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Restoration of the Genus *Paraunisaccoides* Martin, 1973 (Digenea: Haploporidae) and Description of *P. elegans* n. sp. and *Unisaccus halongi* n. sp. from Mugilid Fish in Vietnam

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Abstract: We restore the genus *Paraunisaccoides* (Haploporidae), synonymised earlier with the genus *Skrjabinolecithum*. Adult worms, detected in Vietnamese mullet fish, were highly similar to trematodes described as *P. lobolecithum* via digestive and genital system structures and relative organ arrangement. Differences are expressed as absence and presence of pads on the hermaphrodite duct, respectively, and the disjunction of some metric parameter values, namely body, ovary and eggs. Ribosomal DNA sequences, based on the phylogenetic analysis of Haploporidae, indicates that new worms represent a sister clade to *Unisaccus tonkini*. Genetic divergence between new worms and *Skrjabinolecithum* species can be interpreted as intergeneric. Based on morphological and molecular data, we recognise *Paraunisaccoides* as a valid genus within Waretrematinae and worms from Vietnam as a new species of this genus, *P. elegans* n. sp. Other worms detected in Vietnamese mugilids are morphologically similar to representatives of *Paraunisaccoides* и *Skrjabinolecithum*. However, molecular-based phylogenetic analysis showed that these trematodes are closely related to *Unisaccus tonkini*; the genetic divergence between them is at the interspecific level, despite considerable differences in vitellarium structure as intergeneric character. Accepting the priority of molecular results, we include these new worms into the genus *Unisaccus* as new species, *Unisaccus halongi* n. sp.

Keywords: Haploporidae; *Unisaccus*; *Paraunisaccoides*; DNA sequence; 28S

1. Introduction

Species of Haploporidae Nicoll, 1914, as well as other Haploporoidea, parasitising a wide range of definitive hosts, marine, freshwater, and eurihaline fish species, are cosmopolitan. Species of different subfamilies (except the cosmopolitan Megasoleninae Manter, 1935) are somehow related to certain geographical territories, infecting mainly fish species of Mugilidae Jarocki, 1822 and, rarely, Cyprinidae Rafinesque, 1815. Representatives of three subfamilies, Haploporinae, Waretrematinae Srivastava, 1937 and Pseudohaploporinae Atopkin, Besprozvannykh, Ha, Nguyen, Bguyen & Chalenko, 2018, of the eight known at present, have mainly been detected in the Indo-West Pacific region [1–11]. Most of the trematodes have been extracted from Mugilidae fish species.

We obtained specimens of two hypothetic species of Haploporidae in mullet fish from coastal waters of Halong Bay, Vietnam. In the present study, we performed morphological and molecular analyses to validate these species and to reconstruct the phylogenetic relationships of the family Haploporidae with new data.

2. Materials and Methods

2.1. Collection of Trematodes

Adult worms were collected from intestines of mullet fish (Mugilidae) in coastal waters near Cat Ba Island, Ha Long Bay, Vietnam (20°84′ N, 106°59′ E). Worms were rinsed in saline, previously defined under a microscope using temporal slides preparation technique, killed in hot distilled water and preserved in 70% ethanol. After fixation, they were replaced in 96% ethanol. Whole mounts were made by staining specimens with alum carmine, dehydrating them in graded ethanol series and clearing in clove oil, followed by mounting the specimens in Canada balsam under a coverslip on a glass slide. All measurements are given in micrometres.

2.2. DNA Extraction, Amplification and Sequencing

Three and four adult specimens of *Unisaccus halongi* n. sp. and *Paraunisaccoides elegans* n. sp., respectively, from 96% ethanol were used for molecular analysis (Table 1). Total DNA was extracted from flukes using a "hot shot" technique [12].

28S ribosomal DNA (rDNA) 1200 base pairs (bp) in lenght was amplified by a polymerase chain reaction (PCR) method using the Q5 HF polymerase (New England Biolabs, Ipswich, MA, USA) and the primers 28S-A (5′-TCGATTCGAGCGTGAWTACCCGC-3′) [13] and 1500R (5′-GCTATCCTGAGGGAAACTTCG-3′) [14] with an annealing temperature of 55 °C. The ribosomal ITS1-5.8S-ITS2 fragment 1500 bp in lenght was amplified with the primers ITSF (5′-CGCCCGTCGCTACTACCGATTG-3′) [3] and S4R (5′-TATGCTTAAATTCAGCGGGT-3′) [15] with an annealing temperature of 54 °C. Negative and positive controls using both primer pairs were included. PCR parameters began with a 1 min denaturation at 98 °C, followed by 35 cycles of 10 s at 98 °C, 5 s at 54/55 °C and 30 s at 72 °C, and concluded with a 2 min extension at 72 °C.

PCR products were directly sequenced using an ABI Big Dye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA) as recommended by the manufacturer. The internal sequencing primers for 28S rDNA are described in [14], for ITS they are described in [16]. PCR product sequences were analysed using an ABI 3500 genetic analyser at the FSC of Biodiversity FEB RAS. Sequences were submitted to the GenBank database (Table 1).

2.3. Alignments and Phylogenetic Analysis

Ribosomal DNA sequences were assembled using the 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 v. 7.1 software [17]. The values for genetic p-distances were calculated for the 28S ribosomal DNA fragment. Phylogenetic relationships were obtained using a concatenated data set of partial sequences of the 28S rRNA gene and ITS1-5.8S-ITS2 rDNA. Phylogenetic analysis was performed using the Bayesian algorithm in the MrBayes v. 3.2.6 software [18]. The best nucleotide substitution model, a transversional model with estimates of invariant sites and gamma-distributed among-site variation TVM + G+I [19], was estimated using the jModeltest v. 2.1.5 software [20] for partial 28S rDNA and combined ITS1-5.8S-ITS2-28S rDNA sequence data sets. 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 phylogenetic relationships was estimated using posterior probabilities [18]. Sequences of ITS1-5.8S-ITS2 rDNA and 28S rDNA of *Brachycladium goliath* (van Beneden, 1858) from GenBank were used as the outgroup. The authors of these and other sequences from GenBank [3–11,21–31] and their accession numbers are given in Table 1.

Table 1. List of taxa used for molecular analysis.

Species	N	Definitive Host	Authors	Accession Number in the NCBI	
				28S	ITS1-5.8S-ITS2
		Haploporidae			
		Waretrematinae			
Paraunisaccoides elegans n. sp.	4/2	*Planiliza subviridis*	Present study	KY501639–KY501641	KY501642–KY501644
Unisaccus halongi n. sp.	3/3	*Crenimugil seheli*	Present study	OK644190-OK644192	OK644196-OK644198
Unisaccus tonkini	1/1	*Moolgarda cunnesius*	[6]	MF176843	MF176838
Skrjabinolecithum pyriforme	1/1	*Planiliza haematocheila*	[5]	HE806359	LN864990
Skrjabinolecithum spinosum	1/1	*Planiliza haematocheila*	[6]	MF176834	MF176831
Skrjabinolecithum. spasskii	3/3	*Planiliza haematocheila*	[21]	LN614538, HG530210, HG530207	LK022754, HE806371, HG530228
Parasaccocoelium mugili	1/1	*Planiliza haematocheila*	[22]	MW813991	
Parasaccocoelium armatum	-/2	*Mugil cephalus*	[11]	-	MT298950–MT298951
Parasaccocoelium haematochelium	-/2	*Liza haematocheila*	[4]	-	HF548466–HF548467
Parasaccocoelium polyovum	-/2	*Liza haematocheila*	[4]	-	HF548477–HF548478
Elonginurus mugilus	1/1	*Mugil cephalus*	[9]	MH763766	MH763761
Carassotrema koreanum	3/3	*Carassius gibelio*	[9]	MH763763–MH763765	MH763758–MH763760
Carassotrema sp.	1/1		unpublished	MH285255	
Spiritestis herveyensis	1/1	*Moolgarda seheli*	[2]	KC206500	
Capitimitta costata	1/1	*Selenotoca multifasciata*	[2]	KC206497	
Capitimitta darwinensis	1/1	*Selenotoca multifasciata*	[2]	KC206498	
		Pseudohaploporinae			
Parahaploporus elegantus	10	*Moolgarda seheli*	[10]	MN639712-MN639721	
Pseudohaploporus vietnamensis	6/8	*Osteomugil engeli*	[8]	MF774420–MF774421, MF774423–MF774426	MF774427–MF774429; MF774436–MF774440
Pseudohaploporus vietnamensis	1/1	*Moolgarda seheli*	[8]	MF774422	MF774431
Pseudohaploporus planilizum	3/3	*Planiliza subviridis*	[8]	MF774417–MF774419	MF774433–MF774435
Pseudohaploporus pusitestis	2/2	*Moolgarda seheli*	[10]	MH986037, MH986038	MF774430, MF774432
		Haploporinae			
Saccocoelium brayi	1/1	*Liza saliens*	[23]	FJ211234	FJ211244
Saccocoelium cephali	1/1	*Mugil cephalus*	[23]	FJ211233	FJ211243
Saccocoelium obesum	2/2	*Liza ramada*	[23]	FJ211259–FJ211260	FJ211265–FJ211266
Saccocoelium tensum	2/2	*Liza ramada*	[23]	FJ211257–FJ211258	FJ211263–FJ211264
Dicrogaster contracta	1/1	*Liza aurata*	[23]	FJ211261	FJ211267
Dicrogaster perpusilla	1/1	*Liza ramada*	[23]	FJ211238	FJ211248
Lecithobotrys putrescen	1/1	*Liza saliens*	[23]	FJ211236	FJ211246
Litosaccus_brisbanensis	1/1	*Mugil cephalus*	[3]	KM253765	
Haploporus benedeni	1/1	*Liza ramado*	[23]	FJ211237	FJ211247
Ragaia lizae	1/1	*Liza aurata*	[23]	FJ211235	FJ211245
		Forticulcitinae			
Forticulcita isabelae	1/1	*Mugil curema*	[24]	MT957787	MT957640
Forticulcita macropharyngis	1/1	*Mugil curema*	[25]	MW796513	MW796548
Forticulcita minuta	1/1	*Mugil cephalus*	[24]	MT957822	MT957660
Forticulcita venezuelensis	1/1	*Mugil curema*	[25]	MW796522	MW796549
Forticulcita gibsoni	1/1	*Mugil cephalus*	[24]	FJ211239	FJ211249
F. apiensis	1/1	*Mugil cephalus*	[26]	KP761087	
F. platana	1/1	*Mugil liza*	[26]	KP761086	
Xiha fastigata	1/1	*Mugil cephalus*	[26]	KP761088	
Overstreetoides_pacificus	2/2	*Mugil curema*	[24]	MT957753–MT957754	MT957629–MT957630
Ekuarhuni papillatum	2/2	*Mugil* sp.	[24]	MT957687–MT957688	MT957587–MT957588
Ekuarhuni mexicanum	2/2	*Mugil* sp.	[24]	MW796487–MW796488	MW796524–MW796525

Table 1. *Cont.*

Species	N	Definitive Host	Authors	Accession Number in the NCBI	
Chalcinotrematinae					
Saccocoelioides sp.	1/1	Unidentified molly (Poecilidae)	[27]	EF032696	-
Saccocoelioides beauforti	1/1	*Mugil cephalus*	[28]	MG925104	MG925103
Saccocoelioides elongatus	1/1	*Prochilodus lineatus*	[28]	MG925108	MG925107
Saccocoelioides magnus	1/1	*Cyphocharax voga*	[28]	MG925112	MG925111
Saccocoelioides nanii	1/1	*Prochilodus lineatus*	[28]	MG925114	MG925113
Saccocoelioides orosiensis	1/1	*Poecilia gillii*	[28]	MG925118	MG925117
Saccocoelioides tkachi	1/1	*Astyanax aeneus*	[28]	MG925122	MG925121
Intromugil mugilicolus	1/1	*Mugil cephalus*	[29]	KC430096	
Intromugil alachuaensis	1/1	*Mugil cephalus*	[29]	KC430095	
Hapladeninae					
Hapladena acanthuri	1/1	*Acanthurus chirurgus*	[30]	MH244119	
Hapladena cf. *varia*	1/1	*Acanthurus chirurgus*	[30]	MH244120	
Megasoleninae					
Megasolena hysterospina	1/1	*Archosargus rhomboidalis*	[30]	MH244121	
Megasolena sp. m MA-2018	1/1	*Holacanthus ciliaris*	[30]	MH244122	
Cadenatelinae					
Cadenatella americana	1/1	*Kyphosus sectatrix*	[30]	MH244117	
Cadenatella floridae	1/1	*Kyphosus incisor*	[30]	MH244118	
Atractotrematidae					
Isorchis anomalus	1/1	*Chanos chanos*	[7]	KU873018	
Isorchis currani	1/1	*Selenotoca multifasciata*	[31]	KU873017	
Isorchis megas	1/1	*Selenotoca multifasciata*	[7]	KU873015	
Brachycladiidae					
Brachycladium goliath	1/1	*Balaenoptera acutorostrata*	[32]	KR703279	
Monorchiidae					
Hurleytrematoides chaetodoni	1/1	*Chaetodon striatus*	[30]	MH244116	

N—number of sequences of 28S rDNA/ITS rDNA.

3. Results

3.1. Diagnosis of the Genus *Paraunisaccoides*

Family. Haploporidae Nicoll, 1914; Subfamily.
Waretrematinae Srivastava, 1937; Genus.
Paraunisaccoides Martin, 1973.

Body oval, fusiform or elongate. Eye-spot pigment dispersed. Oral sucker subterminal. Ventral sucker larger than oral sucker in anterior half of body. Prepharynx long, reach level of ventral sucker or posterior to ventral sucker. Pharynx round or transversely oval. Oesophagus short poorly defined. Caecum saccular. Testis single, in posterior end of body. Hermaphroditic sac oval with muscular sphincter at anterior end. External seminal vesicle sac-shaped or another form depending of fullness of sexual products. Internal seminal vesicle elongated. Hermaphroditic ducts with or without pads. Genital pore anterior to ventral sucker. Ovary round or transversal oval, immediately anterior to testis. Uterus short, between anterior margin of testis and hermaphroditic sac. Metraterm short, thin-walled. Eggs unnumerous, unembryonated, operculated. Mehlis'gland sinistrally to ovary. Vitellarium, consists from elongate thin of follicles. Vitelline fields can reach level of posterior edge of ventral sucker and to posterior end of body. Excretory bladder I-shaped with or without muscular sphincter. Type species: *Paraunisaccoides lobolecithum* Martin, 1973.

3.2. Paraunisaccoides elegans n. sp.

3.2.1. Taxonomic Summary

Host. *Planiliza subviridis* (Valenciennes, 1836).

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

Site. Intestine.

Prevalence. 3 of 5 specimens infected.

Intensity. 1–12 worms.

Type-deposition: Type No. 182-Tr, paratype No. 183-187-Tr. This material is held in the collection of the Zoological Museum (Institute of Biology and Soil Sciences, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia); e-mail: petrova@ibss.dvo.ru. Deposited: 29 July 2020.

Etymology: species name associated with trematode body form.

3.2.2. Morphology

Based on 6 adult worms (Figure 1, Table 2).

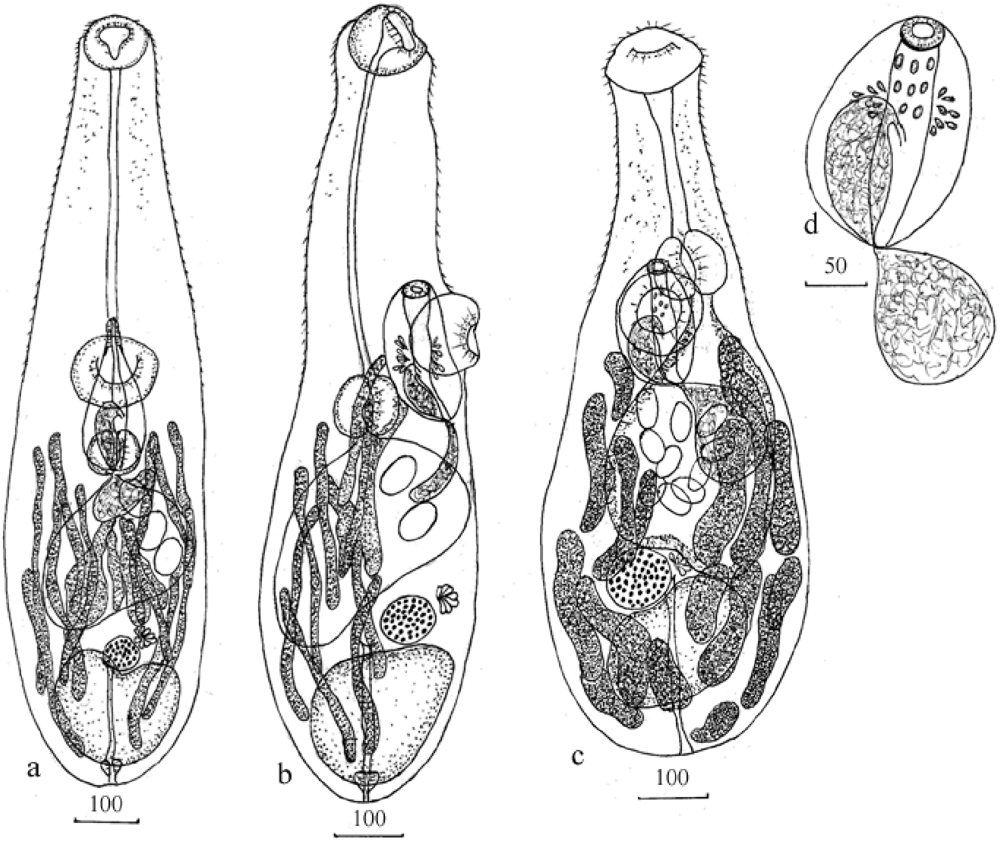

Figure 1. *Paraunisaccoides elegans* n. sp.: (**a**) adult worm ventral, (**b**) adult worm lateral; *Unisaccus halongi* n. sp.: (**c**) adult worms, (**d**) cirrus sac. Measurements are given in μm.

Table 2. Measurements (µm) of adult worms of new species *Paraunisaccus elegans* n. sp. and *Unisaccus halongi* n. sp. and known species *Skrjabinolecithum lobolecithum* and *S. indicum*.

	***Paraunisaccoides elegans* n. sp.**			***Skrjabinolecithum lobolecitum* (Martin, 1973)**	***S. vitellosum* (Martin, 1973)**	***Unisacus halongi* n. sp.**			***S. indicum* (Zhukov, 1972)**
	Holotype	**Range**	**Mean**			**Holotype**	**Range**	**Mean**	
Body length	1217	1124–1232	1170	1850; 2070	510–790	920	920–1390	1170	1000–1200
Body width	354	323–370	347	266; 406	140–300	420	390–500	430	280–370
Body length/width%	29.1	26.0–32.9	29.7	-		45.7	31.7–45.7	36.8	-
Forebody length	501	424–508	469	-		0.347	347–396	364	-
Body/forebody length%	41,2	37.5–44.0	40.1	-		32.2	28.2–37.7	31.1	-
Oral sucker length	96	77–104	92	96; 112	50–80	96	92–127	104	83–120
Oral sucker width	96	92–112	100	100; 112	59–90	131	116–154	139	110–120
Ventral sucker length	123	112–139	125	93; 143	56–74	127	96–142	120	120–150
Ventral sucker width	131	127–135	129	78; 156	56–74	127	112–150	124	140–170
Ventral/oral sucker length ratio	1:1.28	1:1.17–1.71	1:1.36	-		1:1.38	1: 0.91–1.48	1:1.15	-
Ventral/oral sucker width ratio	1:1.36	1:1.13–1.47	1:1.29	-		1:1.09	1: 0.73–1.09	1:1.06	-
Prepharynx length	539	377–566	483	426; 684	90–109	227	227–354	306	120–170
Pharynx length	77	77–116	93	109; 112	44–80	77	77–116	95	110–140
Pharynx width	85	85–123	100	131; 137	59–110	89	89–135	117	80–110
Oesophagus length	15	0–154	-	112; 249	60	58	23–58	49	-
Ovary length	62	46–77	71	109; 140	31–62	69	69–116	96	62–83
Ovary width	62	54–77	64	100; 109	31–62	69	69–116	97	62–83
Testis length	177	162–219	206	239; 345	75–165	150	150–270	180	210–300
Testis width	193	154–235	198	168; 202	50–90	193	139–227	184	120–140
Hermaphroditic sac length	254	181–270	220	258; 286	90	177	154–231	186	-
Hermaphroditic sac width	96	100–123	114	96; 118	50	104	104–173	138	-
Eggs length	62–65	62–65	-	71–93	59–65	62–73	62–73	-	71–79
Eggs width	50–54	50–54	-	56–59	42	42–50	42–50	-	39–43

Body elongate, with narrow forebody, tegument with needle-shaped spines. Eyes-spot pigmentation dispersed in anterior half of forebody. Oral sucker subterminal. Prepharynx long, reach level of ventral sucker or slightly posterior to ventral sucker. Pharynx round or transversely oval. Oesophagus short poorly defined. Caecum saccular, in middle of posterior half of body. Ventral sucker larger than oral sucker, at beginning of middle third of body. Testis single, in posterior end of body, irregular, with anterior recess. Hermaphroditic sac oval, at level ventral sucker and pharynx. Anterior end of hermaphroditic sac with muscular sphincter. External seminal vesicle sac-shaped or another form depending of fullness of sexual products. Internal seminal vesicle elongated. Prostatic cells unnumerous. Pads at hermaphroditic duct not identified. Genital pore opening on midline of body immediately before ventral sucker. Ovary round or transversal oval, adjacent to anterior margin of testis on midline of body. Uterus short, between anterior margin of testis and hermaphroditic sac. Metraterm short, thin-walled. Eggs few, light yellow, unembryonated, operculated. Mehlis'gland sinistrally to ovary. Vitellarium, consists from elongate thin of follicles. Vitelline fields can reach level of posterior edge of ventral sucker and to posterior end of body, merge at median line of body and cover of ovary and testis. Excretory bladder I-shaped with muscular sphincter, pore terminal.

3.2.3. Molecular Data

For four specimens of *P. elegans* n. sp. totals of 1291 and 1604 alignable characters with three (0.23%) and nineteen (1.18%) variable sites were generated for analysis in the 28S rRNA gene and ITS1-5.8S—ITS2 rDNA fragment datasets, respectively.

3.3. Unisaccus halongi n. sp.

3.3.1. Taxonomic Summary

Host. Crenimugil seheli (Fabricius, 1775).

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

Site. Intestine.

Prevalence. 5 of 37 specimens infected.

Intensity of infection. 1–5 worms.

Type-deposition: Type No. 188-Tr, paratype No. 189-193-Tr. This material is held in the collection of the Zoological Museum (Institute of Biology and Soil Sciences, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia); e-mail: petrova@ibss.dvo.ru. Deposited: 29 July 2020.

Etymology: species name is associated with location, where definitive host of these worms has been caught.

3.3.2. Morphology

Based on 6 adult worms (Figure 1, Table 2).

Body elongate, with narrow forebody, tegument with needle-shaped spines. Eyes-spot pigmentation dispersed in forebody. Oral sucker subterminal, transversaly oval. Prepharynx long, slightly does not reach of ventral sucker or reach level of anterior half of ventral sucker. Pharynx at level ventral sucker, spherical, large, slightly smaller of oral sucker. Oesophagus short, poorly defined. Caecum single, saccular, thick-walled, in middle and partly in posterior third of body. Ventral sucker larger than oral sucker, at the border of anterior and posterior third of body. Testis single, in posterior end of body, irregular. Hermaphroditic sac oval, mostly at level ventral sucker. Anterior end of hermaphroditic sac with muscular sphincter. External seminal vesicle sac-shaped. Internal seminal vesicle oval. Size of seminal vesicles depends on fullness of sexual products. Prostatic cells unnumerous. Hermaphroditic duct with pads. Genital pore opening on midline of body immediately before ventral sucker. Ovary round, adjacent to anterior margin of testis. Uterus short, between anterior margin of testis and hermaphroditic sac. Metraterm equal to length hermaphroditic duct. Eggs few, light yellow, unembryonated, operculated. Mehlis'gland sinistrally to ovary. Vitellarium, consists from large elongate, wide of follicles, in close contact with each other. Vitelline fields between ventral sucker to posterior end of body, merge at median line of body and cover of ovary and testis. Excretory bladder I-shaped, pore terminal.

3.3.3. Molecular Data

For three specimens of *S. indicum* totals of 1240 and 972 alignable characters were generated for analysis in the 28S rRNA gene and ITS1-5.8S—ITS2 rDNA fragment datasets, respectively. Sequences within both datasets were identical to each other.

4. Discussion

On the basis of the last known classification of Haploporidae, provided by Overstreet and Curran [1], Vietnamese worms belong to this family by morphological characteristics, including organ topology, single testis, identical hermaphrodite sac structure, and single caecum. The latter characteristic is representative for worms of the genera *Paraunisaccoides* within Haploporidae, as well as *Unisaccus* and *Unisaccoides* (Unisaccinae Martin, 1973, Haploporidae), validated by Martin [33,34]. In 2005, *Paraunisaccoides* and *Unisaccoides* were synonymised with *Skrjabinolecithum* Belous, 1954 (Waretrematinae), and the type genus *Unisaccus* of Unisaccinae was transferred to Haploporinae [1]. This has led to recognising Unisaccinae as a synonym of Haploporinae [1].

Among representatives of Haploporidae, worms ex *Planiliza subviridis* from Vietnam are morphologically most similar with representatives of *Skrjabinolecithum* that can possess double for type species *S. spasskii* Belous, 1954, and also *S. indicum* (Zukov, 1972), *S. puriforme* Besprozvannykh et al., 2017 and *S. spinosum* Besprozvannykh et al., 2018 or single for *S. lobolecitum* (Martin, 1973), *S. vetillosum* (Martin, 1973) caecum. Vietnamese flukes and *S. vetillosum* characterized by hiatus in metric parameters for body, ventral sucker, hermaphrodite sac etc. (Table 2). Worms from our study show highest similarity with trematodes, detected in *Mugil cephalus* Linnaeus, 1758 in Australia [33], denoted as *Paraunisaccoides* (=*Skrjabinolecithum*) *lobolecithum.* These worms differ by the presence pads on the hermaphrodite sac for worms from Martin's study [33] and, according to the figure provided, the presence of a large space between the ventral sucker and anterior end of the vitellarium (description of vitellarium arrangement absent in [33]). Alongside this, there is a disjunction of metric parameter values, namely body length and sizes of ovary and eggs (Table 2), between worms from [33] and those from our study. Although trematode specimens from both studies were detected in mugilids from relatively close geographical regions and with similar morphology, a number of differences, reported above, and the absence of molecular data for specimens from Australia do not allow to conclude that these trematodes belong to the same species. In our opinion, it is rational to currently consider Vietnamese worms as a new species of Haploporidae. A final conclusion of the validity or conspecificity of these species can be made after obtaining molecular data for the Australian worms, reported in [33]. Phylogenetic analyses performed on the basis of 28S rDNA partial sequences 1050 bp in length and concatenated 28S rDNA and 5.8S + ITS2 rDNA (1460 bp overall length) fragments for Haploporidae confirmed the membership of considered here Vietnamese worms to Waretrematinae (Figures 2 and 3). However, within this subfamily, Vietnamese worms formed a common clade with *Unisaccus tonkini* instead of *Skrjabinolecithum* species. At the same time, the genetic distance values between Vietnamese worms, *Unisaccus* and *Skrjabinolecithum*, represent an intergeneric divergence level by both markers: 7.05 ± 0.78%–12.05 ± 1.0% by 28S rDNA and 8.05 ± 0.97%–12.10 ± 1.14% by ITS2 rDNA (alignment length 379 bp). These values are compatible with those between different genera within each subfamily: 3.62 ± 0.56%–13.93 ± 1.03% and 4.21 ± 0.73%–16.38 ± 1.35% for Waretrematinae, 5.9 ± 0.62% and 8.33 ± 0.91% for Pseudohaploporinae, 6.13 ± 0.68% for 10.11 ± 1.08% for Forticulcitinae Blasco-Costa, Balbuena, Kostadinova & Olson, 2009 by 28S rDNA and ITS2 rDNA, respectively [8,23,24]. Based on this, accepting the morphological similarity of Vietnamese worms and Australian described by Martin as *Paraunisaccoides lobolecithum*, we conclude that synonymisation of *Paraunisaccoides* with *Skrjabinolecithum* is unreasonable. In the light of this, we restore the genus *Paraunisaccoides* with the type species *Paraunisaccoides lobolecithum* Martin, 1973 with the inclusion of the new species *Paraunisaccoides elegans* n. sp. from the present study.

New trematodes ex *Crenimugil seheli* from Vietnam, share many morphometrical characteristics with *Paraunisaccoides,* and also with *Skrjabinolecithum*. Alongside this, Vietnamese worms differ from *Paraunisaccoides lobolecithum* by lesser body length along with similar body width, higher ventral sucker width, lesser prepharynx and oesophagus length and lesser hermaphroditic sac length along with its higher width (Table 2). New worms differ from *Paraunisaccoides elegans* n. sp., described above, by presence of pads on hermaphroditic duct, and different structure of vitellarium follicules, and also by metric parameters of body width relative to body length, forebody length and forebody lengh/overall body length ratio and by a number of other characteristics (Table 2). Among *Skrjabinolecithum*, *S. vitellosum* and *S. indicum*, which were firstly detected in *Planiliza subviridis* and *Etroplus surutensis* (Bloch, 1790) from Australia coastal waters and the Arabian Sea, respectively [1,35], are most similar to new trematodes ex Vietnamese *Crenimugil seheli* from our study. However, new worms differ from *S. vitellosum* by presence of pads on hermaphroditic duct, different structure of vitellarium follicules and by most of metric parameters (Table 2). New worms are similar to *S. indicum* by most of metric parameters, except prepharynx length (Table 2). The main morphological difference between these worms is the presence of single and double caeca, and presence and absence of pads on hermaphroditic duct, respectively. Another difference for these trematodes can be found in the vitellarium structure. The vitellarium of *S. indicum* represents two poorly developed follicular fields [35], whereas for Vietnamese worms, the vitellarium is well developed and represents large elongate follicules. On the basis of stated above morphometrical differences between trematodes, we consider that worms ex *Crenimugil seheli* from Vietnam belong to neither *S. vitellosum* nor *S. indicum*. Additional molecular data on *S. vitellosum* and *S. indicum* from type locations are needed to clarify taxonomical and phylogenetic questions of these two species and new Vietnamese trematodes.

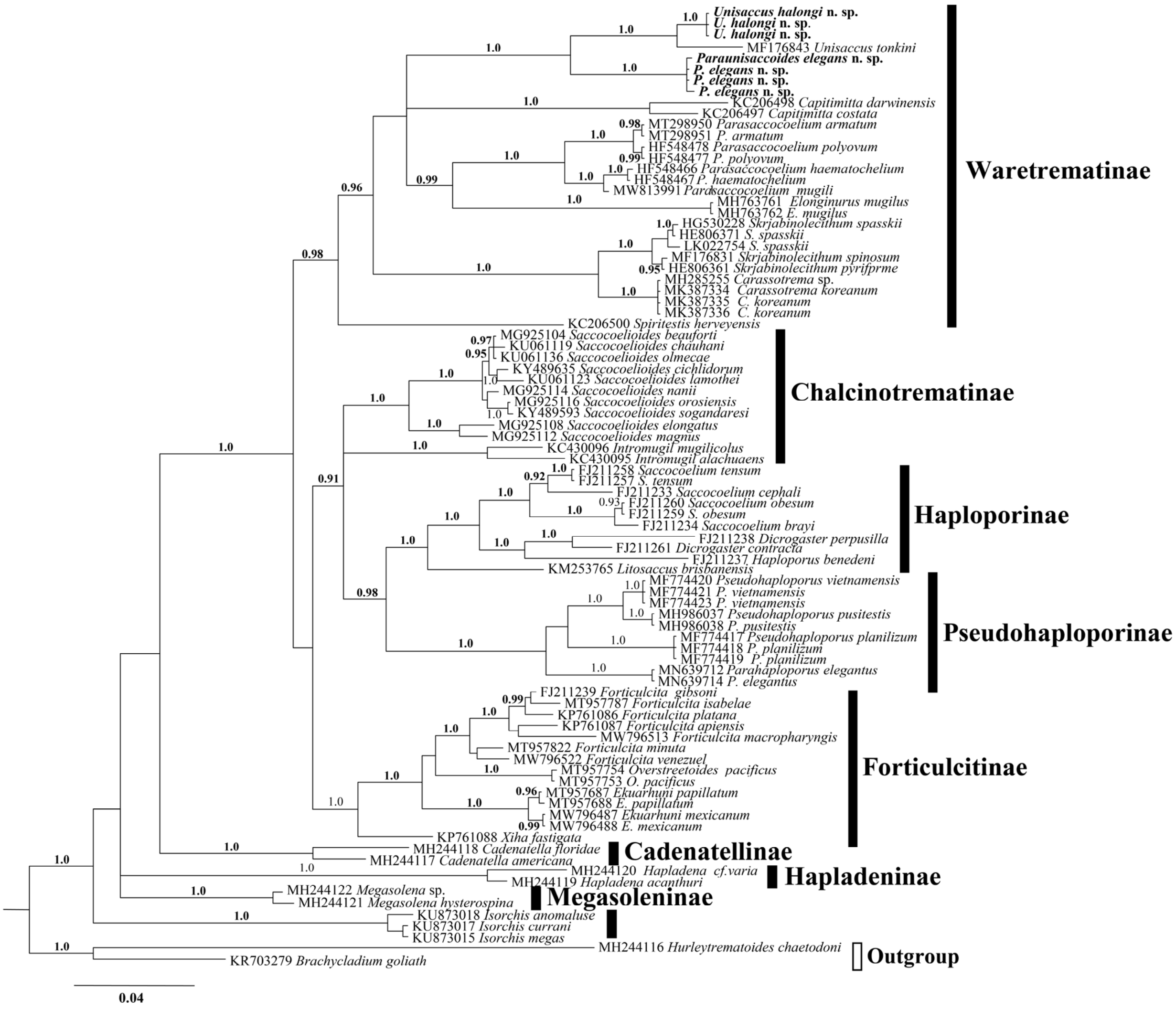

Figure 2. Phylogenetic relationships of the family Haploporidae obtained with Bayesian algorithm based on partial 28S rRNA gene (alignment length 1050 bp). Nodal numbers—posterior probabilities that indicate statistical support of phylogenetic relationships, only significant values (0.9–1.0) are showed.

Molecular results support the membership trematodes ex *Crenimugil seheli* to Waretrematinae and indicate close relationships with *Unisaccus tonkini* (Figures 2 and 3). Alongside this, worms from our material possess both common and uncommon morphological characteristics for *Unisaccus*. The similarity of these worms appears in the structure of genital and digestive systems. Differences of *Unisaccus* spp. and worms from our study observed in following characteristics: body pyriform to fusiform vs. body elongate; eggs embryonated vs. unembryonated; subspherical follicles vs. elongate follicles. Trematodes from our material are similar in terms of the listed characteristics to *Paraunisaccoides elegans* n. *sp.*, which appears as sister clade relative to the *Unisaccus* clade on the Bayesian tree (Figures 2 and 3). Genetic differentiation between these two clades ranged from 6.53 ± 0.76%–7.05 ± 0.5% by 28S rDNA sequence data and 7.94 ± 0.95%–8.05 ± 0.67% by ITS2 rDNA sequence data. These results indicate that trematodes from the two clades represent different genera.

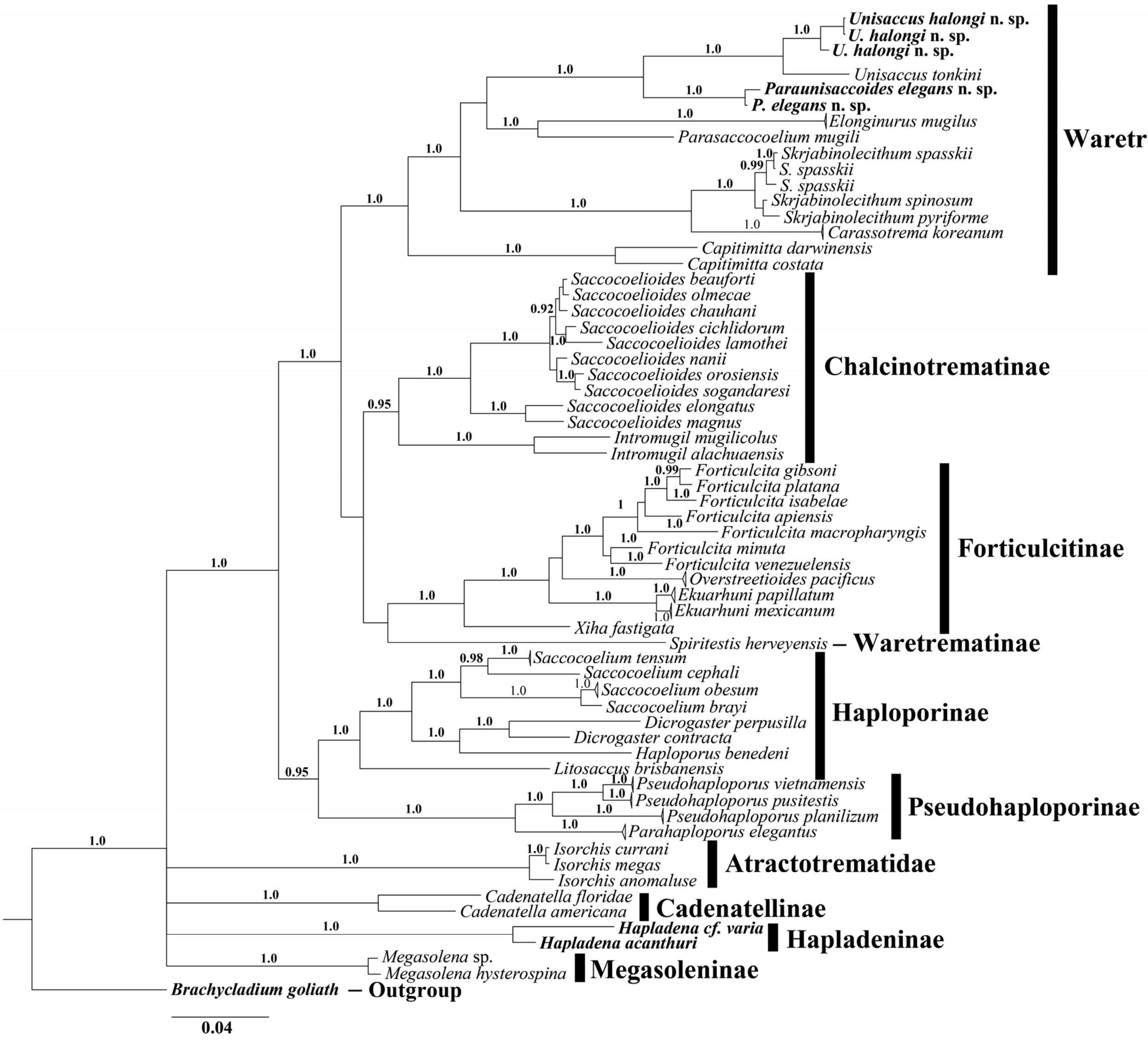

Figure 3. Phylogenetic relationships of the family Haploporidae obtained with Bayesian algorithm based on concatenated ribosomal ITS2 and partial 28S rRNA gene (alignment length 1460 bp). Nodal numbers—posterior probabilities that indicate statistical support of phylogenetic relationships, only significant values (0.9–1.0) are showed.

Within clade, the *p*-distance values between worms ex *Crenimugil seheli* and *U. tonkini* were 2.59 ± 0.52% and 3.54 ± 0.67% by 28S rDNA and ITS2 rDNA, respectively, which is compatible with the interspecific values for other haploporid genera by these molecular markers: 0.43 ± 0.19% to 4.85 ± 0.65% by 28S rDNA and 2.15 ± 0.56% to 5.86 ± 0.87% by 5.8S + ITS2 rDNA [4,8,23,24,36].

These data provide evidence that worms from our material represent distinct valid species. Despite the existence of morphological differences between worms ex Vietnamese *Crenimugil seheli* and representatives of *Unisaccus*, we prefer the molecular results and recognise Vietnamese worms as a new species within *Unisaccus*, *U. halongi* n. sp. However, as we said above, additional morphological and molecular data on *S. vitellosum* and *S. indicum* from type locations are needed to clarify the taxonomical status of these two species and new trematodes from our study.

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References

1. Overstreet, R.M.; Curran, S.S. Family Haploporidae Nicoll, 1914. In *Keys to the Trematoda*; Jones, A., Bray, R.A., Gibson, D.I., Eds.; CABI: Cambridge, MA, USA, 2005; Volume 2, pp. 129–165.
2. Pulis, E.E.; Overstreet, R.M. Review of haploporid (Trematoda) genera with ornate muscularization in the region of the oral sucker, including four new species and a new genus. *Syst. Parasitol.* **2013**, *84*, 167–191. [CrossRef]
3. Andres, M.J.; Pulis, E.E.; Cribb, T.H.; Overstreet, R.M. Erection of the haploporid genus *Litosaccus* n.g. and its phylogenetic relationship within the Haploporidae Nicoll, 1914. *Syst. Parasitol.* **2014**, *89*, 185–194. [CrossRef]
4. Besprozvannykh, V.V.; Atopkin, D.M.; Ermolenko, A.V.; Nikitenko, A.Y. 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.* **2015**, *89*, 565–576. [CrossRef]
5. Besprozvannykh, V.V.; Atopkin, D.M.; Ermolenko, A.V.; Nikitenko, A.Y. Morphometric and molecular analyses for a new species *Skrjabinolecithum pyriforme* n. sp. (Digenea: Haploporidae) in mullet fish from the Primorsky Region, Russia. *J. Helminthol.* **2017**, *91*, 625–632. [CrossRef]
6. Besprozvannykh, V.V.; Atopkin, D.M.; Ngo, H.D.; Ha, N.V.; Tang, N.V.; Beloded, A.Y. 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.* **2018**, *92*, 713–724. [CrossRef]
7. Andres, M.J.; Pulis, E.E.; Overstreet, R.M. Description of three species of *Isorchis* (Digenea: Atractotrematidae) from Australia. *Acta Parasitol.* **2016**, *61*, 590–601. [CrossRef]
8. Atopkin, D.M.; Besprozvannykh, V.V.; Ha, D.N.; Nguyen, V.H.; Nguyen, V.T.; Chalenko, K.P. 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. *Parasitol. Int.* **2018**, *69*, 17–24. [CrossRef]
9. Atopkin, D.M.; Besprozvannykh, V.V.; Ha, D.N.; Nguyen, V.H.; Khamatova, A.Y.; Vainutis, K.S. 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. *Parasitol. Res.* **2019**, *118*, 2129–2137. [CrossRef]
10. Atopkin, D.M.; Besprozvannykh, V.V.; Ha, D.N.; Nguyen, V.H.; Nguyen, V.T. New species and new genus of *Pseudohaploporinae* (Digenea): *Pseudohaploporus pusitestis* sp. n. and *Parahaploporus elegantus* n. g., sp. n. (Digenea: *Pseudohaploporinae*) from Vietnamese mullet fish. *Parasitol. Int.* **2020**, *75*, 102023. [CrossRef]
11. Atopkin, D.M.; Besprozvannykh, V.V.; Ha, D.N.; Nguyen, V.H.; Nguyen, V.T. New species of *Parasaccocoelium* (Haploporidae) and new genus *Pseudohaplosplanchnus* (Haplosplanchnidae) from mullet fish in the Far East of Russia and Vietnam: Morphological and molecular data. *J. Helminthol.* **2020**, *94*, e154. [CrossRef]
12. Truett, G.E. Preparation of genomic DNA from animal tissues. In *The DNA Book: Protocols and Procedures for the Modern Molecular Biology*; Kieleczawa, J., Ed.; Jones & Bartlett Publisher: Burlington, MA, USA, 2006; pp. 33–46.
13. Matejusova, I.; Cunningham, C.O. The first complete monogenean ribosomal RNA gene operon: Sequence and secondary structure of the *Gyrodactylus salaris* Malmberg, 1957, large subunit ribosomal RNA gene. *J. Parasitol.* **2004**, *90*, 146–151. [CrossRef] [PubMed]
14. Tkach, V.V.; Littlewood, D.T.J.; Olson, P.D.; Kinsella, J.M.; Swiderski, Z. Molecular phylogenetic analysis of the Microphalloidea Ward, 1901 (Trematoda: Digenea). *Syst. Parasitol.* **2003**, *56*, 1–15. [CrossRef] [PubMed]
15. Besprozvannykh, V.V.; Tatonova, Y.V.; Shumenko, P.G. Life cycle, morphology of developmental stages of *Metorchis ussuriensis* sp. nov. (Trematoda: Opisthorchiidae), and phylogenetic relationships of other opisthorchiids. *J. Zool. Syst. Evol. Res.* **2019**, *57*, 24–40. [CrossRef]
16. Luton, K.; Walker, D.; Blair, D. Comparisons of ribosomal internal transcribed spacers from two congeneric species of flukes (Platyhelminthes: Trematoda: Digenea). *Mol. Biochem. Parasitol.* **1992**, *56*, 323–327. [CrossRef]

17. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874. [CrossRef]
18. Huelsenbeck, J.P.; Ronquist, F.; Nielsen, R.; Bollback, J.P. Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* **2001**, *294*, 2310–2314. [CrossRef]
19. Posada, D. Using MODELTEST and PAUP* to select a model of nucleotide substitution. *Curr. Protoc. Bioinform.* **2003**, *6*, 6.5.1–6.5.14. [CrossRef]
20. Darriba, D.; Taboada, G.L.; Doallo, R.; Posada, D. jModeltest2: More models, new heuristics and parallel computing. *Nat. Methods* **2012**, *9*, 772. [CrossRef]
21. Atopkin, D.M.; Beloded, A.Y.; Ngo, H.D. Molecular genetic characterization of the far eastern trematode *Skrjabinolecithum spasskii* Belous, 1954, (Digenea, Haploporidae), a parasite of mullets. *Russ. J. Mol. Biol.* **2015**, *49*, 422–429. [CrossRef]
22. Atopkin, D.M.; Semenchenko, A.A.; Solodovnik, D.A.; Ivashko, Y.I.; Vinnikov, K.A. First next-generation sequencing data for Haploporidae (Digenea: Haploporata): Characterization of complete mitochondrial genome and ribosomal operon for *Parasaccocoelium mugili* Zhukov, 1971. *Parasitol. Res.* **2021**, *120*, 2037–2046. [CrossRef]
23. Blasco-Costa, I.; Balbuena, J.A.; Kostadinova, A.; Olson, P.D. Interrelationships of the Haploporinae (Digenea: Haploporidae): A molecular test of the taxonomic framework based on morphology. *Parasitol. Int.* **2009**, *58*, 263–269. [CrossRef] [PubMed]
24. Andrade-Gómez, L.; García-Valera, M. Unexpected morphological and molecular diversity of trematode (Haploporidae: Forticulcitinae) parasites of mullets from the ocean Pacific coasts in Middle America. *Parasitol. Res.* **2021**, *120*, 55–72. [CrossRef]
25. Andrade-Gómez, L.; González-García, M.T.; García-Valera, M. Phylogenetic affinities of Forticulcitinae (Haploporidae) parasites of mullet from the Americas, with the description of three new species and notes on the genera and key species. *Syst. Parasitol.* **2021**, *98*, 455–476. [CrossRef]
26. Andres, M.J.; Curran, S.S.; Fayton, T.J.; Pulis, E.E.; Overstreet, R.M. An additional genus and two additional species of Forticulcitinae (Digenea: Haploporidae). *Folia Parasitol.* **2015**, *62*, 025. [CrossRef] [PubMed]
27. Curran, S.S.; Tkach, V.V.; Overstreet, R.M. A review of *Polylekithum* Arnold, 1934 and its familial affinities using morphological and molecular data, with description of *Polylekithum catahoulensis* sp. nov. *Acta Parasitologica* **2006**, *51*, 238–248. [CrossRef]
28. Curran, S.S.; Pulis, E.E.; Andres, M.J.; Overstreet, R.M. 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.* **2018**, *104*, 221–239. [CrossRef]
29. Pulis, E.; Fayton, T.; Curran, S.; Overstreet, R. A new species of *Intromugil* (Digenea: Haploporidae) and redescription of *Intromugil mugilicolus*. *J. Parasitol.* **2013**, *99*, 501–508. [CrossRef]
30. Andres, M.J.; Pulis, E.E.; Curran, S.S.; Overstreet, R.M. On the systematics of some marine haploporids (Trematoda) with the description of a new species of *Megasolena* Linton, 1910. *Parasitol. Int.* **2018**, *67*, 805–815. [CrossRef]
31. Huston, D.C.; Cutmore, S.C.; Cribb, T.H. *Isorchis cannoni* n. sp. (Digenea: Atroctotrematidae) from Great Barrier Reef rabbitfishes and the molecular elucidation of its life cycle. *J. Helmointhology* **2018**, *92*, 604–611. [CrossRef]
32. Briscoe, A.G.; Bray, R.A.; Brabec, J.; Littlewood, D.T. The mitochondrial genome and ribosomal operon of *Brachycladium goliath* (Digenea: Brachycladiidae) recovered from a stranded minke whale. *Parasitol. Int.* **2016**, *65*, 271–275. [CrossRef]
33. Martin, W.E. A new genus and species of haploporid trematode (Haploporidae: Trematoda) from Australian mullet. *Bull. South. Calif. Acad. Sci.* **1973**, *72*, 166–168.
34. Martin, W.E. A new subfamily, two new genera, and three new species of haploporid trematodes. *Proc. Helminthol. Soc.* **1973**, *40*, 112–117.
35. Zhukov, E.V. New genera of trematodes from marine fishes of India. *Parazitologya* **1972**, *6*, 346–350.
36. Andrade-Gómez, L.; Pinacho-Pinacho, C.D.; Hernández-Orts, J.S.; Sereno-Uribe, A.L.; García-Valera, M. Morphological and molecular analyses of a new species of Saccocoelioidea Szidat, 1954 (Haploporidae Nicoll, 1914) in the fat sleeper Dominator maculates (Blosh) (Perciformes: Eleotridae) from the Gulf of Mexico. *J. Helminthol.* **2017**, *91*, 504–516. [CrossRef]