> , 2013 | | KC430095 |
| <i>Spiritestis herveyensis</i> | 1 | <i>Moolgarda seheli</i> | Pulis <i>et al.</i> , 2013 | | KC206500 |
| <i>Capitimitta costata</i> | 1 | <i>Selenotoca multifasciata</i> | Pulis <i>et al.</i> , 2013 | | KC206497 |
| <i>Capitimitta darwinensis</i> | 1 | <i>Selenotoca multifasciata</i> | Pulis <i>et al.</i> , 2013 | | KC206498 |
| Haploporinae | | | | | |
| <i>Saccocoelium brayi</i> | 1 | <i>Liza saliens</i> | Blasco-Costa <i>et al.</i> , 2009 | FJ211234 | FJ211244 |
| <i>S. cephalii</i> | 1 | <i>Mugil cephalus</i> | Blasco-Costa <i>et al.</i> , 2009 | FJ211233 | FJ211243 |
| <i>S. obesum</i> | 2 | <i>Liza ramada</i> | Blasco-Costa <i>et al.</i> , 2009 | FJ211259 | – |
| <i>S. tensus</i> | 2 | <i>Liza ramada</i> | Blasco-Costa <i>et al.</i> , 2009 | FJ211257 | FJ211263 |
| <i>Dicrogaster contracta</i> | 2 | <i>Liza aurata</i> | Blasco-Costa <i>et al.</i> , 2009 | FJ211261 | FJ211267 |
| <i>D. perpusilla</i> | 1 | <i>Liza ramada</i> | Blasco-Costa <i>et al.</i> , 2009 | FJ211238 | FJ211248 |
| <i>Lecithobotrys putrescens</i> | 1 | <i>Liza saliens</i> | Blasco-Costa <i>et al.</i> , 2009 | FJ211236 | FJ211246 |
| <i>Litosaccus brisbanensis</i> | 1 | <i>Mugil cephalus</i> | Andres <i>et al.</i> , 2014 | | KM253765 |
| <i>Haploporus benedeni</i> | 1 | <i>Liza ramada</i> | Blasco-Costa <i>et al.</i> , 2009 | FJ211237 | FJ211247 |
| <i>Ragaia lizae</i> | 1 | <i>Liza aurata</i> | Blasco-Costa <i>et al.</i> , 2009 | FJ211235 | FJ211245 |
| Forticulcitinae | | | | | |
| <i>Forticulcita gibsoni</i> | 1 | <i>Mugil cephalus</i> | Blasco-Costa <i>et al.</i> , 2009 | FJ211239 | FJ211249 |
| <i>F. apiensis</i> | 1 | <i>Mugil cephalus</i> | Andres <i>et al.</i> , 2015 | | KP761087 |
| <i>F. platana</i> | 1 | <i>Mugil liza</i> | Andres <i>et al.</i> , 2015 | | KP761086 |
| <i>Xiha fastigata</i> | 1 | <i>Mugil cephalus</i> | Andres <i>et al.</i> , 2015 | | KP761088 |
| Chalcinotrematinae | | | | | |
| <i>Saccocoelioides</i> sp. | 1 | Unidentified molly (Poeciliidae) | Curran <i>et al.</i> , 2006 | EF032696 | – |
| Megasoleninae | | | | | |
| <i>Hapladena nasonis</i> | 1 | <i>Naso unicornis</i> | Olson <i>et al.</i> , 2003 | AY222265 | – |
| Atractotrematidae | | | | | |
| <i>Pseudomegasolena ishigakiense</i> | 1 | <i>Scarus rivulatus</i> | Olson <i>et al.</i> , 2003 | AY222266 | – |
| Paragonimidae | | | | | |
| <i>Paragonimus westermani</i> | 1 | Unknown | Narain <i>et al.</i> , 2006, direct submission | DQ836244 | DQ836243 |

n, number of sequences.

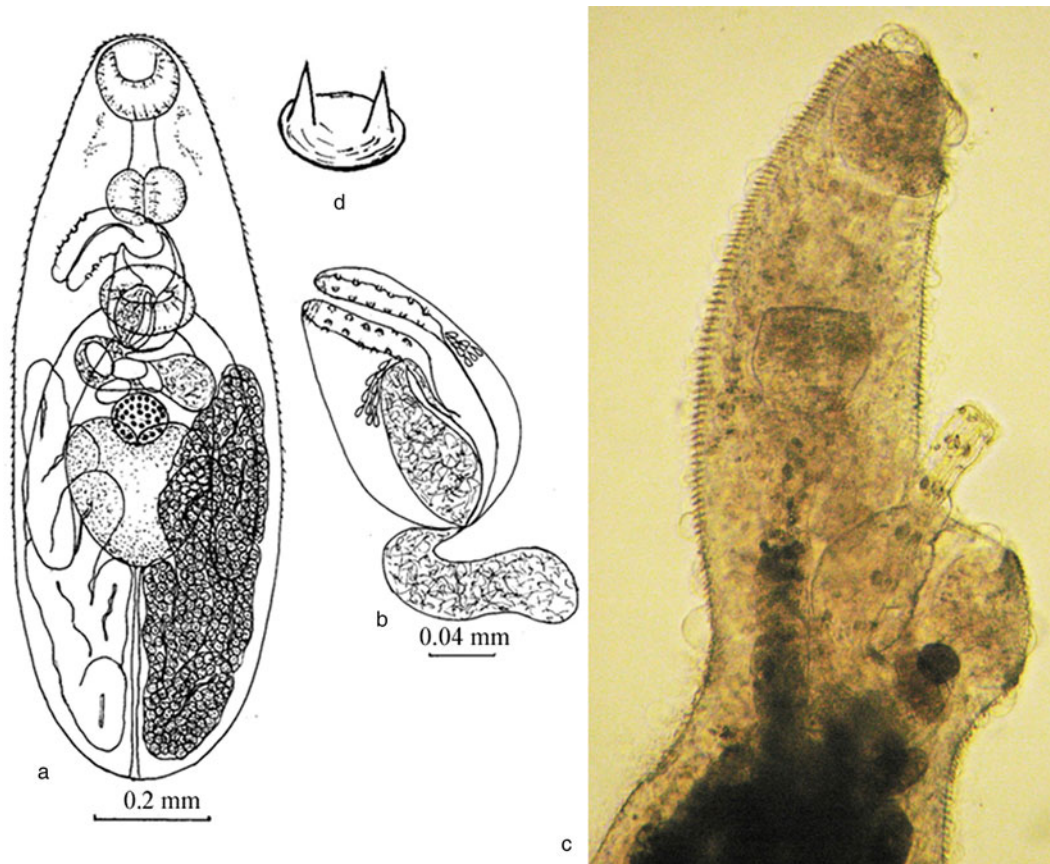


Fig. 1. *Skrjabinolecithum spinosum* n. sp.: (a) adult worm, (b) hermaphroditic sac, (c) hermaphroditic duct with pads, (d) spines on a reticular sclerotized base.

of a tape and consists of small, round follicles closely adjacent to each other. Both lateral fields are connected on the midline of the body and cover the ovary, testis and caeca. Ovary round or transversely oval, adjacent to anterior margin of the testis left from the midline of the body. Uterus is short, placed between the hermaphroditic sac and anterior margin of the testis, and contains unembryonated eggs. Eggs are white–yellow, operculated. Excretory bladder is I-shaped.

Molecular data

For *S. spinosum* n. sp., there were 1311 and 1184 alignable characters available for analysis in the 28S rRNA gene and ITS1–5.8S–ITS2 rDNA fragment datasets, respectively. Intraspecific variation of ribosomal DNA fragments of *S. spinosum* n. sp. extremely low. The 28S rRNA gene fragment was conservative, and only one variable site was detected for the ITS1–5.8S–ITS2 rDNA fragment. The sequences were submitted to the NCBI database with accession numbers MF176829–MF176834.

Unissacus tonkini n. sp.

Taxonomic summary

Type host. *Moolgarda cunnesius*.

Other host. *Moolgarda seheli*.

Type-locality. Coastal water of Cat Ba Island, Tonkin Bay, northern Vietnam (20°84'N, 106°59'E).

Site. Intestine.

Type deposition. Type No. 94-Tr, paratype No. 95-103-Tr. This material is held in the collection of the Zoological Museum (Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia); e-mail: petrova@ibss.dvo.ru. Deposited 29 July 2015.

Etymology. The specific name refers to Tonkin Bay.

Material investigated. Ten flukes.

Description

Adult worm. Shown in [fig. 2](#) and described in [table 3](#). Body is saccular, with the cuticle spinous near to the posterior end. Eye-spot pigment is dispersed at the forebody. Oral sucker is sub-terminal. Prepharynx is long, extending to level with the genital pore. Pharynx is transversely oval, oesophagus short, caeca single, saccular, situated equatorially. Acetabulum is pre-equatorial. A single testis is transversely oval, in the posterior one-third of the body. External seminal vesicle is saccular. Hermaphroditic sac is saccular, dorsal to ventral sucker. Posterior end of the hermaphroditic sac does not cross the posterior margin of the ventral sucker. Internal seminal vesicle is oval, and its size depends on the fullness of the sexual products. Prostatic cells are few. Hermaphroditic duct is with pads, each pad with two spines on a reticular sclerotized base. Genital pore is on the midline of the body, immediately before the ventral sucker. Ovary is spherical, adjacent to testis at the midline of the body. Receptaculum seminis was not observed. Uterus is from near the posterior end of the body to the acetabulum. Metraterm is short with muscular walls. Vitellarium in two distinct lateral groups of follicles, at the level of the ovary/testis.

Table 2. Measurements (mm) of adult worms of *Skrjabinolecithum*.

| | <i>S. spinosum</i> n. sp. | | | <i>S. spasskii</i> (Besprozvannykh et al., 2015)** | <i>S. indicum</i> (Zhukov, 1972) | <i>S. lobolectum</i> (Martin, 1973a) | <i>S. vitellosum</i> (Martin, 1973b) | <i>S. pyriforme</i> (Besprozvannykh et al., 2017) |
|-------------------------------------|---------------------------|-------------|--------|--|-------------------------------------|---|---|---|
| | Holotype | Range | Mean | | | | | |
| Body length | 1.294 | 0.801–1.294 | 1.019 | 0.939–1.510 | 1.00–1.20 | 1.85, 2.07 | 0.510–0.790 | 0.878–1.090 |
| Body width | 0.462 | 0.323–0.462 | 0.370 | 0.292–0.430 | 0.28–0.37 | 0.266, 0.406 | 0.140–0.300 | 0.493–0.755 |
| Body length/ width (%) | 35.7 | 26.6–40.0 | 33.7 | 27.8–33.3* | – | – | – | 48–76 |
| Forebody length | 0.358 | 0.293–0.408 | 0.343 | 0.270–0.447 | – | – | – | 0.270–0.362 |
| Body/forebody length ratio (%) | 27.7 | 26.6–40.0 | 33.7 | 27–30.8* | – | – | – | 27.4–41 |
| Oral sucker length | 0.139 | 0.100–0.139 | 0.119 | 0.065–0.131 | 0.083–0.120 | 0.096, 0.112 | 0.050–0.080 | 0.142–0.158 |
| Oral sucker width | 0.146 | 0.119–0.146 | 0.130 | 0.080–0.146 | 0.110–0.120 | 0.100, 0.112 | 0.059–0.090 | 0.142–0.166 |
| Ventral sucker length | 0.127 | 0.104–0.135 | 0.119 | 0.104–0.177 | 0.120–0.150 | 0.093, 0.143 | 0.056–0.074 | 0.135–0.185 |
| Ventral sucker width | 0.162 | 0.116–0.162 | 0.134 | 0.108–0.177 | 0.140–0.170 | 0.078, 0.156 | 0.056–0.074 | 0.166–0.185 |
| Ventral/oral sucker length ratio | 1:0.91 | 1:0.91–1.16 | 1:1.0 | 1:1.13–1.64 | – | – | – | 1:0.90–1.17* |
| Ventral/oral sucker width ratio | 1:1.11 | 1:0.94–1.13 | 1:1.03 | 1:1.03–1.33 | – | – | – | 1:1.02–1.41* |
| Prepharynx | 0.077 | 0.019–0.131 | 0.071 | 0.035–0.058 | 0.120–0.170 | 0.426, 0.684 | 0.090–0.109 | 0.035–0.046 |
| Pharynx length | 0.100 | 0.077–0.100 | 0.089 | 0.039–0.096 | 0.110–0.140 | 0.109, 0.112 | 0.040–0.080 | 0.154–0.173 |
| Pharynx width | 0.135 | 0.085–0.135 | 0.109 | 0.042–0.096 | 0.080–0.110 | 0.131, 0.137 | 0.059–0.110 | 0.146–0.177 |
| Oesophagus length | 0.131 | 0.123–0.158 | 0.139 | 0.050–0.244 | – | 0.112, 0.249 | 0.060 | 0.092–0.173 |
| Ovary length | 0.096 | 0.069–0.116 | 0.091 | 0.054–0.100 | 0.062–0.083 | 0.109, 0.140 | 0.031–0.062 | 0.069–0.096 |
| Ovary width | 0.096 | 0.081–0.123 | 0.095 | 0.054–0.085 | 0.062–0.083 | 0.100, 0.109 | 0.031–0.062 | 0.096–0.123 |
| Testis length | 0.258 | 0.173–0.262 | 0.221 | 0.112–0.231 | 0.21–0.30 | 0.239, 0.345 | 0.075–0.165 | 0.192–0.239 |
| Testis width | 0.254 | 0.119–0.254 | 0.164 | 0.092–0.239 | 0.12–0.14 | 0.168, 0.202 | 0.050–0.090 | 0.162–0.223 |
| Hermaphroditic sac length | 0.231 | 0.162–0.262 | 0.217 | 0.154–0.285 | – | 0.258, 0.286 | 0.090 | 0.227–0.277 |
| Hermaphroditic sac width | 0.135 | 0.081–0.135 | 0.099 | 0.100–0.177 | – | 0.096, 0.118 | 0.050 | 0.116–0.142 |
| Posterior end of testis | 0.366 | 0.154–0.366 | 0.258 | 0.270–0.500 | – | – | – | 0.119–262 |
| Eggs, length | 0.058–0.065 | 0.058–0.065 | – | 0.050–0.065 | 0.071–0.079 | 0.071–0.093 | 0.059–0.065 | 0.054–0.060 |
| Eggs, width | 0.035–0.039 | 0.035–0.039 | – | 0.031–0.042 | 0.039–0.043 | 0.056–0.059 | 0.042 | 0.033–0.035 |

*From type location, ** additional data to earlier studies (Besprozvannykh et al., 2015, 2017).

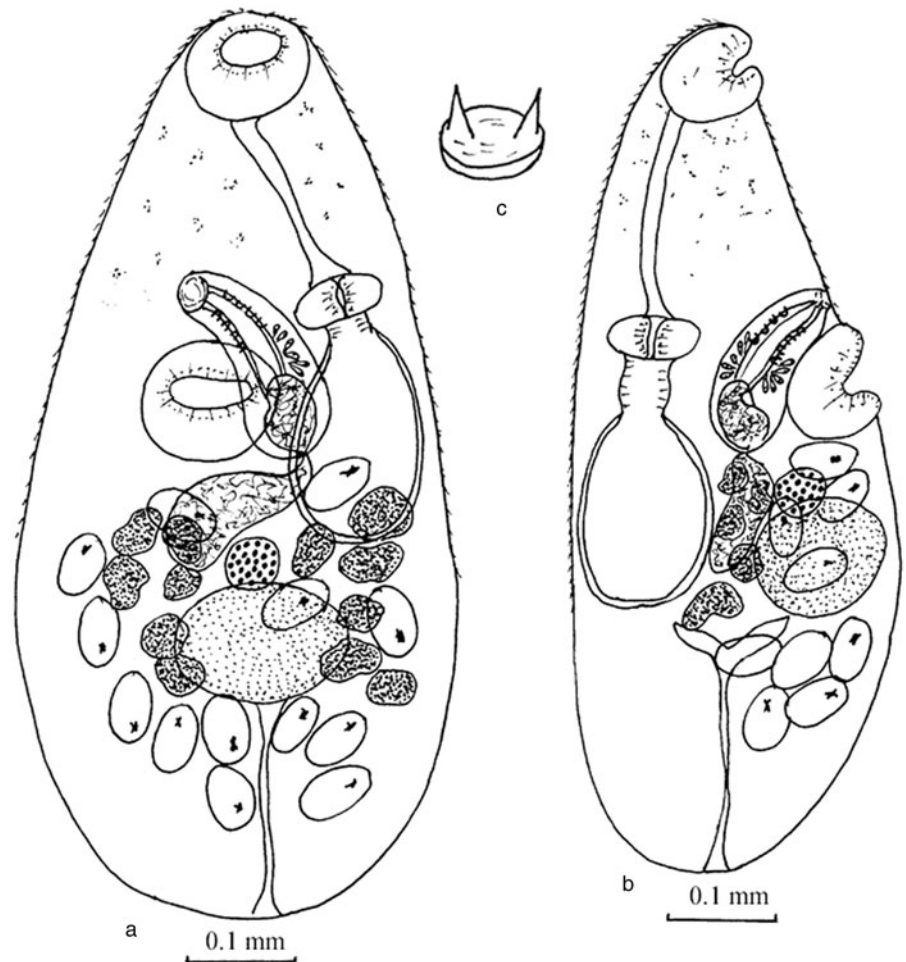


Fig. 2. Adult worm *Unissacus tonkini* n. sp.: (a) ventrally, (b) laterally, (c) spines on a reticular sclerotized base.

Eggs are large, operculate, embryonated, miracidia with two fused eye-spots. Excretory vesicle is Y-shaped.

Molecular data

For *U. tonkini* n. sp. there were 1007 and 1287 alignable characters available for analysis in the 28S rRNA gene and ITS1–5.8S–ITS2 rDNA fragment datasets, respectively. Within *U. tonkini* n. sp., four variable sites were detected for the 28S rRNA gene fragment and four variable sites were detected for the ITS1–5.8S–ITS2 rDNA fragment. The sequences were submitted to the NCBI database with accession numbers MF176835–MF176844.

Remarks

Morphological characteristics, including vitellaria, testes and organ rearrangement, indicate that the investigated trematodes from Russian mullet belong to the genus *Skrjabinolectithum* (Overstreet & Curran, 2005, Besprozvannykh *et al.*, 2015). In terms of metric parameters, specimens of *S. spinosum* n. sp. collected from *M. cephalus* in southern Far-East Russia have a larger body size, ventral and oral suckers size in comparison with *S. vitellosum*; a smaller pharynx size and body length/width ratio in comparison with *S. pyriforme*; smaller body length and prepharynx length in comparison with *S. lobolectitum*; and smaller pharynx length and egg size in comparison with *S. indicum* and *S. lobolectitum* (table 2). According to testis localization and form, specimens of *S. spinosum* n. sp. differ from *S. indicum*,

S. vitellosum and *S. lobolectitum*. The testis has a V-shaped localization in the middle part of the body of *S. spinosum* n. sp. and is spherical to elongate at the posterior end of the body of *S. indicum*, *S. vitellosum* and *S. lobolectitum*. Moreover, *S. spinosum* n. sp. possesses a two-branched intestine, whereas *S. vitellosum*, and *S. lobolectitum* are characterized by the presence of one intestinal branch. In terms of metric parameters (table 2), arrangement and form of organs, *S. spinosum* n. sp. is similar to *S. spasskii*. However, *S. spinosum* n. sp., unlike *S. spasskii*, has an armed hermaphroditic duct. This single morphological character allows us to distinguish between these two species. *Skrjabinolectithum spinosum* n. sp. is a second species within the genus *Skrjabinolectithum* to possess an armed hermaphroditic duct. The presence of a denticulate pad was noted for *S. lobolectitum* (Martin, 1973a). Nevertheless, these worms are different valid species based on other morphometric characters, as mentioned above.

Molecular data confirmed membership of the studied trematodes in the genus *Skrjabinolectithum*. Bayesian phylogenetic analysis based on both partial 28S rRNA gene and ITS2 + 28S rDNA sequence data show that the new species is nested within the genus *Skrjabinolectithum* with high nodal support. Within this clade, *S. spinosum* n. sp. was closely related to *S. pyriforme* by 28S rRNA gene sequence and to *S. spasskii* by ITS2 + 28S rDNA sequence. Three variants (genotypes) of rDNA *S. spasskii*, reported earlier (Atopkin *et al.*, 2015), were included in the phylogenetic analyses. These sequences were more closely related to each other than to the new species. Genetic p-distance values for *S. spinosum*

Table 3. Measurements (mm) of adult worms of *Unisaccus*.

| | <i>U. tonkini</i> n. sp. | | | <i>U. sprengi</i> (Martin, 1973a) | <i>U. spinosus</i> (Martin, 1973a) | <i>U. brisbanensis</i> (Martin, 1973a) | <i>U. mugilis</i> (Rekharani & Madhavi, 1985) | <i>U. martini</i> (Ahmad, 1986) |
|-------------------------------------|--------------------------|-------------|--------|--------------------------------------|---------------------------------------|---|--|------------------------------------|
| | Holotype | Range | Mean | | | | | |
| Body length | 0.847 | 0.570–0.847 | 0.698 | 0.660–0.755 | 0.500–0.774 | 0.700–0.840 | 0.394–0.407 | 0.865–1.365 |
| Body width | 0.416 | 0.262–0.416 | 0.318 | 0.135–0.182 | 0.146–0.399 | 0.260–0.400 | 0.135–0.195 | 0.380–0.510 |
| Body length/ width (%) | 49.0 | 39.2–49.0 | 45.6 | – | – | – | – | – |
| Forebody length | 0.316 | 0.250–0.316 | 0.273 | – | – | – | 0.170–0.175 | – |
| Body/forebody length ratio (%) | 37.3 | 35.1–45.3 | 39.1 | – | – | – | – | – |
| Oral sucker length | 0.100 | 0.073–0.112 | 0.089 | 0.080–0.103 | 0.112–0.188 | 0.073–0.090 | 0.039–0.051 | 0.100–0.150 |
| Oral sucker width | 0.112 | 0.073–0.112 | 0.090 | 0.090–0.106 | 0.130–0.217 | 0.064–0.100 | 0.041–0.054 | 0.100–0.150 |
| Ventral sucker length | 0.112 | 0.089–0.112 | 0.100 | 0.079–0.100 | 0.097–0.160 | 0.080–0.084 | 0.054–0.058 | 0.068–0.090 |
| Ventral sucker width | 0.123 | 0.092–0.123 | 0.104 | 0.080–0.105 | 0.097–0.160 | 0.080–0.084 | 0.039–0.054 | 0.068–0.090 |
| Ventral/oral sucker length ratio | 1.12 | 1:0.96–1.26 | 1:1.12 | – | – | – | – | – |
| Ventral/oral sucker width ratio | 1.10 | 1:1.10–1.26 | 1:1.16 | – | – | – | 1 :1.0–1.20 | 1:0.60–0.68 |
| Prepharynx | 0.158 | 0.077–0.193 | 0.124 | 0.060 | 0.195–0.290 | 0.170–0.280 | 0.058–0.089 | 0.108–0.150 |
| Pharynx length | 0.042 | 0.042–0.054 | 0.047 | 0.060–0.070 | 0.072–0.116 | 0.022–0.044 | 0.027–0.031 | 0.085–0.105 |
| Pharynx width | 0.085 | 0.073–0.100 | 0.084 | 0.060–0.090 | 0.130–0.200 | 0.030–0.068 | 0.039–0.058 | 0.075–0.082 |
| Oesophagus length | 0.031 | 0.031–0.100 | 0.052 | 0.180 | 0.072–0.116 | 0.170–0.280 | 0.038–0.058 | 0.105–0.145 |
| Ovary length | 0.046 | 0.042–0.054 | 0.045 | 0.073–0.106 | 0.043–0.073 | 0.013–0.018 | 0.035–0.046 | 0.075–0.110 |
| Ovary width | 0.046 | 0.042–0.046 | 0.047 | 0.050–0.080 | 0.043–0.073 | 0.012–0.022 | 0.035–0.039 | 0.075–0.110 |
| Testis length | 0.116 | 0.085–0.116 | 0.102 | 0.113–0.246 | 0.073–0.206 | 0.097–0.147 | 0.062–0.078 | 0.140–0.207 |
| Testis width | 0.154 | 0.100–0.154 | 0.126 | 0.060–0.077 | 0.080–0.210 | 0.056–0.101 | 0.039–0.058 | 0.140–0.207 |
| Hermaphroditic sac length | 0.189 | 0.123–0.189 | 0.157 | 0.220–0.290 | 0.203–0.300 | 0.106–0.166 | 0.099–0.117 | 0.110–0.160 |
| Hermaphroditic sac width | 0.077 | 0.065–0.096 | 0.080 | 0.113–0.133 | 0.130–0.260 | 0.090–0.190 | 0.054–0.060 | 0.070–0.098 |
| Post-testicular length | 0.204 | 0.131–0.239 | 0.170 | | | | 0.054–0.078 | 0.046–0.070 |
| Eggs, length | 0.062–0.069 | 0.062–0.069 | – | 0.060–0.077 | 0.090–0.106 | 0.046–0.064 | 0.078–0.079 | 0.095–0.110 |
| Eggs, width | 0.039–0.042 | 0.039–0.042 | – | 0.033–0.040 | 0.040–0.066 | 0.024–0.029 | 0.031–0.037 | 0.055–0.067 |

Table 4. Genetic p-distances between Haploporidae species based on partial 28S rRNA gene sequences. Below diagonal, p-distance values (%); above diagonal, standard error values (%).

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|------------------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 1 <i>Unisaccus</i> | | 1.07 | 1.07 | 1.08 | 1.06 | 1.01 | 1.05 | 1 | 1.05 | 0.98 | 1.07 | 1.05 | 1.08 | 1.08 |
| 2 <i>S. spinosum</i> | 13.8 | | 0.03 | 0.3 | 1.03 | 1.02 | 1.02 | 1.03 | 1.07 | 1.01 | 1.07 | 1.07 | 1.04 | 1.03 |
| 3 <i>S. pyriforme</i> | 13.8 | 0.03 | | 0.3 | 1.03 | 1.02 | 1.02 | 1.03 | 1.07 | 1.01 | 1.07 | 1.07 | 1.04 | 1.03 |
| 4 <i>S. spasskii</i> | 14.3 | 0.94 | 0.96 | | 1.04 | 1.03 | 1.02 | 1.03 | 1.07 | 1.03 | 1.1 | 1.08 | 1.03 | 1.01 |
| 5 <i>P. haematocheilum</i> | 12.5 | 10.8 | 10.8 | 11.2 | | 0.51 | 0.21 | 0.98 | 0.93 | 0.96 | 1.05 | 1.05 | 0.99 | 0.98 |
| 6 <i>P. polyovum</i> | 12.8 | 11.6 | 11.6 | 12.0 | 3.12 | | 0.49 | 0.98 | 0.94 | 0.99 | 1.04 | 1.02 | 1.02 | 1.0 |
| 7 <i>P. mugili</i> | 12.3 | 10.6 | 10.6 | 10.9 | 0.42 | 3.01 | | 0.96 | 0.91 | 0.94 | 1.05 | 1.05 | 0.99 | 0.96 |
| 8 <i>C. costata</i> | 13.0 | 13.1 | 13.1 | 13.2 | 11.3 | 12.1 | 10.9 | | 0.57 | 0.96 | 0.99 | 1 | 0.94 | 0.91 |
| 9 <i>C. darwinensis</i> | 13.7 | 13.8 | 13.9 | 13.7 | 10.8 | 12.1 | 10.6 | 3.22 | | 0.96 | 1.04 | 1.07 | 1 | 0.96 |
| 10 <i>Spiritestis herviensis</i> | 12.2 | 11.9 | 11.9 | 12.1 | 11.6 | 11.7 | 11.2 | 13.0 | 13.4 | | 0.97 | 1.01 | 0.96 | 0.93 |
| 11 <i>Intromugil alahua</i> | 13.2 | 12.2 | 12.2 | 12.6 | 12.8 | 12.5 | 12.4 | 11.3 | 12.8 | 11.2 | | 0.56 | 0.94 | 0.92 |
| 12 <i>I. mugilicolus</i> | 13.5 | 11.5 | 11.6 | 11.6 | 13.1 | 12.9 | 12.7 | 12.3 | 13.7 | 11.3 | 3.43 | | 0.93 | 0.92 |
| 13 <i>Forticulcita apiensis</i> | 13.8 | 11.7 | 11.8 | 11.7 | 11.6 | 12.0 | 11.2 | 12.2 | 12.8 | 11.1 | 9.56 | 9.67 | | 0.37 |
| 14 <i>F. plantata</i> | 13.5 | 11.7 | 11.8 | 11.7 | 11.9 | 12.2 | 11.4 | 11.7 | 12.6 | 10.7 | 9.04 | 9.15 | 1.56 | |
| 15 <i>F. gibsoni</i> | 13.6 | 11.6 | 11.7 | 11.6 | 11.3 | 11.6 | 11.1 | 11.6 | 12.5 | 10.8 | 9.15 | 9.25 | 1.46 | 0.52 |
| 16 <i>Xiha fastigata</i> | 12.7 | 11.2 | 11.2 | 11.4 | 10.9 | 10.7 | 10.5 | 10.8 | 11.4 | 9.25 | 8.32 | 8.52 | 5.51 | 5.61 |
| 17 <i>Saccocoeloides</i> sp. | 13.6 | 11.7 | 11.8 | 12.1 | 11.6 | 11.5 | 11.2 | 11.6 | 12.0 | 10.6 | 7.9 | 8.32 | 8.42 | 8.52 |
| 18 <i>Lecithobotrys putrescens</i> | 13.5 | 12.7 | 12.7 | 12.8 | 11.1 | 11.7 | 11.2 | 13.0 | 12.8 | 11.0 | 9.98 | 9.88 | 9.67 | 9.67 |
| 19 <i>D. contracta</i> | 13.7 | 12.7 | 12.7 | 13.0 | 11.9 | 12.0 | 11.7 | 11.7 | 12.3 | 11.0 | 8.73 | 8.52 | 9.15 | 9.36 |
| 20 <i>D. perpusilla</i> | 14.2 | 13.7 | 13.7 | 14.3 | 11.9 | 12.0 | 11.9 | 13.5 | 13.7 | 11.2 | 11.2 | 11.0 | 10.3 | 10.8 |
| 21 <i>Haploporus benedeni</i> | 13.8 | 12.9 | 12.9 | 12.8 | 11.5 | 11.9 | 11.4 | 13.7 | 13.6 | 12.1 | 10.1 | 10.3 | 9.46 | 9.77 |
| 22 <i>Ragaia lizae</i> | 13.5 | 12.6 | 12.6 | 12.9 | 11.6 | 12.0 | 11.5 | 12.5 | 12.8 | 10.1 | 8.73 | 8.73 | 9.46 | 9.67 |
| 23 <i>Saccocoelium brayi</i> | 13.6 | 12.6 | 12.6 | 13.0 | 11.9 | 11.9 | 11.5 | 12.1 | 12.3 | 10.6 | 8.84 | 8.73 | 9.77 | 9.67 |
| 24 <i>S. cephalii</i> | 13.5 | 12.5 | 12.5 | 12.9 | 11.9 | 12.2 | 11.5 | 11.9 | 11.9 | 10.3 | 9.15 | 9.46 | 8.84 | 9.04 |
| 25 <i>S. obesum</i> | 13.3 | 12.4 | 12.4 | 12.8 | 11.4 | 11.6 | 11.1 | 11.7 | 12.0 | 10.2 | 8.63 | 8.52 | 9.25 | 9.15 |
| 26 <i>S. tensum</i> | 13.0 | 12.3 | 12.3 | 12.6 | 11.1 | 11.9 | 11.0 | 11.4 | 11.3 | 9.88 | 8.52 | 8.73 | 8.94 | 9.15 |
| 27 <i>Litosaccus brisbanensis</i> | 13.0 | 11.9 | 11.9 | 12.2 | 11.9 | 12.1 | 11.7 | 11.2 | 12.1 | 9.77 | 7.8 | 8.0 | 9.04 | 8.94 |
| 28 <i>Hapladena nasonis</i> | 15.6 | 15.3 | 15.3 | 15.6 | 13.4 | 13.3 | 13.2 | 15.8 | 15.6 | 13.8 | 13.0 | 13.4 | 12.4 | 12.4 |

| | | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
|----|---------------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| 1 | <i>Unisaccus</i> | 1.09 | 1.04 | 1.08 | 1.12 | 1.1 | 1.09 | 1.1 | 1.11 | 1.08 | 1.05 | 1.07 | 1.01 | 1.06 | 1.2 |
| 2 | <i>S. spinosum</i> | 1.03 | 1.04 | 1.08 | 1.09 | 1.05 | 1.11 | 1.07 | 1.07 | 1.06 | 1.06 | 1.05 | 1.03 | 1.07 | 1.16 |
| 3 | <i>S. pyriforme</i> | 1.03 | 1.04 | 1.08 | 1.09 | 1.06 | 1.11 | 1.07 | 1.07 | 1.06 | 1.06 | 1.05 | 1.03 | 1.07 | 1.16 |
| 4 | <i>S. spasskii</i> | 1.01 | 1.05 | 1.1 | 1.1 | 1.09 | 1.15 | 1.07 | 1.09 | 1.08 | 1.1 | 1.07 | 1.06 | 1.1 | 1.17 |
| 5 | <i>P. haematocheilum</i> | 0.98 | 0.96 | 0.99 | 0.99 | 0.95 | 1.0 | 0.97 | 0.98 | 0.99 | 1.03 | 0.97 | 0.97 | 1.01 | 1.12 |
| 6 | <i>P. polyovum</i> | 1.0 | 0.93 | 0.99 | 1.01 | 0.96 | 0.97 | 1.01 | 0.95 | 0.97 | 1.0 | 0.96 | 0.97 | 1.0 | 1.08 |
| 7 | <i>P. mugili</i> | 0.97 | 0.95 | 0.99 | 1.0 | 0.96 | 1.0 | 0.97 | 0.98 | 0.98 | 1.03 | 0.96 | 0.97 | 1.02 | 1.12 |
| 8 | <i>C. costata</i> | 0.92 | 0.9 | 1.0 | 0.99 | 0.95 | 1.02 | 1.02 | 0.94 | 0.97 | 0.95 | 0.94 | 0.92 | 0.98 | 1.09 |
| 9 | <i>C. darwinensis</i> | 0.98 | 0.95 | 1.01 | 1.03 | 1.02 | 1.07 | 1.03 | 0.99 | 1.01 | 0.98 | 1.0 | 0.95 | 1.04 | 1.05 |
| 10 | <i>Spiritistes herviensis</i> | 0.95 | 0.88 | 0.96 | 0.98 | 0.94 | 0.95 | 1.0 | 0.95 | 0.91 | 0.91 | 0.9 | 0.9 | 0.93 | 1.07 |
| 11 | <i>Intromugil alahua</i> | 0.92 | 0.91 | 0.87 | 0.96 | 0.83 | 1.01 | 0.95 | 0.87 | 0.86 | 0.9 | 0.86 | 0.87 | 0.87 | 1.06 |
| 12 | <i>I. mugilicolus</i> | 0.91 | 0.88 | 0.85 | 0.97 | 0.84 | 1.02 | 0.97 | 0.86 | 0.84 | 0.92 | 0.82 | 0.88 | 0.83 | 1.04 |
| 13 | <i>Forticulcita apiensis</i> | 0.36 | 0.73 | 0.87 | 0.92 | 0.87 | 0.93 | 0.96 | 0.92 | 0.93 | 0.95 | 0.9 | 0.92 | 0.89 | 1.02 |
| 14 | <i>F. plantata</i> | 0.21 | 0.72 | 0.86 | 0.91 | 0.88 | 0.97 | 0.96 | 0.9 | 0.9 | 0.92 | 0.87 | 0.91 | 0.87 | 1.01 |
| 15 | <i>F. gibsoni</i> | | 0.73 | 0.86 | 0.93 | 0.86 | 0.95 | 0.96 | 0.9 | 0.91 | 0.94 | 0.88 | 0.91 | 0.87 | 1.02 |
| 16 | <i>Xiha fastigata</i> | 5.51 | | 0.81 | 0.84 | 0.82 | 0.88 | 0.91 | 0.85 | 0.83 | 0.84 | 0.8 | 0.84 | 0.84 | 1.0 |
| 17 | <i>Saccocoeloides</i> sp. | 8.42 | 7.07 | | 0.93 | 0.86 | 0.95 | 0.98 | 0.86 | 0.91 | 0.93 | 0.89 | 0.89 | 0.84 | 1.05 |
| 18 | <i>Lecithobotrys putrescens</i> | 9.56 | 8.63 | 10.1 | | 0.75 | 0.79 | 0.67 | 0.74 | 0.78 | 0.78 | 0.78 | 0.76 | 0.88 | 1.04 |
| 19 | <i>D. contracta</i> | 9.25 | 8.32 | 9.04 | 5.82 | | 0.66 | 0.79 | 0.65 | 0.71 | 0.73 | 0.67 | 0.69 | 0.81 | 1.04 |
| 20 | <i>D. perpusilla</i> | 10.7 | 9.15 | 10.2 | 7.07 | 4.37 | | 0.87 | 0.75 | 0.8 | 0.79 | 0.77 | 0.77 | 0.89 | 1.12 |
| 21 | <i>Haploporus benedeni</i> | 9.67 | 8.84 | 10.8 | 4.37 | 6.24 | 7.48 | | 0.78 | 0.83 | 0.84 | 0.82 | 0.77 | 0.88 | 0.98 |
| 22 | <i>Ragaia lizae</i> | 9.56 | 8.32 | 9.04 | 5.61 | 4.37 | 5.82 | 6.24 | | 0.7 | 0.73 | 0.68 | 0.68 | 0.77 | 1.02 |
| 23 | <i>Saccocoelium brayi</i> | 9.56 | 8.32 | 9.46 | 7.07 | 5.93 | 7.48 | 8.11 | 5.72 | | 0.59 | 0.25 | 0.57 | 0.82 | 0.97 |
| 24 | <i>S. cephalii</i> | 8.94 | 7.69 | 9.15 | 6.44 | 6.03 | 7.17 | 7.9 | 5.82 | 4.26 | | 0.58 | 0.44 | 0.77 | 1.03 |
| 25 | <i>S. obesum</i> | 9.04 | 7.8 | 8.94 | 6.96 | 5.51 | 7.07 | 7.8 | 5.3 | 0.73 | 4.05 | | 0.55 | 0.79 | 0.96 |
| 26 | <i>S. tensum</i> | 9.04 | 7.69 | 8.73 | 6.24 | 5.2 | 6.65 | 7.17 | 5.09 | 3.74 | 2.18 | 3.33 | | 0.74 | 1.01 |
| 27 | <i>Litosaccus brisbanensis</i> | 8.94 | 7.69 | 7.38 | 7.9 | 6.96 | 8.63 | 8.21 | 6.13 | 7.48 | 6.34 | 6.86 | 5.72 | | 1.04 |
| 28 | <i>Hapladena nasonis</i> | 12.3 | 11.2 | 12.4 | 13.0 | 12.6 | 13.8 | 12.2 | 12.5 | 12.1 | 12.4 | 11.9 | 12.2 | 12.5 | |

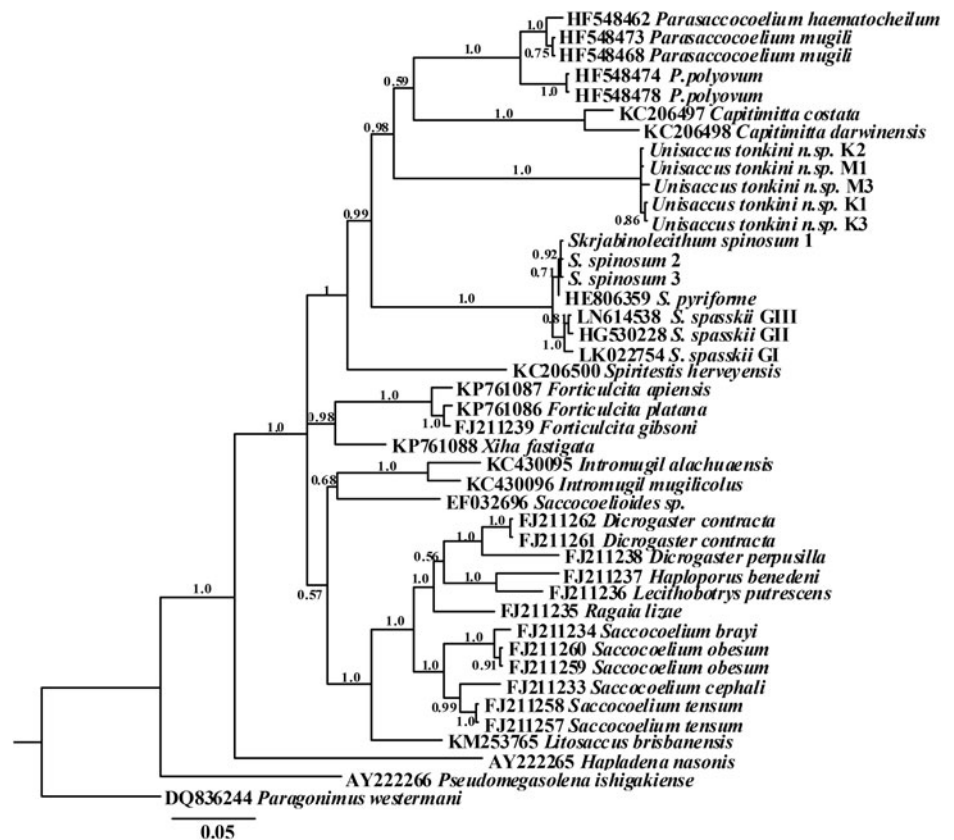


Fig. 3. Phylogenetic relationships of the family Haploporidae obtained by Bayesian algorithm based on partial 28S rRNA gene sequences. Nodal numbers are posterior probabilities that indicate statistical support of phylogenetic relationships.

n. sp. were 0.03% and $0.94 \pm 0.3\%$ with *S. pyriforme* and *S. spasskii*, respectively, by the partial 28S rRNA gene fragment (table 4), and $0.4 \pm 0.02\%$ and $3.3 \pm 0.6\%$ with *S. spasskii* and *S. pyriforme*, respectively, by the ITS rDNA fragment. Our results indicate that species of *Skrjabinolectithum* form a reciprocally monophyletic group, highly supported through molecular phylogenetic analysis. Nothing more can be said yet on the real process of divergence occurring at the molecular level until all congeneric species are sequenced and included in the analysis.

Mature worms detected in mullet fish from Vietnam agreed with morphological characteristics of *Unisaccus*. These worms possess the same organ localizations, single testis, saccular caeca and armed hermaphroditic duct, among other traits (Overstreet & Curran, 2005). Vietnamese trematodes are close to *U. spinosus*, *U. brisbanensis* and *U. overstreeti* by body size (table 3). However, Vietnamese trematodes differ from *U. spinosus* and *U. mugilis* by sizes of oral sucker and pharynx; from *U. brisbanensis*, *U. mugilis* and *U. overstreeti* by size of the ventral sucker; from *Unisaccus* species by ovary size, with the exception of *U. spinosus* and *U. mugilis* for which values of ovary length overlap; from *U. sprengi*, *U. spinosus* and *U. mugilis* by hermaphroditic duct size; from *U. mugilis*, *U. martini* and *U. overstreeti* by post-testicular length; from *Unisaccus* species by egg size, with the exception of *U. sprengi* and *U. brisbanensis*; and from *U. martini* by values of oral sucker/ventral sucker rate ratio (table 3).

Based on these morphometric data we assume that mature worms of the genus *Unisaccus* collected from Vietnamese mullet fish are representatives of a new species, *U. tonkini* n. sp. Molecular results indicate the validity of the genus *Unisaccus* (figs 3 and 4; table 4). Genetic p-distances calculated by 28S rDNA sequence data between *Unisaccus* and other genera of

different subfamilies ranged from $12.2 \pm 0.98\%$ (*Spiritestis herveyensis*, Waretrematinae) to $14.3 \pm 1.1\%$ (*S. spasskii*, Waretrematinae). Mean values of genetic p-distances between *Unisaccus* and different subfamilies of the Haploporidae ranged from $13.5 \pm 1.003\%$ (Forticulcitinae) to $13.8 \pm 0.932\%$ (Chalcinotrematinae), within standard error. Genetic differentiation using ITS2 rDNA sequence data between *Unisaccus* and other genera of Haploporidae ranged from $13.4 \pm 1.7\%$ (*Spiritestis* and *Intrromugil*, Waretrematinae) to $19.6 \pm 2.1\%$ (*Lecithobotrys*, Haploporinae). Mean values of genetic p-distances between *Unisaccus* and different subfamilies of the Haploporidae ranged from $14.1 \pm 1.7\%$ (Waretrematinae) to $19.3 \pm 2.04\%$ (Haploporinae). These data show genetic closeness of *Unisaccus* to the Waretrematinae better than the 28S rDNA data. These values correspond to divergence level, calculated for different subfamilies of Haploporidae (table 5): $8.3 \pm 0.8\%$ to $12.2 \pm 0.78\%$ and $15.7 \pm 1.45\%$ to $18.5 \pm 1.65\%$ by 28S and ITS2 rDNA sequence data, respectively. Phylogenetic tree topologies based on partial 28S rRNA gene sequence data and ITS2 + 28S rDNA sequence data showed that trematodes of *Unisaccus* are closer to representatives of Waretrematinae, except the genus *Intrromugil*, which clustered with *Saccocoelium* sp. by 28S rDNA (fig. 3) and formed a highly distant single branch by ITS2 + 28S rDNA sequence data (fig. 4).

Genetic p-distance values and phylogenetic reconstructions show that *Unisaccus* belongs to a large heterogeneous group that includes different representatives of Waretrematinae. Other subfamilies included in our analysis, Haploporinae and Forticulcitinae, represent more compact distinct clusters with a relatively bounded range of p-distance values. Thus our molecular data can be interpreted at least in two ways. The genus *Unisaccus* can be considered as a member of the Waretrematinae subfamily, as long as polyphyly of this trematode group, mentioned previously (Atopkin et al., 2015), has

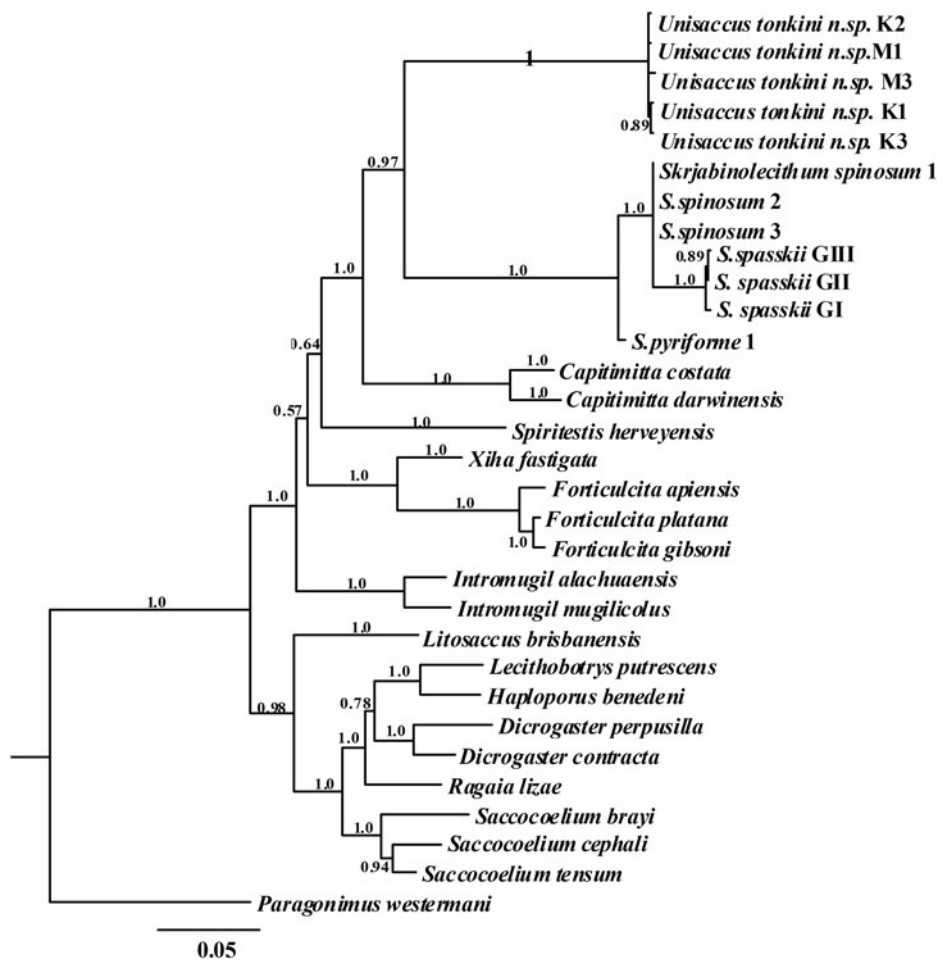


Fig. 4. Phylogenetic relationships of the family Haploporidae obtained by Bayesian algorithm based on a concatenated nucleotide sequence dataset of ITS2 rDNA and partial 28S rRNA gene sequence. Nodal numbers are posterior probabilities that indicate statistical support of phylogenetic relationships.

Table 5. Genetic p-distances between *Unisaccus tonkini* n. sp. and Haploporidae subfamilies based on 28S rDNA sequences. Below diagonal, p-distance values (%); above diagonal, standard error values (%).

| | 1 | 2 | 3 | 4 | 5 | 6 |
|-----------------------------------|-------|-------|-------|-------|-------|-------|
| 1 <i>Unisaccus tonkini</i> n. sp. | | 0.87 | 1.003 | 1.041 | 0.932 | 1.186 |
| 2 Waretrematinae | 13.59 | | 0.778 | 0.811 | 0.775 | 0.992 |
| 3 Forticulcitinae | 13.53 | 11.37 | | 0.803 | 0.72 | 1.033 |
| 4 Chalcinotrematinae | 13.83 | 11.5 | 8.308 | | 0.741 | 1.126 |
| 5 Haploporinae | 13.76 | 12.19 | 9.392 | 9.465 | | 1.027 |

been resolved. However, in consideration of the high molecular differentiation of *Unisaccus* and other haploporids, we can't exclude the possibility that the genus *Unisaccus* belongs to a distinct subfamily. Final conclusions will be possible with additional morphological and molecular data on closely related species of *Unisaccus*.

Financial support. This study was supported by grant from the Russian Foundation for Basic Research (no. 16-34-00222).

Conflict of interest. None.

References

Ahmad J (1986) Studies on digenetic trematodes of marine fishes the Arabian Sea off the Panjim coast, Goa, India. Part 50. On two new haploporid trematodes (Digenea: Haploporidae). *Revista Iberica de Parasitologica* **46**, 27–33.

Ahmad J (1987) On seven new digenetic trematodes of marine fishes from the Arabian Sea, off the Panjim coast, Goa. *Pakistan Journal of Zoology* **19**, 321–340.

Andres MJ, Pulis EE, Cribb TH and Overstreet RM (2014) Erection of the haploporid genus *Litosaccus* n. g. and its phylogenetic relationship within the Haploporidae Nicoll, 1914. *Systematic Parasitology* **89**, 185–194.

Andres MJ, Curran SS, Fayton TJ, Pulis EE and Overstreet RM (2015) An additional genus and two additional species of Forticulcitinae (Digenea: Haploporidae). *Folia Parasitologica* **62**, 025.

Atopkin DM, Beloded AY and Ngo HD (2015) Molecular genetic characterization of the far eastern trematode *Skrjabinolecithum spasskii* Belous, 1954, (Digenea, Haploporidae), a parasite of mullets. *Russian Journal of Molecular Biology* **49**, 373–379.

Belous E (1954) Contribution to the systematics of trematode Family Haploporidae Nicoll, 1914. *Trudy Hel'mintologicheskoi laboratorii Akademija Nauk SSSR* **7**, 277–281 (in Russian).

- Besprozvannykh VV, Atopkin DM, Ngo HD, Beloded AYu, Ermolenko AV, Ha NV and Tang NV** (2015) *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. *Russian Journal of Marine Biology* **41**, 267–275.
- Besprozvannykh VV, Atopkin DM, Ermolenko AV and Beloded AYu** (2017) Morphometric and molecular analyses for a new species *Skrjabinolecithum pyriforme* n. sp. (Digenea: Haploporidae) in mullet fish from the Primorsky Region, Russia. *Journal of Helminthology* doi:10.1017/S0022149X16000626.
- Blasco-Costa I, Montero FE, Gibson DI, Balbuena JA, Raga JA and Kostadinova AA** (2009) Revision of the Haploporinae Nicoll, 1914 (Digenea: Haploporidae) from mullets (Mugilidae): *Saccocoelium* Looss, 1902. *Systematic Parasitology* **72**, 159–186.
- Curran SS, Tkach VV and Overstreet RM** (2006) A review of *Polylekithum* Arnold, 1934 and its familial affinities using morphological and molecular data, with description of *Polylekithum catahouleensis* sp. nov. *Acta Parasitologica* **51**, 238–248.
- Darriba D, Taboada GL, Doallo R and Posada D** (2012) jModeltest2: more models, new heuristics and parallel computing. *Nature Methods* **9**, 772.
- Huelsenbeck JP, Ronquist F, Nielsen R and Bollback JP** (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* **294**, 2310–2314.
- Luton K, Walker D and Blair D** (1992) Comparisons of ribosomal internal transcribed spacers from two congeneric species of flukes (Platyhelminthes: Trematoda: Digenea). *Molecular and Biochemical Parasitology* **56**, 323–327.
- Madhavi R** (1979) Digenetic trematodes from marine fishes of Waltair Coast, Bay of Bengal. Families Haplosporinae and Haploporidae. *Rivista Iberica de Parasitologia* **40**, 237–248.
- Martin WE** (1973a) A new genus and species of haploporid trematode (Haploporidae: Trematoda) from Australian mullet. *Bulletin of the Southern California Academy of Sciences* **72**, 166–168.
- Martin WE** (1973b) A new subfamily, two new genera, and three new species of haploporid trematodes. *Proceedings of the Helminthological Society of Washington* **40**, 112–118.
- Martin WE** (1973c) Life history of *Saccocoelioides pearsoni* n. sp. and the description of *Lecithobotrys sprenti* n. sp. (Trematoda: Haploporidae). *Transactions of the American Microscopical Society* **92**, 80–95.
- Olson PD, Cribb TH, Tkach VV, Bray RA and Littlewood DTJ** (2003) Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *International Journal for Parasitology* **33**, 733–755.
- Overstreet R and Curran S** (2005) Family Haploporidae Nicoll, 1914. pp. 129–165 in Gibson DI, Jones A and Bray RA (Eds) *Keys to the Trematoda*, vol. 2. Wallingford, UK, CAB International.
- Pulis E, Fayton T, Curran S and Overstreet R** (2013) A new species of *Intromugil* (Digenea: Haploporidae) and redescription of *Intromugil mugilicolus*. *Journal of Parasitology* **99**, 501–508.
- Rekharani Z and Madhavi R** (1985) Digenetic trematodes from mullets of Visakhapatnam (India). *Journal of Natural History* **19**, 929–951.
- Tamura K, Stecher G, Peterson D, Filipinski A and Kumar S** (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* **30**, 2725–2729.
- Tavare S** (1986) Some probabilistic and statistical problems on the analysis of DNA sequences. *Lectures on Mathematics in the Life Sciences* **17**, 57–86.
- Tkach VV, Littlewood DTJ, Olson PD, Kinsella JM and Swiderski Z** (2003) Molecular phylogenetic analysis of the Microphalloidea Ward, 1901 (Trematoda: Digenea). *Systematic Parasitology* **56**, 1–15.
- Truett GE** (2006) Preparation of genomic DNA from animal tissues. pp. 33–46 in Kieleczawa J (Ed.) *The DNA book: protocols and procedures for modern molecular biology*. Burlington, Massachusetts, USA, Jones & Bartlett.
- Zhukov EV** (1972) New genera of trematodes from marine fishes of India. *Parazitologiya* **6**, 346–350 (in Russian).