

Research Paper

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# Morphological and molecular data on *Pseudozoogonoides ugui* Shimazu, 1974 (Digenea: Microphalloidea: Zoogonidae) ex *Pseudaspius hakonensis* (Günther, 1877) and taxonomic problems in Zoogoninae genera

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## Abstract

New morphological and molecular data were generated for trematodes recovered from the intestines of the fish *Pseudaspius hakonensis* from two locations in the south of the Russian Far East. Morphologically, these trematodes are identical to *Pseudozoogonoides ugui* (Microphalloidea: Zoogonidae) from Japan. According to results of phylogenetic analysis based on 28S rDNA sequence data, *P. ugui* was closely related to *Zoogonoides viviparus*, and *P. subaequiporus* appears as a sister taxon to these two species. Genetic distance values, calculated based on both 28S rDNA and ITS2 rDNA, between *P. ugui* and *Z. viviparus* represents an interspecific differentiation level. Our results have an ambiguous explanation, indicating that the implication of the presence of one or two compact vitellaria aggregations for the differentiation of *Zoogonoides* and *Pseudozoogonoides* should be reconsidered or that our results open up the question of the taxonomical status of trematodes previously denoted as *Z. viviparus* and *P. subaequiporus*.

## Introduction

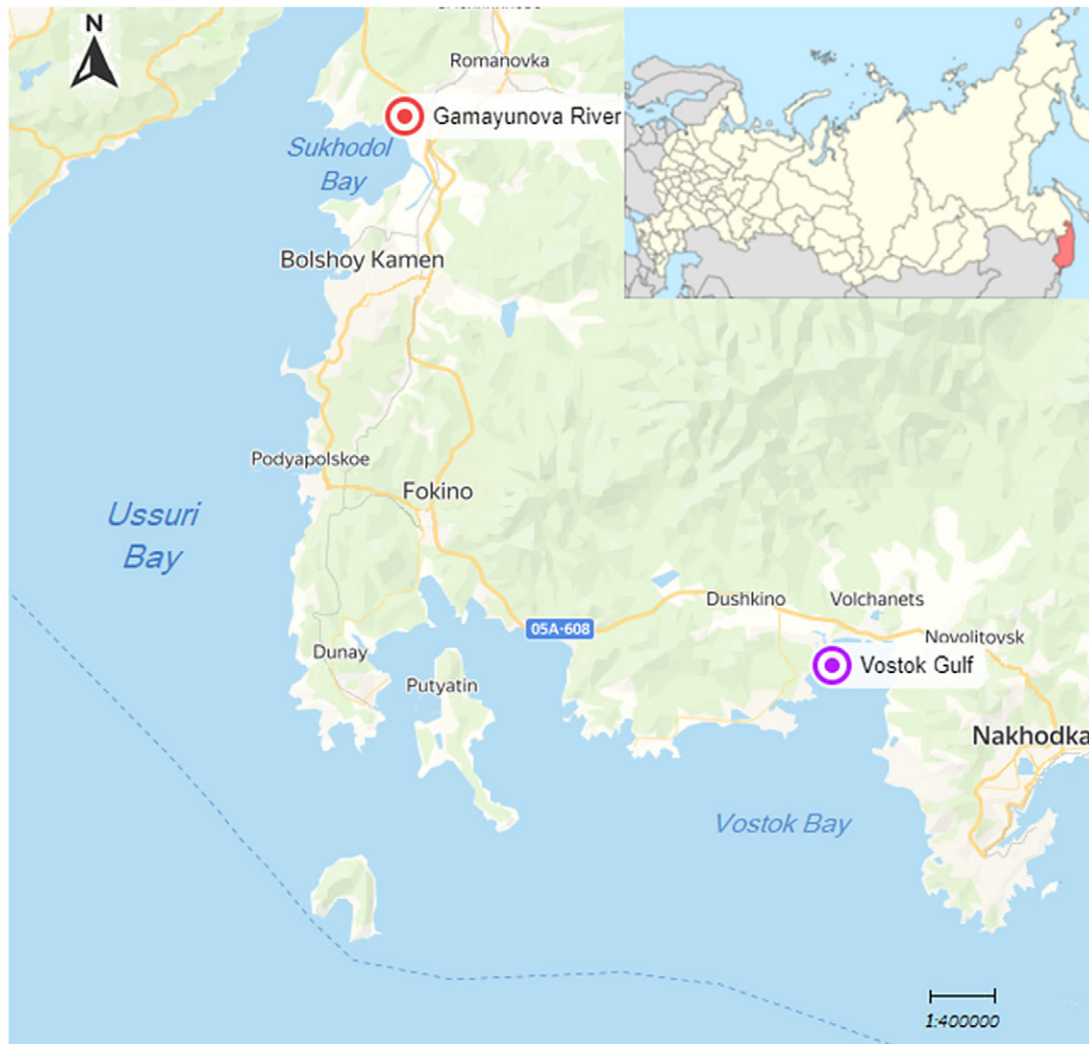
The family Zoogonidae Odhner, 1902 is one of the taxonomically problematic groups of trematodes, which was periodically revised on the basis of the morphological and biological features of its representatives (Bray and Gibson, 1986; Bray, 1986, Bray, 2008, Blend *et al.*, 2020; Sokolov *et al.*, 2021a, Kremnev *et al.*, 2023). Bray (2008) recognised two subfamilies within the family Zoogonidae: Zoogoninae Odhner, 1902 and Lepidophyllinae Stossich, 1903. Recently, the subfamilies Cephaloporinae Yamaguti, 1934 and Lecithostaphyllinae Odhner, 1911 were resurrected within Zoogonidae (Blend *et al.*, 2020; Sokolov *et al.*, 2021a). According to Bray (2008), the subfamily Zoogoninae comprises nine genera of intestinal trematodes of marine, anadromous, and amphidromic fishes. Among representatives of Zoogoninae, long-term studies mainly concern two species, *Zoogonoides viviparus* (Olsson, 1868) and *Pseudozoogonoides subaequiporus* (Odhner, 1911), which are type species for respective genera (Bray and Gibson, 1986, Bray, 1986). These species have a wide and similar composition of definitive host species and their areas are overlapping. Morphologically, representatives of these two genera differs by vitellaria structure and arrangement and extension of caeca (Bray, 1986). At present, according to the World Register of Marine Species database (<https://www.marinespecies.org/>), there are eight and two species represented within *Zoogonoides* and *Pseudozoogonoides*, respectively. The systematic of the subfamily Zoogoninae as well as the family Zoogonidae is mainly based on the morphology of adult worms. Molecular data for representatives of Zoogoninae are available only for *Pseudozoogonoides subaequiporus*, *Zoogonoides viviparus*, and *Diptherostomum* sp. (Olson *et al.*, 2003; Kremnev *et al.*, 2023). Results of several phylogenetic studies based on molecular data show that Zoogonidae is non-monophyletic, but formed strongly supported monophyly with representatives of Faustulidae; the problem actively discussed and still unresolved (Hall *et al.*, 1999; Sun *et al.*, 2014; Sokolov *et al.*, 2021a; 2021b).

In the present study, we provide new morphological and molecular data for trematodes of Zoogonidae recovered from fish *Pseudaspius hakonensis* (Günther, 1877) caught in Gamayunova River estuary and Vostok Gulf shell waters, south of the Russian Far East (Figure 1). Using these data, we performed species identification and reconstruction of the phylogenetic relationships of the studied trematodes within the Zoogonidae.

## Material and Methods

### Material collection

Three and ten fish specimens of *Pseudaspius hakonensis* were caught in the Gamayunova River estuary (43°13'01.9"N 132°22'47.7"E) and Vostok Bay shell waters (42°54'18.4"N 132°43'30.5"E),



**Figure 1.** The localities of collection of *Pseudaspis hakonensis* specimens on the south of Russian Far East territory. This map was prepared with the Yandex Map Constructor web service.

respectively. Adult trematodes were found in two and three fish specimens from respective localities. Infection intensity was 9 and 3 from fishes from the Gamayunova River estuary, and from 5 to 35 from the Vostok Bay fish specimens. Overall, 52 trematode specimens were collected.

### **Morphological analysis**

Worms were defined under a microscope using temporal slides preparation technique, rinsed in pure 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 in graded ethanol series and clearing in clove oil, followed by mounting the specimens in Canada balsam under coverslips on glass slides. All measurements of trematode morphometrics (range values) are given in micrometres.

### **DNA extraction, amplification and sequencing**

Overall, nine specimens of *Pseudozoogonoides ugui*, including seven from Vostok Bay and two from Gamayunova River, were

analysed with molecular approach. Total DNA of *Pseudozoogonoides ugui* was extracted from adult 96% ethanol-fixed specimen using a DNeasy Blood and Tissue kit (Qiagen, Toronto, ON, Canada) per the manufacturer's instructions. The polymerase chain reaction (PCR) amplification volume amounted to 10  $\mu$ L containing 5  $\mu$ L GoTaq Green Master Mix, 1  $\mu$ L each primer, 1  $\mu$ L DNA template and 3  $\mu$ L sterile deionised water. 28S ribosomal DNA (rDNA) was amplified with the primers 28SA (5'-TCG ATT CGA GCG TGA WTA CCC GC-3') (Matejusova and Cunningham, 2004) 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 primers were included. Products were sequenced using the internal sequencing primers described by Tkach *et al.* (2003) for 28S rDNA and Luton *et al.* (1992) for the ITS2 rDNA fragment. PCR products were directly sequenced using an ABI Big Dye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, USA) following the manufacturer's

recommendations. PCR product sequences were determined using an ABI 3500 genetic analyzer at the Federal Scientific Center of the East Asia Terrestrial Biodiversity FEB RAS. The sequences were submitted to the GenBank database (NCBI) (Table 1).

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

Species	Reference	Accession numbers
<b>Zoogonidae</b>		
Zoogoninae		
<b><i>Pseudozoogonoides ugui</i> (n = 9)</b>	<b>This study</b>	<b>PP317542 – PP317550</b>
<i>Pseudozoogonoides subaequiporus</i> (n = 6)	Kremnev <i>et al.</i> , 2023	OP956068– OP956073
<i>Zoogonoides viviparus</i> (n = 1)	Olson <i>et al.</i> , 2003	AY222271
<i>Zoogonoides viviparus</i> (n = 5)	Kremnev <i>et al.</i> , 2023	OP956058–OP956059, OP956061–OP956063
<i>Diptherostomum</i> sp. (n = 1)	Olson <i>et al.</i> , 2003	AY222272
Cephaloporinae		
<i>Plectognathotrema kamegii</i> (n = 1)	Cutmore <i>et al.</i> , 2014	KM505035
Lecithostaphylinae		
<i>Deretrema nahaense</i> (n = 1)	Olson <i>et al.</i> , 2003	AY222273
<i>Lecithostaphylus brayi</i> (n = 3)	Cabañas–Granillo <i>et al.</i> , 2020	MT704137– MT704139
<i>Lecithostaphylus halongi</i> (n = 2)	Atopkin <i>et al.</i> , 2022a	OK636406–OK636407
<i>Proctophantastes gillissi</i> (n = 2)	Sokolov <i>et al.</i> , 2016	KU163452– KU163453
<i>Steganoderma eamiqtrema</i> (Lecithostaphylinae s.l. <i>sensu</i> Sokolov <i>et al.</i> , 2021, n = 1)	Sokolov <i>et al.</i> , 2021a	MW264135
Lepidophyllinae		
<i>Lepidophyllum cameroni</i> (n = 1)	Sograna <i>et al.</i> , 2019, direct submission (unpublished)	MN217107
<i>Lepidophyllum steenstrupi</i> (n = 1)	Lockyer <i>et al.</i> , 2003	AY157175
<b>Faustulidae s. lato</b>		
<i>Antorchis pomacanthi</i> (n = 1)	Cribb <i>et al.</i> , 2015	KR149729
<i>Bacciger astyanactis</i> (n = 1)	Pérez–Ponce de León and Hernández–Mena, 2019	MK648283
<i>Bacciger major</i> (n = 1)	Cutmore <i>et al.</i> , 2018	MH476358
<i>Bacciger minor</i> (n = 1)	Cutmore <i>et al.</i> , 2018	MH476357

(Continued)

**Table 1.** (Continued)

Species	Reference	Accession numbers
<i>Bacciger lesteri</i> (n = 1)	Olson <i>et al.</i> , 2003	AY222269
<i>Bacciger potamaris</i> (n = 1)	Curran <i>et al.</i> , 2022	OM654762
<i>Pseudobacciger cheneyae</i> (n = 1)	Sun <i>et al.</i> , 2014	KJ648919
<i>Trigonocryptus conus</i> (n = 1)	Olson <i>et al.</i> , 2003	AY222270
<i>Pronoprimum petrowi</i> (n = 1)	Sokolov <i>et al.</i> , 2021b	MT872204
<i>Pronoprimum ventricosa</i> (n = 2)	Sokolov <i>et al.</i> , 2021b, Belousova <i>et al.</i> , 2023	MT872203, n/a
<b>Eucotylidae</b>		
<i>Paratanaisia bragai</i> (n = 1)	Unwin <i>et al.</i> , 2013	JX231099
<i>Tanaisia fedtschenkoi</i> (n = 1)	Olson <i>et al.</i> , 2003	AY116870
<b>Renicolidae</b>		
<i>Renicola keimahuri</i> (n = 1)	Galaktionov <i>et al.</i> , 2023	ON650720
<i>Renicola thapari</i> (n = 1)	Pérez–Ponce de León and Hernández–Mena, 2019	MK648311
<i>Renicola</i> sp. (n = 1)	Olson <i>et al.</i> , 2003	AY116871
<i>Renicola</i> sp. (n = 1)	O’Dwyer <i>et al.</i> , 2014	KJ868215
<b>Prosthogonimidae</b>		
<i>Prosthogonimus cuneatus</i> (n = 1)	Tkach <i>et al.</i> , 2003	AY220634
<i>Schistogonimus rarus</i> (n = 1)	Tkach <i>et al.</i> , 2003	AY116869
<b>Microphallidae</b>		
<i>Maritrema subdolum</i> (n = 1)	Tkach <i>et al.</i> , 2000	AF151926
<i>Microphallus similis</i> (n = 1)	Tkach <i>et al.</i> , 2003	AY220625
<b>Plagiariochidae</b>		
<i>Neoglyphe sobolevi</i> (n = 1)	Tkach <i>et al.</i> , 2001	AF300329
<i>Plagiariochis elegans</i> (n = 1)	Zikmundová <i>et al.</i> , 2014	KJ533393
<b>Hemiuroidea (outgroup)</b>		
<i>Aphanurus mugilis</i> (n = 1)	Atopkin <i>et al.</i> , 2017	LT607807

n/a, not yet available.

### Alignments and phylogenetic analysis

Ribosomal DNA sequences were assembled with SeqScapev.2.6 software provided by Applied Biosystems. Alignment and estimations of the number of variable sites and sequence differences were performed using MEGA 7.0 software (Kumar *et al.*, 2016). After the first alignment procedure, all used data were processed with the Gblocks Server ([http://phylogeny.lirmm.fr/phylo.cgi/one\\_](http://phylogeny.lirmm.fr/phylo.cgi/one_)

task.cgi?task\_type=gblocks). The values of genetic *p*-distances were calculated for the 28S rDNA and ITS2 rDNA fragments data set. The ITS2 sequence region had a length of 241 bp.

Phylogenetic analysis of the Zoogonidae was performed on the basis of the 28S rDNA dataset 1262 bp in length using the Maximum Likelihood (ML) and Bayesian Inference (BI) algorithms with the PhyML 3.1 and MrBayes 3.2.6. software, respectively (Guindon and Gascuel, 2003; Ronquist *et al.*, 2012). The best nucleotide substitution models, the TVM+I+G and GTR+I+G (Posada and Crandall, 1998), were estimated with jModeltest v. 2.1.5 software (Darriba *et al.*, 2012) for 28S rDNA for Bayesian (BIC criterion) and ML (AIC criterion) algorithms, respectively (Ronquist *et al.*, 2012; Akaike, 1974). Bayesian analysis was used with the following parameters: nst = 6, rates = gamma, Revmatpr = estimate, statefreqpr = estimate, shapepr = estimate and ngen = 1000000 via four simultaneous Markov Chain Monte Carlo chains (nchains = 4) with every 100th tree saved (samplefreq = 100) and two independent runs with the standard deviation of split frequencies at 0.0035. Summary parameters and the phylogenetic tree were calculated with a burn-in of 250,000 generations. Nodal support was estimated as posterior probabilities in the Bayesian inference analyses (Huelsenbeck *et al.*, 2001) and an approximate likelihood-ratio test (Anisimova and Gascuel, 2006) for the ML algorithm. Accession numbers, authority, and supporting information about 28S rDNA sequences from GenBank used for the phylogenetic analyses are provided in Table 1. *Plagiorchis elegans* and *Neoglyphe sobolevi* (Plagiorchioidea) were used as an outgroup. Accession numbers of the ITS2 sequences used for genetic distance calculation are: OP956013-OP956014, OP956017-OP956019 for *Z. viviparus* and OP956023-OP956028 for *P. subaequiporus*. The nucleotide sequences of ITS2 and 28S rDNA for trematodes *P. ugui* were submitted to the NCBI database with following accession numbers: PP317537-PP317541 and PP317542-PP317550, respectively.

## Results

### Description

*Pseudozoogonoides ugui* Shimazu, 1974

HOST: *Pseudaspis hakonensis* (Cyprinidae)

SITE: Intestine

LOCALITIES: Gamayunova River estuary, Primorsky Region, south of the Russian Far East, 43°13'01.9"N 132°22'47.7"E; Vostok Bay shell waters, Primorsky Region, south of the Russian Far East, 42°54'18.4"N 132°43'30.5"E.

Intensity of infection: 3–35 worms per fish

Extensiveness of infection: 30–66%

Materials deposited. Materials no. 243-248-Tr are deposited in the parasitological collection of the Zoological Museum (deposited 20 November 2023, Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia; e-mail: [petrova@biosoil.ru](mailto:petrova@biosoil.ru)).

### Adult worm (material examined: six specimens) (Table 2, Fig. 2)

Body fusiform. Tegument with spines. Oral sucker subterminal, round. Ventral sucker round, larger than oral sucker, equatorial. Prepharynx short. Pharynx round. Oesophagus long, bifurcating anteriorly to ventral sucker at level of midway between pharynx and ventral sucker. Caeca extending usually to level of posterior edge of ventral sucker. Testes opposite, longitudinally oval, at level of posterior half of ventral sucker and can be partially covered by

ventral sucker. Cirrus sac curved, expanded proximally, reaching to level of middle of ventral sucker. Internal seminal vesicle bipartite. Pars prostatica vesicular, prostate gland cells numerous, fill up most of cirrus sac. Ejaculatory duct with spines. Genital atrium shallow. Diverticle of genital atrium present, saccular. Genital pore sinistrally lateral at level of intestinal bifurcation. Ovary spherical, at level of testes, slightly dextral to median line and more or less covered by ventral sucker. Seminal receptacle just posterior to vitellarium. Uterus occupying all posttesticular space. Metraterm tubular, without spines, anterior to ventral sucker. Eggs oval, with very thin capsules. Vitellarium composed of two symmetrical compact masses between ovary and seminal receptacle. Excretory vesicle saccate, pore terminal.

### Molecular results

Results of ML and Bayesian phylogenetic analysis based on the 28S rDNA sequence data set showed similar tree topologies (Figure 3). *Pseudozoogonoides ugui* was within the clade together with *Z. viviparus* and *P. subaequiporus*. These three species formed a monophyletic clade with *Diptherostomum* sp., representing the subfamily Zoogoninae. Genetic *p*-distance values between *P. ugui* and *Z. viviparus* were 0.85±0.27% based on 28S sequence data, whereas genetic differentiation by ITS2 sequence data between these two species *P. ugui* and *Z. viviparus* was 1.39±0.59%. Based on 28S rDNA sequence dataset, *P. subaequiporus* had 7.19±0.7% and 6.94±0.67% differences with *P. ugui* with *Z. viviparus*, respectively. These results corresponded to results based on ITS2 rDNA sequence dataset: *p*-distance values were 10.89±1.6% and 9.78±1.54% for *P. subaequiporus*/*P. ugui* and *P. subaequiporus*/*Z. viviparus* species pairs, respectively.

## Discussion

### Taxonomical status of Zoogonoides and Pseudozoogonoides

The morphological characteristics of the adult worms detected in Japanese dace *P. hakonensis* in our study correspond to those of the Zoogonidae diagnosis. In particular, these specimens are most similar to *Zoogonoides* and *Pseudozoogonoides* species. Representatives of these two genera have compatible morphology but differ from each other by the presence of one or two compact vitellarial aggregations, respectively (Bray, 2008). Based on this characteristic, worms from our material belong to the genus *Pseudozoogonoides* and are morphologically similar to *P. subaequiporus* and *P. ugui*. However, morphometrical characteristics of these worms are not efficient enough for species delimitation, except for ventral sucker size and oral/ventral sucker size ratio, which are larger for *P. ugui* than for *P. subaequiporus* (Table 2). Worms obtained in this study and *P. ugui* have no differences in most metric parameters; there are only minor discrepancies in some points of the metrics presented (measurements of body, testes and vitellarium). The conspecificity of these trematodes is confirmed by the presence of a spined ejaculatory duct, whereas in *P. subaequiporus* the ejaculatory duct has no spines. Based on these results, we conclude that trematodes recovered from *P. hakonensis* in the south of the Russian Far East belong to *Pseudozoogonoides ugui*. Moreover, *P. ugui* previously discovered in the River Nukui near Gabino, Hokkaido, Japan, by Shimazu (1974) was obtained in the same definitive host species, and this locality is close to coastal waters of the Japan Sea of the East Asian region where our trematodes were collected. Contrarily, *Pseudozoogonoides subaequiporus* is known to infect fish species

**Table 2.** Measurements ( $\mu\text{m}$ ) of adult worms of *Pseudozoogonoides* species

	<i>Pseudozoogonoides ugui</i> (This study)	<i>Pseudozoogonoides ugui</i> (Shimazu, 1974)	<i>Pseudozoogonoides subaequiporus</i> (Bray and Gibson, 1986)*
Body length	816–939	1020–1460	650–1040
Body width	370–416	440–540	200–400
Oral sucker length	150–169	120–170	90–180
Oral sucker width	154–169	150–190	90–170
Forebody	277–390	—	—
Ventral sucker length	219–270	210–250	100–180
Ventral sucker width	250–266	200–270	100–180
Prepharynx	0–12	—	—
Pharynx length	50–54	40–70	34–55
Pharynx width	50–54	40–70	34–55
Oesophagus length	73–100	90–180	20–75
Ovary length	95–119	110–170	60–130
Ovary width	95–116	90–150	50–110
Testis length right	123–142	150–220	60–150
Testis width right	77–116	90–170	40–110
Testis length left	123–135	120–190	60–150
Testis width left	73–116	100–130	40–110
Cirrus sac length	231–308	200–340	210–480
Cirrus sac width	69–77	60–80	35–80
Metroterm	73–96	—	—
Vitellarium length right	46–50	50–80	—
Vitellarium width right	46–55	60–140	—
Vitellarium length left	39–46	—	—
Vitellarium width left	42–58	—	—
Posttesticular fields length	289–370	—	—
Eggs length	62–77	76–93	53–80
Eggs width	27–35	29–35	20–32
Length/width (eggs), %	40.7–49.0	—	—
Forebody/body length ratio, %	33.9–42.9	—	1:0.27–0.38
Suckers' length ratio	1:1.46–1.60	1: 1.28–1.78	1:0.98–1.30
Suckers' width ratio	1.48–1.73	—	—

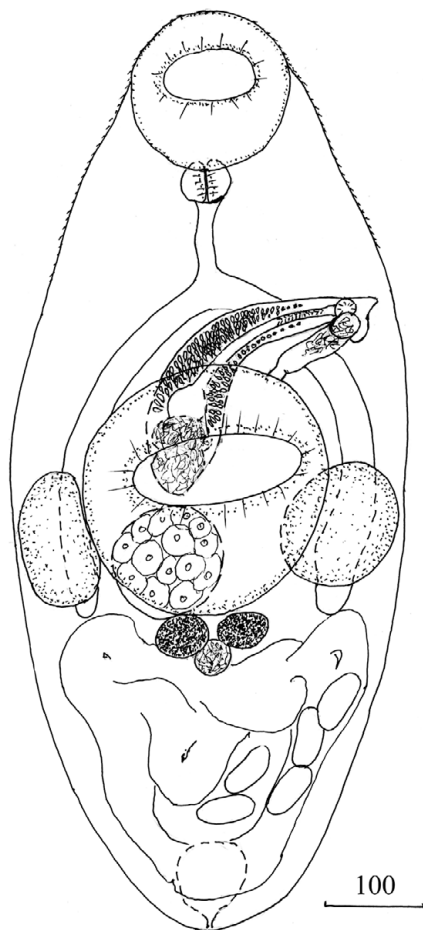
\*Unificated data.

of Anarhichadidae Bonaparte, 1832 from the North-Eastern Atlantic. Thus, these species have both different hosts and are geographically isolated.

Results of the phylogenetic analysis and genetic distance calculation indicate that *P. ugui* and *Z. viviparus* have interspecific differentiation level. Moreover, these species should be considered members of the same genus. In contrast, both species significantly differed from *P. subaequiporus* both on 28S rDNA and the ITS2 rDNA fragment; the differentiation values between these taxa corresponded to intergeneric level. However, according to generic diagnostic characteristics from Bray (2008), namely, vitellarium structure, these trematodes belong to the different genera, and *P. ugui* was expected to be closely related to *P. subaequiporus*. Thus,

there is an obvious disagreement between morphological and molecular data in the question of the generic taxonomy of the studied trematodes.

The first molecular data for *Zoogonoides* were presented by Olson *et al.* (2003), who provided the 28S rDNA sequence for *Zoogonoides viviparus* within the phylogenetic analysis of Digenea. Later, 28S rDNA and ITS2 rDNA sequence data were provided for *Z. viviparus* and *Pseudozoogonoides subaequiporus* from the White Sea basin (Kremnev *et al.*, 2023). Unfortunately, Olson *et al.* (2003) did not describe morphological characteristics to confirm that the worm they analysed belonged to *Z. viviparus*. Kremnev and co-authors (2023) carried out a detailed molecular analysis of representatives of different developmental stages for species



**Figure 2.** Adult worm of *Pseudozoogonoides ugui* ex *Pseudaspis hakonensis* from the Gamayunova River estuary, Primorsky Region, Russia. Scale bars:  $\mu\text{m}$ .

recovered from the first and second intermediate hosts and were denoted as *Z. viviparus* and *P. subaequiporus*. Morphological characterization for sporocysts and cercariae and metric parameters for cercariae, metacercariae and adult worms for both species were provided as well. However, morphological characteristics for adult worms that confirm their membership to *Zoogonoides* or *Pseudozoogonoides* were not presented. We do not exclude that in the study of Kremnev *et al.* (2023) species identification of adult worms denoted as *Z. viviparus* was performed based on the identity of 28S rDNA sequence data with that provided by Olson *et al.* (2003) for this species. Accepting the high morphological similarity of *Zoogonoides* and *Pseudozoogonoides*, which only differ in the structure of vitellarium, it is difficult to estimate the generic membership of trematodes, as reported in Olson *et al.* (2003) and Kremnev *et al.* (2023) because of the absence of morphological data on adult worms in their studies. Accepting these results and the molecular data based taxonomical validity of trematodes denoted as *P. subaequiporus*, reported by Kremnev *et al.* (2023), the presence of one or two compact vitellarial aggregations cannot be accepted as a reliable character for the differentiation of *Zoogonoides* and *Pseudozoogonoides*. In this case, differentiation of *Zoogonoides* and *Pseudozoogonoides* can be implemented only based on the molecular data. Based on the assumption that Olson *et al.* (2003) and Kremnev *et al.* (2023) performed incorrect taxonomical identification of zoogonid trematodes, the question of the species and generic taxonomical status of trematodes denoted as *Z. viviparus*

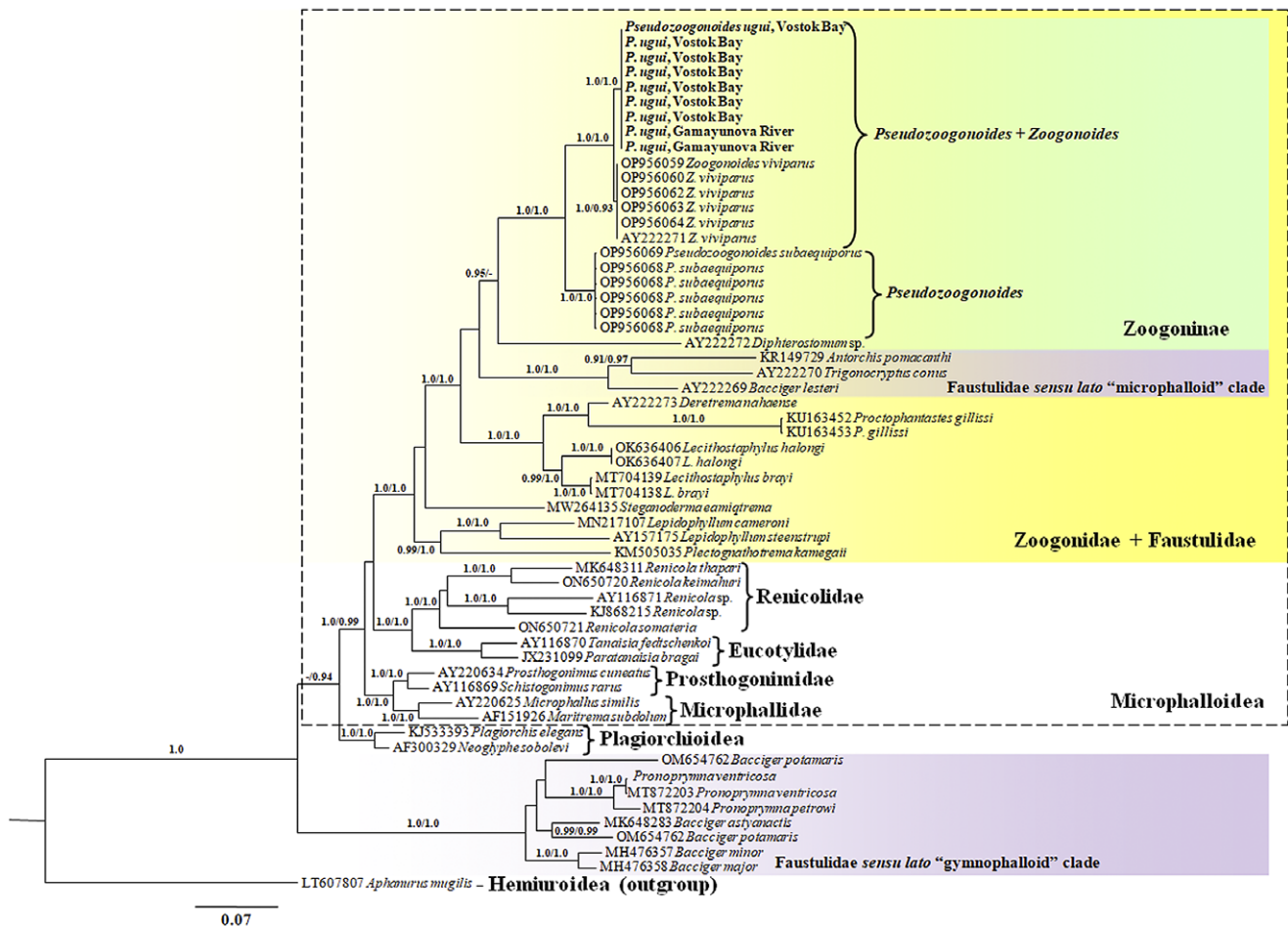
and *P. subaequiporus* remains open. A final conclusion with respect to this issue can only be made by analysing combined morphological and molecular data for trematodes *Z. viviparus* and *P. subaequiporus* from their hosts caught in type localities.

### Phylogenetic relationships of Microphalloidea

Results of ML and BI phylogenetic analysis based on the 28S rDNA sequence data set showed that Microphalloidea Ward, 1901 is polyphyletic; members of this superfamily were divided into two clades. Most Microphalloidea specimens were gathered within a large monophyletic clade, which includes five families of true Microphalloidea and members of the 'microphalloid' clade of the family Faustulidae Poche, 1926 *sensu lato*, which was closely related to the Zoogoninae with poor support on the both ML and BI trees. Families Rencolidae Dollfus, 1939 and Eucotyliidae Skrjabin, 1924 were closely related to each other within a separate subclade, as well as Microphallidae Ward, 1901 and Prosthogonimidae Lühe, 1909.

The representatives of the 'gymnophalloid' clade of Faustulidae *s. lato*, the species of the genus *Pronoprymna* Poche, 1926, three species of the genus *Bacciger* Nicoll, 1914, and *Pseudobacciger cheneyae* Sun, Bray, Yong, Cutmore and Cribb, 2014, formed separate clade that was highly divergent from Microphalloidea.

Results of phylogenetic analysis based on the 28S rDNA sequence data set repeated the polyphyly for the family Faustulidae *s. lato*, as revealed in the previous studies (Sun *et al.*, 2014; Cutmore *et al.*, 2018; Sokolov *et al.*, 2021b; Belousova *et al.*, 2023). The polyphyly was expressed in the existing two distant clades of this family: the 'microphalloid' clade, which is closer to Microphalloidea, and the 'gymnophalloid' clade, which is closer to Gymnophalloidea. The problem of the polyphyly of the Faustulidae *s. lato* directly concerns the polyphyly of the genus *Bacciger*, whose members appeared both within the 'microphalloid' (*B. lesteri*) and 'gymnophalloid' (*B. major*, *B. minor*, *B. astyanactis*) clades. De Montaudouin *et al.* (2014) and later Cutmore *et al.* (2018) provided conclusive evidence that *Bacciger* species from the 'gymnophalloid' clade are close to the type species *Bacciger bacciger* (Rudolphi, 1819) Nicoll, 1914 based on the ITS2 rDNA sequence data set. Sokolov *et al.* (2021b) discussed in detail the polyphyletic genus *Bacciger sensu lato*, concluding that there are not enough molecular data to be representative for adequate phylogenetic analysis and taxonomical interpretations for both 'gymnophalloid' and 'microphalloid' clades of Faustulidae *sensu lato*. Nevertheless, Curran *et al.* (2022) proposed a concept of taxonomical status *incertae cedis* for *Bacciger lesteri* Bray, 1982 within Microphalloidea, removing *Bacciger sensu stricto* and *Pseudobacciger* Nahhas & Cable, 1964 from the Faustulidae and transferring them to the Gymnophalloidea with status *incertae cedis*. Moreover, according to the viewpoint of Curran *et al.* (2022), *B. lesteri* and close species *Antorchis pomacanthi* (Hafeezullah and Siddiqi, 1970) Machida, 1975 from the 'microphalloid' clade have shared morphological features. In our view, the problem is hidden in the absence of morphological description of trematodes for which molecular data were provided. Namely, the validity of *Bacciger lesteri* and *Trigonocryptus conus* Martin, 1958 from the study of Olson *et al.* (2003) was not confirmed morphologically. For this reason, we cannot know for certain with what species we deal. Such confusion was observed in the previous phylogenetic studies of Bucephalidae Poche, 1907 and Lissorchiidae Magath, 1917 (Atopkin *et al.*, 2022b; 2023). We propose that final conclusions about the taxonomical status of the 'microphalloid' and 'gymnophalloid' clades of the Faustulidae *s. lato*, as well as familiar membership of species from the 'microphalloid' clade, can



**Figure 3.** Phylogenetic relationships reconstruction of the Microphalloidea, based on partial 28S rRNA gene sequence dataset. Nodal numbers - posterior probabilities that indicate statistical support of phylogenetic relationships for the Maximum Likelihood/the Bayesian algorithms; only significant values (0.9–1.0) are shown. Scale: average number of nucleotide substitution per site.

be reached after the availability of complex morphological and molecular data on all species from these two clades, especially *B. lesteri*, and a detailed comparative morphological analysis of this species with representatives of the genus *Antorchis* Linton, 1911.

## Conclusion

Results of this study show that taxonomical status of the two zoogonid genera *Zoogonoides* and *Pseudozoogonoides* are unclear. The taxonomical problem of *Faustulidae s. lato* is far from being resolved as well. In our view, these questions arise because of fragmental and discrete data on general morphology and nucleotide sequences are available for representatives of previously mentioned taxa, and these data are not mutually complementary. In this respect we completely agree with the point of Sokolov *et al.* (2021b). Final conclusions can be made after morphological and molecular data are revised simultaneously for respective trematode species from type localities and hosts.

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## References

- Akaike H (1974) A new look at the statistical model identification. *IEEE Transactions and Automatic Control* **19**, 716–723.
- Anisimova M and Gascuel O (2006) Approximate likelihood-ratio test for Branches: a fast, accurate and powerful alternative. *Systematic Biology* **55**, 539–552.
- Atopkin DM, Besprozvannykh VV, Beloded AY, Ngo HD, Ha NV and Tang NV (2017) Phylogenetic relationships of Hemiuridae (Digenea: Hemiuroidea) with new morphometric and molecular data of *Aphanurus mugilis* Tang, 1981 (Aphanurinae) from mullet fish of Vietnam. *Parasitology International* **66**, 824–830.
- Atopkin DM, Besprozvannykh VV, Ha ND, Nguyen VH and Nguyen VT (2022a) New trematode species *Lecithostaphyllus halongi* n. sp. (Zoogonidae, Microphalloidea) and *Gymnoterhestia strongyluri* n. sp. (Fellodistomidae, Gymnophalloidea) from beloniform fishes in Vietnam. *Journal of Helminthology* **96**, e15, 1–9.
- Atopkin DM, Shedko MB, Rozhkovan KV, Nguyen HV and Besprozvannykh VV (2022b) *Rhipidocotyle husi* n. sp. and three known species of Bucephalidae Poche, 1907 from the East Asian Region: morphological and molecular data. *Parasitology* **149**, 774–785.
- Atopkin DM, Ivashko YI, Besprozvannykh VV and Zhokhov AE (2023) New species of Asymphyllodorinae Szidat, 1943 (Digenea: Lissorchiidae), fish

- parasites from the East Asian Region: morphological and molecular data. *Systematics and Biodiversity* **21**, 2286947.
- Belousova YV, Atopkin DM and Vodiadova EA** (2023) The first modern morphological description of *Cercaria pennata* and molecular evidence of its synonymy with *Pronoprymna ventricosa* in the Black Sea. *Journal of Helminthology* **97**, e12, 1–8.
- Blend CK, Racz GR and Gardner SL** (2020) *Gaharitrema droneni* n. gen., n. sp. (Digenea: Zoogonidae) from the pudgy cuskeel, *Spectrunculus grandis* (Ophidiiformes: Ophidiidae), from deep waters off Oregon, with updated keys to Zoogonid subfamilies and genera. *Journal of Parasitology* **106**, 235–246.
- Bray RA** (2008) Family Zoogonidae Odhner, 1902. Pp. 605–629 in Bray R.A., Gibson D.I. and Jones A. (Eds) *Keys to the Trematoda*. Vol. 3. CABI Publishing and Natural History Museum, Wallingford.
- Bray RA** (1986) A revision of the family Zoogonidae Odhner, 1902 (Platyhelminthes: Digenea): introduction and subfamily Zoogoninae. *Systematic Parasitology* **9**, 3–28.
- Bray RA and Gibson DI** (1986). The Zoogonidae (Digenea) of fishes from the north-east Atlantic. *Bulletin of the British Museum (Natural History) (Zoology Series)* **51**, 127–206.
- Cabañas-Granillo J, Solórzano-García B, Mendoza-Garfias B and Pérez-Ponce de León G** (2020) A new species of *Lecithostaphylus* Odhner, 1911 (Trematoda: Zoogonidae) from the Pacific needlefish, *Tylosurus pacificus*, off the Pacific coast of Mexico, with a molecular assessment of the phylogenetic position of this genus within the family. *Marine Biodiversity* **50**, 83.
- Cribb TH, Bray RA, Hall KA and Cutmore SC** (2015) A review of the genus *Antorchis* Linton, 1911 (Trematoda: Faustulidae) from Indo-West fishes with the description of a new species. *Systematic Parasitology* **92**, 1–11.
- Cutmore SC, Miller TL, Bray RA and Cribb TH** (2014) A new species of *Plectognathotrema* Layman, 1930 (Trematoda: Zoogonidae) from an Australian monacanthid, with a molecular assessment of the phylogenetic position of the genus. *Systematic Parasitology* **89**, 237–246.
- Cutmore SC, Bray RA and Cribb TH**. (2018) Two new species of *Bacciger* Nicoll, 1914 (Trematoda: Faustulidae) in species of *Herklotsichthys* Whitley (Clupeidae) from Queensland waters. *Systematic Parasitology* **95**, 645–654.
- Curran SS, Warren B and Bullard S** (2022) Description of a new species of *Bacciger* (Digenea: Gymnophalloidea) infecting the American gizzard shad, *Dorosoma cepedianum* (Lesueur, 1818), and molecular characterization of *Cercaria rangiae* Wardle, 1983, with molecular phylogeny of related Digenea. *Comparative Parasitology* **89**, 9–29.
- Darriba D, Taboada GL, Doallo R and Posada D** (2012) jModeltest2: more models, new heuristics and parallel computing. *Nature Methods* **9**, 772.
- De Montaudouin X, Bazairi H, Mlik, KA and Gonzalez P** (2014) *Bacciger bacciger* (Trematoda: Fellodistomidae) infection effects on wedge clam *Donax trunculus* condition. *Diseases of Aquatic Organisms* **111**, 259–267.
- Galaktionov KV, Solovyeva AI, Blakeslee AMH and Skirnisson K** (2023) Overview of renicolid digeneans (Digenea, Rencolidae) from marine gulls of northern Holarctic with remarks on their species statuses, phylogeny and phylogeography. *Parasitology* **150**, 55–77.
- Guindon S and Gascuel O** (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology* **52**, 696–704.
- Hall KA, Cribb TH and Barker SC** (1999) V4 region of small subunit rDNA indicates polyphyly of the Fellodistomidae (Digenea) which is supported by morphology and life-cycle data. *Systematic Parasitology* **43**, 81–92.
- Huelsenbeck JP, Ronquist F, Nielsen R and Bollback JP** (2001) Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* **294**, 2310–2314.
- Kremnev G, Gonchar A, Uryadova A, Krapivin V, Skobkina O, Gubler A and Krupenko D** (2023) No tail no fail: life cycles of the Zoogonidae (Digenea). *Diversity* **15**, 121.
- Kumar S, Stecher G and Tamura K** (2016) MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. *Molecular Biology and Evolution* **33**, 1870–1874.
- Lockyer AE, Olson PD, and Littlewood DTJ** (2003) Utility of complete large and small subunit rDNA genes in resolving the phylogeny of the Neodermata (Platyhelminthes): implications and a review of the cercomer theory. *Biological Journal of the Linnean Society*, **78**, 155–171.
- 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.
- Matejusova I and Cunningham CO** (2004) The first complete monogenean ribosomal RNA gene operon: sequence and secondary structure of the *Gyrodactylus salaris* Malmberg, 1957, large subunit ribosomal RNA gene. *Journal of Parasitology* **90**, 146–151.
- O'Dwyer K, Blasco-Costa I, Poulin R and Faltynkova A** (2014) Four marine digenean parasites of *Austrolittorina* spp. (Gastropoda: Littorinidae) in New Zealand: morphological and molecular data. *Systematic Parasitology* **89**, 133–152
- 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.
- Pérez-Ponce de León G and Hernández-Mena DI** (2019) Testing the higher-level phylogenetic classification of Digenea (Platyhelminthes, Trematoda) based on nuclear rDNA sequences before entering the age of the 'next-generation' Tree of Life. *Journal of Helminthology* **93**, 260–276.
- Posada D and Crandall KA** (1998) Modeltest: testing te model of DNA substitution. *Bioinformatics* **14**, 817–818.
- Ronquist F, Teslenko M, Mark PVD, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA and Huelsenbeck JP** (2012) MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**, 539–542.
- Shimazu T** (1974) *Pseudozoogonoides ugu* sp. nov., a new digenetic trematode from the dace, *Tribolodon hakonensis*, from Hokkaido, Japan (Trematoda: Zoogonidae). *Bulletin of the Japanese Society of Scientific Fisheries*, **40**: 433–438.
- Sokolov S, Gordeev I and Lebedeva D** (2016) Redescription of *Proctophantases gillissi* (Overstreet et Pritchard, 1977) (Trematoda: Zoogonidae) with discussion on the systematic position of the genus *Proctophantases* Odhner, 1911. *Acta Parasitologica* **61**, 529–536.
- Sokolov S, Shchenkov S, Gordeev I and Ryazanova T** (2021a) Description of a metacercaria of a zoogonid trematode *Steganoderma* cf. *eamiqtrema* Blend and Racz, 2020 (Microphalloidea: Zoogonidae), with notes on the phylogenetic position of the genus *Steganoderma* Stafford, 1904, and resurrection of the subfamily Lecithostaphylinae Odhner, 1911. *Parasitology Research* **120**, 1669–1676.
- Sokolov SG, Shchenkov, SV and Gordeev II** (2021b) A phylogenetic assessment of *Pronoprymna* spp. (Digenea: Faustulidae) and Pacific and Antarctic representatives of the genus *Steringophorus* Odhner, 1905 (Digenea: Fellodistomidae), with description of a new species. *Journal of Natural History* **55**, 867–887.
- Sun D, Bray RA, Yong R Q-Y, Cutmore SC and Cribb TH** (2014) *Pseudo-bacciger cheneyae* n. sp. (Digenea: Gymnophalloidea) from Weber's chromis (*Chromis weberi* Fowler & Bean) (Perciformes: Pomacanthidae) at Lizard Island, Great Barrier Reef, Australia. *Systematic Parasitology* **88**, 141–152.
- Tkach VV, Pawlowski J and Matiaux J** (2000) Phylogenetic analysis of the suborder Plagiorchiata (Platyhelminthes, Digenea) based on partial lsrDNA sequences. *International Journal for Parasitology* **30**, 83–93.
- Tkach V, Grabda-Kazubska B and Swiderski Z** (2001) Systematic position and phylogenetic relationships of the family Omphalometridae (Digenea, Plagiorchiida) inferred from partial lsrDNA sequences. *International Journal for Parasitology* **31**, 81–85.
- 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.
- Unwin S, Chantrey J, Chatterton J, Aldhoun JA and Littlewood DT** (2013) Renal trematode infection due to *Paratanaisia bragai* in zoo housed Columbigiformes and a red bird-of-paradise (*Paradisaea rubra*). *International Journal for Parasitology Parasites Wild life* **2**, 32–41.
- Zikmundová J, Georgieva S, Faltynková A, Soldánová M and Kostadinova A** (2014) Species diversity of *Plagiorchis* Lühe, 1899 (Digenea: Plagiorchiidae) in lymnaeid snails from freshwater ecosystems in central Europe revealed by molecules and morphology. *Systematic Parasitology* **88**, 37–54.