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ORIGINAL PAPER

Microphallus ochotensis sp. nov. (Digenea, Microphallidae) and relative merits of two-host microphallid life cycles

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Abstract

A new digenean species, *Microphallus ochotensis* sp. nov., was described from the intestine of Pacific eiders (*Somateria mollissima v-nigrum*) from the north of the Sea of Okhotsk. It differs from other microphallids in the structure of the metraterm, which consists of two distinct parts: a sac with spicule-like structures and a short muscular duct opening into the genital atrium. *Mi. ochotensis* forms a monophyletic clade together with other congeneric species in phylograms derived from the 28S and ITS2 rRNA gene. Its dixenous life cycle was elucidated with the use of the same molecular markers. Encysted metacercariae infective for birds develop inside sporocysts in the first intermediate host, an intertidal mollusc *Falsicingula kurilensis*. The morphology of metacercariae and adults was described with an emphasis on the structure of terminal genitalia. Considering that *Falsicingula* occurs at the Pacific coast of North America and that the Pacific eider is capable of trans-continental flights, the distribution of *Mi. ochotensis* might span the Pacific coast of Alaska and Canada. The range of its final hosts may presumably include other benthos-feeding marine ducks as well as shorebirds. We suggest that a broad occurrence of two-host life cycles in microphallids is associated with parasitism in birds migrating along sea coasts. The chances that migrating birds would stop at a site where both first and second intermediate hosts occur are relatively low. The presence of a single molluscan host in the life cycle increases the probability of transmission.

Keywords Digenea · Microphallidae · Trematoda · Marine parasites · Life cycle · Molecular phylogeny · Pacific distribution · Marine ducks

Introduction

The Microphallidae is a unique family of digenetic trematodes. Their evolution has been closely linked with marine coastal ecosystems and migratory birds (Belopol'skaya 1963; Deblock 1971; Galaktionov 1993). The biology of these birds, in particular the fact that many of them stop at coastal sites for a short time only, has shaped the adaptations of microphallids. All stages of their life cycles are characterised by a high degree of juvenilisation (a general simplification of morphology and physiology) and miniaturisation (Galaktionov and Dobrovolskij 2003). The miracidium hatches only after the egg gets into the gut of the molluscan host. Its germinal material contains one or two germ cells and several non-differentiated cells. The mother generation of parthenitae is represented by stolon-like germinal masses developing from the germinal material of the miracidium (Galaktionov and Dobrovolskij 1985; Galaktionov 1993). The germinal masses produce numerous daughter sporocysts. Cercariae developing in the sporocysts have a simple excretory formula, an underdeveloped ventral sucker and an undifferentiated germinal primordium but possess a specialised set of provisional structures for searching and penetrating the second intermediate host (tail with striated musculature, differentiated penetration glands and tegumental glands, the stylet) (reviewed in Galaktionov 1991a; Galaktionov and Dobrovolskij 2003; Galaktionov and Skírnisson 2007).

In the second intermediate host, mostly represented by crustaceans, the larvae undergo a complex and prolonged morphogenesis (Belopol'skaya 1963; Galaktionov and

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Malkova 1993, 1994, 1995; Galaktionov et al. 1996; Galaktionov 1991a, 1993; Galaktionov and Dobrovolskij 2003). The metacercariae develops a complete, but yet non-functioning, reproductive system. Thus, the adult can start egg production as early as few hours after the infection of the final host, dispersing eggs in the same biotope where the host has been infected (Saville and Irwin 1991; Galaktionov 1993; Field et al. 1998). The lifespan of adults in the host is short (5–10 days), which is due to their extreme juvenilisation and miniaturisation (reviewed in Belopol'skaya 1983; Galaktionov 1993; Galaktionov and Dobrovolskij 2003). However, it is sufficient for the worms to complete their life cycle at stopovers of migrating birds, minimising losses due to their removal with the bird hosts outside the region of possible transmission (Belopol'skaya 1983; Galaktionov 1993).

In general, three-host (trixenous) life cycles are typical of trematodes. However, several phylogenetic lineages of microphallids have independently evolved a truncation of transmission pathways resulting in two-host (dixenous) life cycles. This means that cercariae do not leave the molluscan host. They transform (encysting or not encysting) into metacercariae infective for the final host directly in the mollusc (Belopol'skaya 1963; Deblock 1977, 1980; Galaktionov 1991b; Montoliu et al. 1992; Galaktionov and Skírnisson 2007).

Microphallids are a dominant group of digeneans in the coastal waters of Palaearctic seas, demonstrating the greatest species diversity and the highest infection levels in final and intermediate hosts (Galaktionov 1993). Nevertheless, while in Atlantic seas their fauna and life cycles have been extensively investigated (e.g. Reimer 1963; James 1968, 1969; Deblock 1980; Lauckner 1983; Galaktionov 1983, 1984, 1988, 1989; Montoliu et al. 1992), similar studies in the North Pacific are scarce. Until recently, the only such studies on Asian coasts have been those by Tsimbalyuk and colleagues dating back to the 1960–1970s (Tsimbalyuk et al. 1968a, b, 1978; Kulikov et al. 1970; Pois et al. 1974). Ching (reviewed in Ching 1991) carried out her research at approximately the same time at the coast of the British Columbia. There are just a handful of more recent studies on digeneans circulating in the coastal waters of the North Pacific (Miura et al. 2005; Galaktionov 2007; Galaktionov et al. 2010, 2012). This is all the more regrettable, as the biodiversity of digeneans in this region is high. Furthermore, the centres of origin of several taxa, including the Microphallidae, are hypothesised to be associated with this region (Belopol'skaya 1983). From there, they may have expanded into the North Atlantic after the opening of the Bering Strait in late Pliocene and the following mass flow of the Pacific marine fauna into the Atlantic (Belopol'skaya 1983; Hoberg and Adams 2000; Galaktionov et al. 2012). In the light of this, studies on biodiversity and movements of digeneans in the North Pacific remain a promising area of research with an enduring value, while the elucidation of life cycles has become considerably easier with the advance of molecular methods (Blasco-Costa and Poulin 2017). Besides, the accumulation of molecular data on trematodes from the North Pacific and the North Atlantic offers tempting possibilities for phylogeographic reconstructions. They will make it possible to elucidate the pathways of geographic expansion of the parasites to the north of the Palaearctic in the past and to forecast the impact of the ongoing climate changes on this process (Galaktionov 2017).

This study continues the series of our studies of biodiversity and life cycles of trematodes circulating in the coastal ecosystems of the Sea of Okhotsk employing classical and molecular-biological approaches (Galaktionov et al. 2012; Gonchar and Galaktionov 2017). Herein, we describe a new microphallid species with a truncated two-host life cycle and an unusual structure of the terminal genitalia in adults.

Materials and methods

Material collection and treatment

The material was collected at the southern coast of the Pyagina Peninsula, the Sea of Okhotsk (North Pacific) in the Vnutrenn'aya Bay and the Shkiperov Bay in July–August 2008 and 2012 (Table 1). Gastropod molluscs *Falsicingula kurilensis* (Pilsbry, 1905) were collected in the intertidal and the upper subtidal zone. Pacific common eiders *Somateria mollissima v-nigrum* Bonaparte, 1855 were obtained by shooting in accordance with local regulations. The molluscs were dissected under a stereomicroscope to identify those infected with sporocysts containing metacercariae of the undescribed species. Metacercariae were excysted with the help of preparation needles. Some of them were studied in vivo, while others were fixed in 70% ethanol under a slight pressure of a coverslip. Eiders were dissected in the field, and the microphallid individuals with noticeable spicule-like structure in the genital region (see "Results") and individuals of *Levinseniella* sp. (identified in accordance with Deblock (2008) by presence of several accessory atrial sacs of type I associated with one sac of type II) were selected from all the adult microphallids in their intestine under a stereomicroscope. These adults were fixed in 70% ethanol following the same procedure as in the case of metacercariae. Samples of metacercariae and adults were stored in 70 and 95% ethanol for further morphological and molecular analysis correspondingly.

Carmine-stained whole mounts were used for morphological studies and to make drawings. Measurements were made on 17 metacercariae and 22 adults. Additionally, one of the adults fixed in 70% ethanol was used to make a temporary preparation in a mixture of glycerine and lactic acid (10:3). This medium, which is used for clarification of nematodes, also yields satisfactory results for the morphological analysis

Table 1 Taxa included in the phylogenetic analyses and GenBank accession codes for each sequence. Accession codes in bold correspond to the newly sequenced specimens

Classification/species	GenBank accession numbers	
	28S rDNA	ITS2
Microphalloidea		
Lecithodendriidae		
Paralecithodendrium parvouterus	AY220617	
Pycnoporus heteroporus	AF151918	
Microphallidae		
Longiductotrema tethepae	KX712084	KX712086
Maritrema arenaria	AY220629	HM584171
Maritrema brevisacciferum	KT355818	KT355824
Maritrema corai	KT880222	
Maritrema deblocki	KJ144173	
Maritrema eroliae	JF826247	HQ650132
Maritrema heardi	AY220632	
Maritrema madrynense		KF575167
Maritrema neomi	AF151927	
Maritrema novaezealandense	KJ144178	KJ540203
Maritrema oocysta	AY220630	HM584170
Maritrema poulini	KJ144175	
Maritrema prosthometra	AY220631	
Maritrema subdolum	AY151926	HM584172
Maritrema sp.		KC222023–KC222024
Maritrema sp. 1		KC012521
Maritrema sp. 2		KC222022
Microphallus abortivus	AY220626	HM5841731
Microphallus basodactylophallus	AY220628	
Microphallus calidris	HM584125	HM5841831
Microphallus fusiformis	AY220633	
Microphallus kurilensis	HM584140	HM5841851
Microphallus minutus	KT355822	KT355828
Microphallus ochotensis sp. nov., adult	**MG783586–MG783587**	**MG783581–MG783582**
Mi. ochotensis sp. nov., metacercaria	**MG783588–MG783589**	**MG783583–MG783584**
Microphallus piriformes	HM584122	HM5841811
Microphallus primas	AY220627	
Microphallus pseudopygmaeus	HM584126	HM5841981
Microphallus pygmaeus	HM584133	HM5841901
Microphallus similis	HM584138	HM5841781
Microphallus triangulatus	HM584139	HM5841951
Microphallus sp.	HM584142	HM5841751
Probolocoryphe uca		GQ377842
Microphallidae gen. sp.	KT355820	KT355826
Levinseniella sp.	**MG783585**	**MG783580**
Pleurogenidae		
Parabascus duboisi	AY220618	
Pleurogenes claviger	AF151925	
Prosthogonimidae		
Prosthogonimus ovatus	AF151928	
Plagiorchioidea		
Haematoloechidae		
Haematoloechus longiplexus	AF387801	

Table 1 (continued)

Classification/species	GenBank accession numbers	
	28S rDNA	ITS2
Plagiorchiidae		
Plagiorchis vespertilionis	AF151931	
Telorchiidae		
Telorchis assula	AF151915	

of trematodes. Three metacercariae fixed in 70% ethanol were prefixed in Bouin fluid and analysed histologically. Then, they were placed in paraffin wax after dehydration, according to the standard technique and used to make longitudinal serial sections 5 μm thick with the help of a microtome (Leica RM2245). The sections were stained with Bömer haematoxylin. The morphology of metacercariae and adults was studied in vivo in the field using an Amplival microscope (Karl Zeiss, Jena). Microphotographs were made with the help of an amateur Sony Cyber-shot camera DSC-W100 connected to the eyepiece of the Amplival microscope. Stained total mounts of metacercariae and adults and sections of metacercariae were studied in the laboratory of the Zoological Institute under the Olympus CH40 compound microscope equipped with an Olympus XC-30 digital camera. All measurements presented in the paper are in micrometres, with the mean in parentheses. Drawings were made with the aid of a *camera lucida*.

Molecular data generation

One adult specimen of *Levinseniella* sp. and two adult microphallid specimens with noticeable spicule-like structures in the genital region from Pacific common eider, *So. mollissima v-nigrum*, and two metacercariae specimens from individual molluscs, *F. kurilensis*, were characterised molecularly (Table 1). Genomic DNA was extracted from ethanol-fixed isolates in 200 μl of a 5% suspension of Chelex® in deionised water and containing 0.1 mg/ml proteinase K, followed by incubation at 56 °C for 5 h, boiling at 90 °C for 8 min and centrifugation at 14,000*g* for 10 min. Partial fragments of the ribosomal RNA gene were amplified using the following primers: the large ribosomal subunit (28S) [1800 bp; primers U178F: 5′-GCA CCC GCT GAA YTT AAG-3′ and L1642R: 5′-CCA GCG CCA TCC ATT TTC A-3′; Lockyer et al. 2003] and the internal transcribed spacer 2 (ITS2) [500 bp; primers 3S: 5′-GTA CCG GTG GAT CAC GTG GCT AGT G-3′ and ITS2·2: 5′-CCT GGT TAG TTT CTT TTC CTC CGC-3′]. Polymerase chain reaction (PCR) amplifications were performed in 20 μl reactions containing 2 μl of extraction supernatant (~ 10–20 ng of template DNA), 2× MyFi™ Mix (Bioline France, France; containing DNA Polymerase, dNTPs, $MgCl_2$ and enhancers at optimal concentrations) and 0.4 μM of each primer combination. Thermocycling conditions used for amplification of the rDNA regions followed Galaktionov et al. (2012). PCR amplicons were purified prior to sequencing using exonuclease I and shrimp alkaline phosphatase enzymes (Werle et al. 1994). Amplicons were cycle-sequenced from both strands using PCR primers and an internal primer for the 28S fragment (L1200R: 5′-GCA TAG TTC ACC ATC TTT CGG-3′; Littlewood et al. 2000) at the commercial facility Macrogen (Amsterdam, The Netherlands). Contiguous sequences were assembled and edited using Geneious® (v. 8.1 Biomatters Ltd., Auckland, New Zealand) and submitted to GenBank (see accession numbers in Table 1).

Molecular analyses

Five newly generated sequences for the 28S rDNA and the ITS2 fragments were aligned in two independent datasets together with the published sequences of other microphallids from GenBank (see accession numbers in Figs. 7 and 8 and Table 1). The sequences were aligned using default parameters of MAFFT implemented in Guidance (Sela et al. 2015), and the extremes of the alignment were trimmed to match the shortest sequences. The 28S dataset (1281 bp long) included 13 representative sequences of *Microphallus* spp., 12 of *Maritrema* spp., one of *Levinseniella* and one of *Longiductotrema* retrieved from GenBank (Table 1). Additionally, an unidentified microphallid; five sequences of species belonging to sister families of the Microphallidae, i.e. Lecithodendriidae, Pleurogenidae and Prosthogonimidae in the Microphalloidea; and three sequences of species in the Plagiorchioidea were retrieved from GenBank and included as outgroups. The ITS2 dataset (394 bp long) included ten representative sequences of *Microphallus* spp.; 11 sequences of *Maritrema*; one of *Levinseniella*, *Probolocoryphe* and *Longiductotrema*; and one of an unidentified microphallid. The phylogenetic analyses were run on the two datasets individually under the maximum likelihood (ML) and Bayesian inference (BI) criteria, employing the nucleotide substitution model GTR+Γ. ML analyses were conducted using the program RAxML v. 7.3 (Stamatakis 2006; Stamatakis et al. 2008). All model parameters and bootstrap nodal support

values (1000 repetitions) were estimated using RAxML. BI trees were constructed using MrBayes v. 3.2 (Ronquist et al. 2012), running two independent MCMC runs of four chains for 10^7 generations and sampling tree topologies every 10^3 generation. Burn-in periods were set automatically to 25% generations ensuring the remaining trees were obtained after values for standard deviation of split frequencies were < 0.01. A consensus topology and nodal support estimated as posterior probability values (Huelsenbeck et al. 2001) were calculated from the remaining trees. All MrBayes and RAxML analyses were performed on the computational resource CIPRES (Miller et al. 2010). Genetic divergences amongst taxa were calculated as uncorrected *p*-distances for each gene region using MEGA v. 6 (Tamura et al. 2011).

Results

Molecular characterisation of adult microphallids with spicule-like structures in the genital region and the metacercariae ex. *F. kurilensis* allowed us to link the two life stages (see "Molecular results" for details) of the new species. We provide a formal morphological description for both the adult and the metacercaria, below.

Description

Family Microphallidae Ward, 1901

Supersubfamily Microphallidi Ward, 1901

Subfamily Microphallinae Ward, 1901

Tribe Microphallini Ward, 1901

Genus *Microphallus* Ward, 1901

Microphallus ochotensis sp. nov.

Type-final host: Pacific common eider *Somateria mollissima v-nigrum* Bonaparte, 1855 (Anatidae).

Type-locality: Shelikhov Bay and bays of Pyagina Peninsula (north of the Sea of Okhotsk).

First intermediate host: *Falsicingula kurilensis* (Pilsbry, 1905) (Caenogastropoda, Falsicingulidae).

Site in host: Final host: intestine; first intermediate host: hepatopancreas and gonad.

Prevalence: *So. mollissima v-nigrum*: in 4 of 6 dissected birds (male, female and 2 ducklings).

F. kurilensis: Vnutrenn'aya Bay (entrance 3.3%, $N = 30$; Chayachiy islet 2.2%, $N = 186$), Shkiperov Bay 4.6%, $N = 87$.

Intensity in *So. mollissima v-nigrum*: varied from tens to thousand.

Type-material: Holotype (slide # 3714-1), 21 paratypes (slides #3714-2–3714-5) and 17 metacercariae vouchers (slides # 3715-1–3715-4) deposited in the Collection of helminthes, section Trematoda in Zoological Institute of the Russian Academy of Sciences (Saint Petersburg); four paratypes (MHNG-PLAT-99644) and 5 metacercariae vouchers (MHNG-PLAT-99645) deposited in the Muséum d'Histoire Naturelle de Genève (Geneva, Switzerland).

Representative DNA sequences in GenBank: adults MG783581–MG783582; MG783586–MG783587, metacercariae MG783583–MG783584; MG783588–MG783589.

ZooBank registration: To comply with the regulations set out in article 8.5 of the amended 2012 version of the *International Code of Zoological Nomenclature*, details of the new species have been submitted to ZooBank. The Life Science Identifier (LSID) for *Mi. ochotensis* nov. sp. is urn:lsid:zoobank.org:act:DC77B715-755D-4C4B-B621-EDC44BE712C2.

Etymology: The species was named after the site where it was discovered, the Sea of Okhotsk.

Adult

[Measurements based on whole mounts of 22 specimens (Figs. 1, 2 and 3).] Worms pyriform, with powerful posterolateral projections filled with glands (Fig. 1a). Body 330–529 (369) long. Body width at mid-level of oesophagus 100–186 (129) and at level of testes 200–429 (267). Body covered with spines, larger in anterior region. Oral sucker 24–36 × 29–43 (31 × 36), somewhat shorter than ventral sucker 29–42 × 28–43 (35 × 35), the oral sucker width/ventral sucker width ratio is approx. 1:1. Prepharynx when visible very short, 4–11 (6). Pharynx oval, 14–25 (21) × 14–22 (19). Oesophagus length greatly variable, 72–194 (106), mainly resulting from varying contraction condition of fixed worms.

Testes irregular to oval, 43–97 (62) × 25–54 (38). Seminal vesicle and prostatic glands enclosed in a thin-walled membrane-like structure. Seminal vesicle highly variable depending on degree of filling with sperm (Fig. 2), 25–94 (54) × 29–72 (39). Prostatic part well developed, the ducts of prostatic cells enveloping distal part of seminal vesicle and ejaculatory duct and opening into ejaculatory duct at phallus base. Ducts and pores of prostatic cells clearly visible in live specimens serving as good diagnostic character during preliminary examination of material in the field. Phallus somewhat elongated along transverse axis, 17–25 (21) × 14–25 (18). Genital atrium muscular, genital pore sinistral to ventral sucker (Fig. 1b).

Ovary dextral to ventral sucker, adjacent to or slightly overlapping ventral sucker laterally, transversely elongate, approximately same size as testes, 36–101 (60) × 25–68 (38). Uterus in hindbody, reaching to level of ventral sucker, often overlapping testes ventrally. Metraterm complex, consisting of two structurally different parts (Fig. 1b).

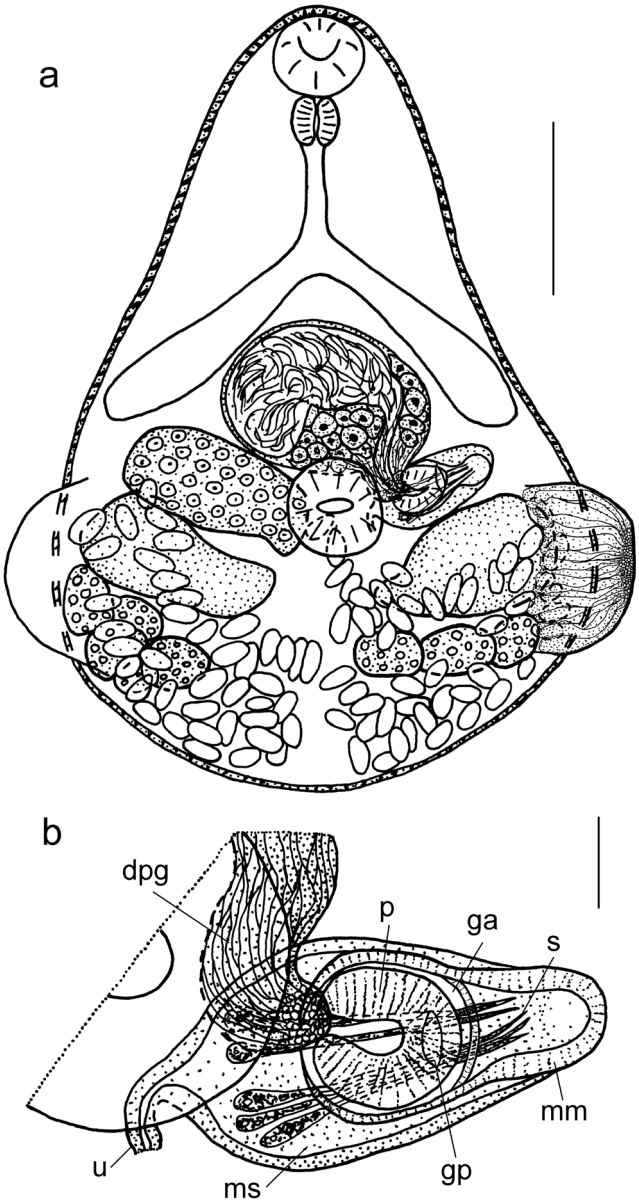


Fig. 1 Adult of *Microphallus ochotensis* (ventral view). **a** General morphology, *bar* 100 μm. **b** Terminal genitalia, *bar* 20 μm. *dpg* ducts of prostatic gland cells, *ga* genital atrium, *gp* genital pore, *mm* muscular part of metraterm, *ms* metratermal sac, *p* phallus, *s* spicule-like structures, *u* uterus

Distal muscular part of metraterm opening into sinistral wall of genital atrium by broad aperture. Proximal part, connecting with uterus (Fig. 3), forms large sac-like extension, which we refer to as metratermal sac, 31–76 (48) × 11–32 (21), dorsal and somewhat posterior to genital atrium. Spicule-like structures arranged into two longitudinal bundles visible in metratermal sac of fully formed adults (Fig. 1b and 2). Pointed ends of spicules entering muscular part of metraterm, sometimes extending into genital atrium. Vitellarium represented by two compact groups of 3–6 follicles posterior to testes. Eggs small, 14–22 (19) × 7–14 (10).

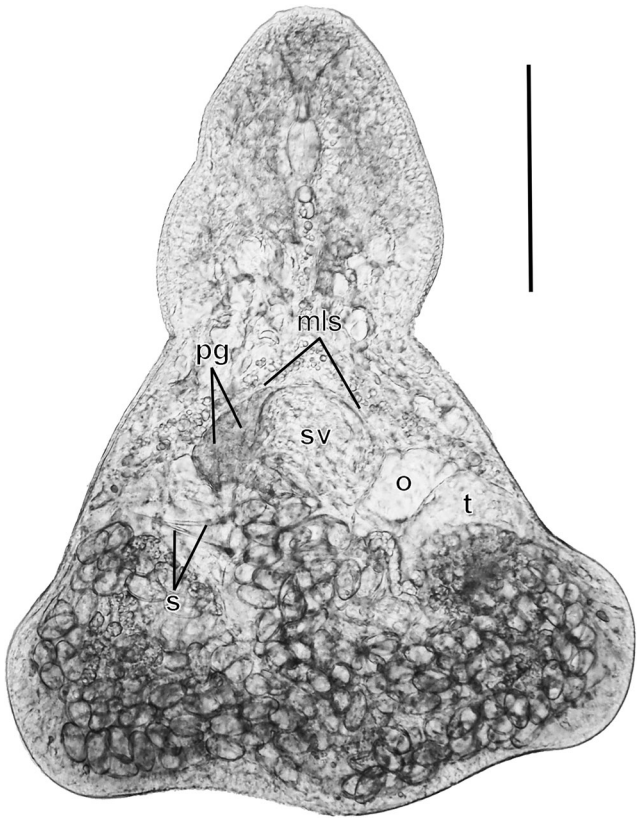


Fig. 2 Microphotograph of living adult of *Microphallus ochotensis* (dorsal view), *bar* 100 μm. *mls* membrane-like structure, *pg* prostatic gland cells, *o* ovary, *s* spicule-like structures, *sv* seminal vesicle, *t* testis

Metacercaria

[Measurements based on whole mounts of 17 specimens (Figs. 4, 5 and 6).] Fully formed metacercariae enclosed in oval cysts (Fig. 4a), 132–182 (160) × 119–172 (145). Larvae extracted from cysts tongue-shaped, with marked posterolateral projections (Fig. 5a). Body length 157–257 (209); body width 86–129 (106) at mid-oesophagus level and 100–172 (140) at testes level. Oval oral sucker 20–33 × 23–38 (28 × 31), somewhat greater than round ventral sucker 20–28 × 20–28 (25 × 26), oral sucker width/ventral sucker width ratio is approx. 1.2:1. Prepharynx not present, pharynx oval 14–21 (18) × 10–17 (14), oesophagus length 28–56 (42). Ducts of gland cells, arranged in 3 longitudinal rows on ventral and dorsal planes, opening at oral sucker margin and on forebody. Nucleus-containing cell bodies of gland cells concentrated in body regions anterior to intestinal caeca. Agglomeration of gland cells forms posterolateral body projections and opens at the tip of projection. Flame-cell formula 2[(2 + 2) + (2 + 2)] = 16.

Testes oval, 25–59 (38) × 15–38 (24), left testis often located posteriorly to the right one. Seminal vesicle rather small, 15–28 (21) × 13–21 (17). Seminal vesicle and prostatic glands enclosed in thin-walled membrane-like structure. Prostatic part well developed, prostatic cells agglomerated near distal

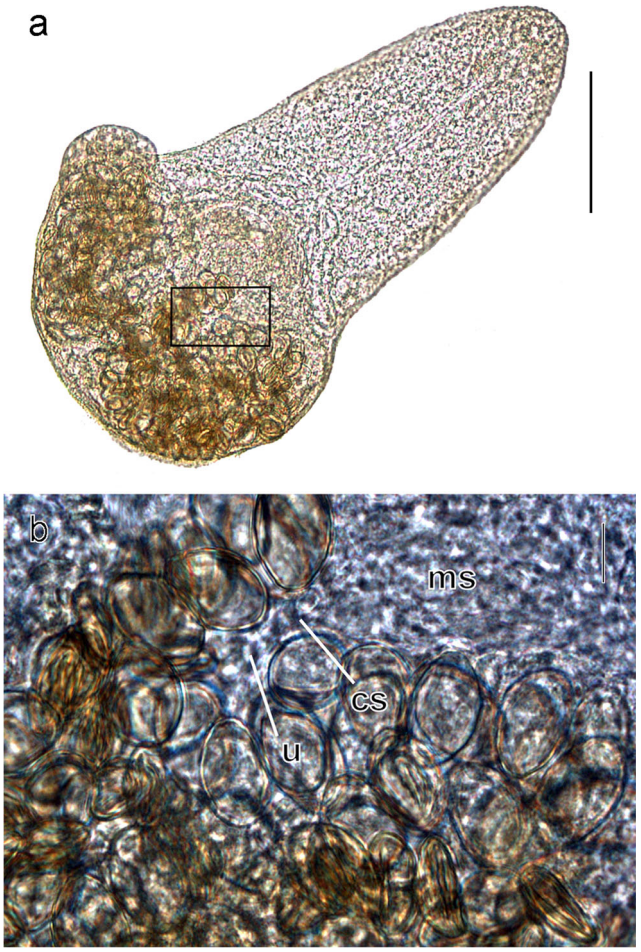


Fig. 3 Microphotographs of an adult of *Microphallus ochotensis* placed on a temporary mount in a mixture of lactic acid and glycerine. **a** General view, boxed area—body part enlarged in **b**, *bar* 100 μm. **b** Body part at the site of connection between the metratermal sac and the uterus, *bar* 10 μm. *cs* connection site of the metratermal sac with the uterus; *ms* metratermal sac, *u* uterus

part of seminal vesicle and around proximal part of ejaculatory duct. Ducts of prostatic cells enveloping distal part of ejaculatory duct opening into it at the phallus base (Fig. 5b). Expanded distal parts of ducts of prostatic cells and pores clearly visible in live metacercariae (Fig. 4a, b). Phallus somewhat elongated along transverse axis, 14–21 (18) × 8–17 (13), accommodated in muscular genital atrium.

Ovary subtriangular, 18–34 (26) × 13–21 (17). Metratermal sac, 29–50 (38) × 11–28 (19), localised dorsally and somewhat posteriorly of genital atrium (Figs. 5b and 6). Posterior shift of left testis (see above) associated with position of metratermal sac. Longitudinal cords of amorphous fibrous material (developing spicule-like structures) visible in metratermal sac (Fig. 6). Cords tapering distally and entering muscular distal part of metraterm opening into sinistral wall of genital atrium with broad aperture (Fig. 4b and 6). Vitellarium forms two compact groups of 3–5 follicles behind testes.

Hosts

Adults of *Mi. ochotensis* were found in most eiders examined from the Sea of Okhotsk. The highest infection intensity (more than 1000 individuals) was recorded in a duckling and a female from the Shkiperov Bay, and single individuals of *Mi. ochotensis* (identified based on molecular markers—see above) were found in a male from the area of Cape Taygonos.

The prevalence of *Mi. ochotensis* sporocysts in molluscs *F. kurilensis* examined in the Shkiperov Bay and the Vnutrenn'aya Bay was low at 3–5%. Mature encysted metacercariae were enclosed in thin-walled sporocysts. When molluscs are dissected, the body wall of sporocysts bursts and metacercariae pour out, creating a false impression that the encysted larvae lie freely in the molluscan tissues. In some sporocysts, earlier developmental stages were found alongside mature metacercariae. These developing larvae had a reduced tail. No stylet nor penetration glands were seen in them.

Molecular results

The two adults with spicule-like structures in the genital region and the two metacercariae isolates ex. *F. kurilensis*

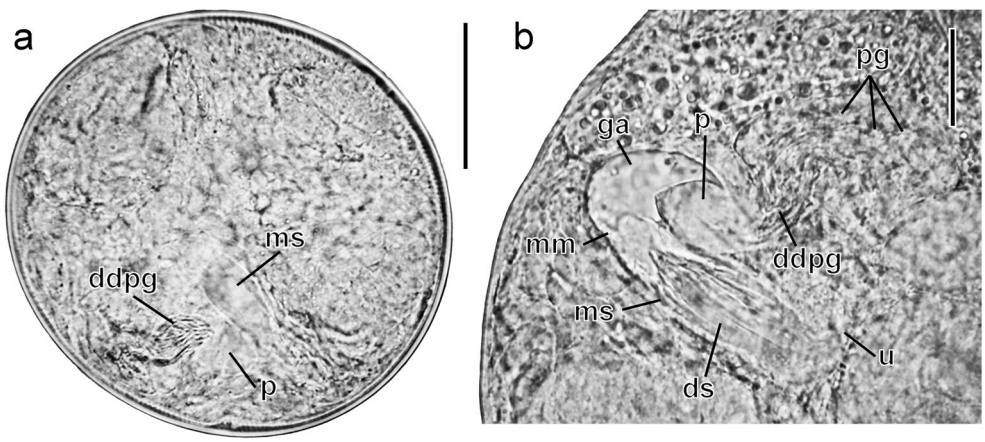
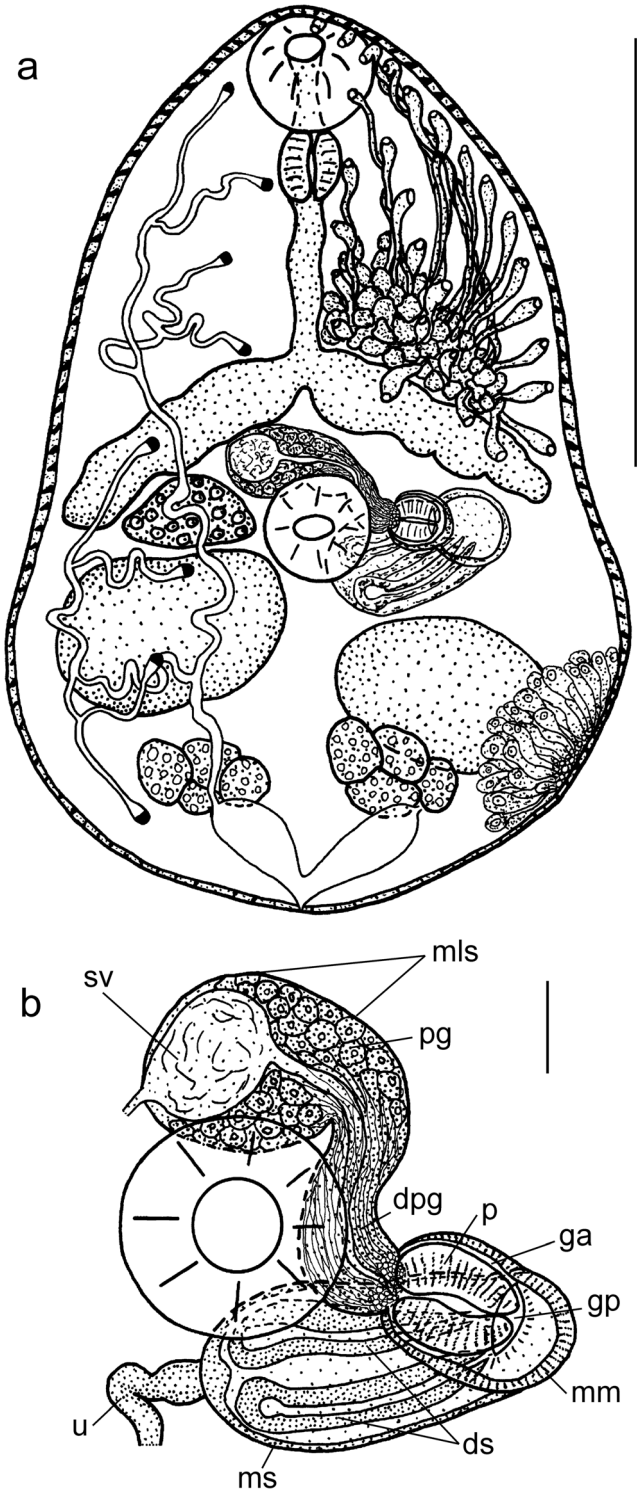


Fig. 4 Microphotographs of living metacercaria of *Microphallus ochotensis*. **a** Encysted metacercaria, *bar* 50 μm. **b** The body area of metacercaria strongly pressed by cover glass showing terminal genitalia, *bar* 20 μm. *ddpg* ducts of prostatic glands (distal parts), *ds* developing spicule-like structures, *ga* genital atrium, *mm* muscular part of metraterm, *ms* metratermal sac, *p* phallus, *pg* prostatic gland cells



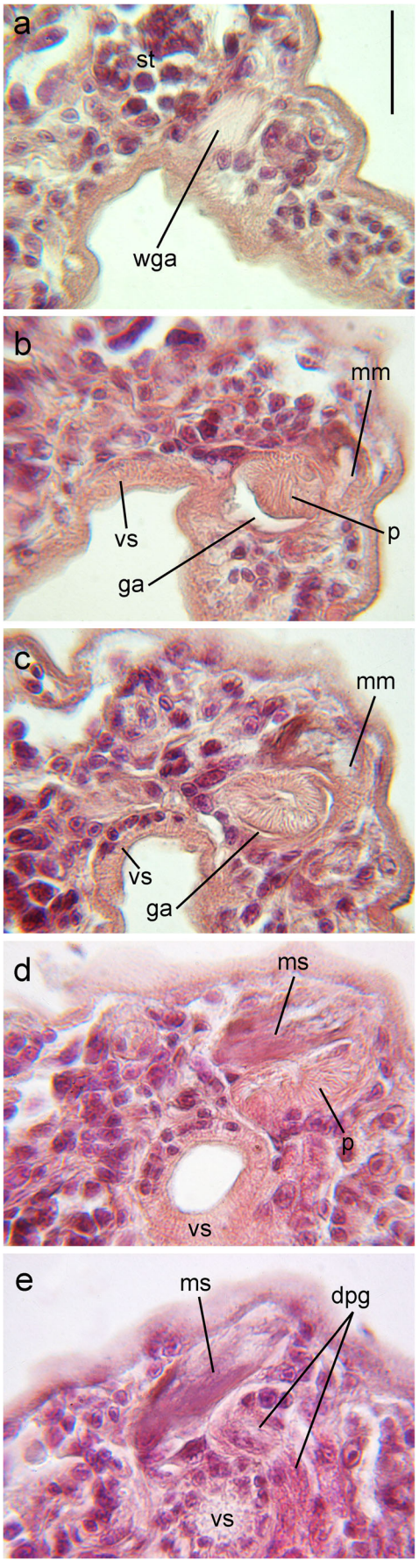


Fig. 5 Metacercaria of *Microphallus ochotensis* (ventral view). **a** General morphology, *bar* 100 μm. **b** Terminal genitalia, *bar* 20 μm. *ds* developing spicule-like structures, *dpg* ducts of prostatic gland cells, *ga* genital atrium, *gp* genital pore, *mls* membrane-like structure, *mm* muscular part of metraterm, *ms* metratermal sac, *sv* seminal vesicle, *p* phallus, *pg* prostatic gland cells, *u* uterus

◀ **Fig. 6** A series of longitudinal sections (5 μm) (**a**–**e**) in the frontal plane (from the ventral side towards the dorsal) through the genital part of the body of *Microphallus ochotensis* metacercaria, *bar* 20 μm. *dpg* ducts of prostatic gland cells, *ga* genital atrium, *mm* muscular part of metraterm, *ms* metratermal sac, *p* phallus, *st* sinistral testis; *vs* ventral sucker, *wga* wall of genital atrium

characterised molecularly showed identical sequences for each of the 28S and ITS2 rDNA regions sequenced (Figs. 7 and 8), which allowed us to match the two life cycle stages of the new species. Their sequences also matched with 100% identity to that of a previously sequenced adult from the same host in Cape Taygonos, north Sea of Okhotsk, Russia (GenBank accession codes HM584142, HM584175; Galaktionov et al. 2012). Interspecific genetic divergences within *Microphallus* ranged from 0.4 to 8.6% in the 28S rDNA fragment, whereas our specimens showed a genetic divergence of 5.1–7.4% from other *Microphallus* spp. The lowest genetic divergence was found between *Microphallus pygmaeus* (Levinsen, 1881) and *Microphallus kurilensis* Galaktionov et al., 2010, and between *Microphallus calidris* Belopol'skaja & Ryjikov, 1963 and *Microphallus minutus* Johnston, 1948, and the highest between *Microphallus abortivus* Deblock, 1974 and *Microphallus pseudopygmaeus* Galaktionov, 2009. The range of interspecific genetic divergence amongst *Microphallus* spp. was similar to that amongst *Maritrema* spp. (0.6–9.2%). Average intergeneric divergence amongst microphallids (*Microphallus*, *Maritrema*, *Levinseniella* and *Longiductotrema*) ranged from 7.0 to 10.3% in the 28S rDNA region. The sequence of an unidentified microphallid (KT355820) and *Microphallus fusiformis*

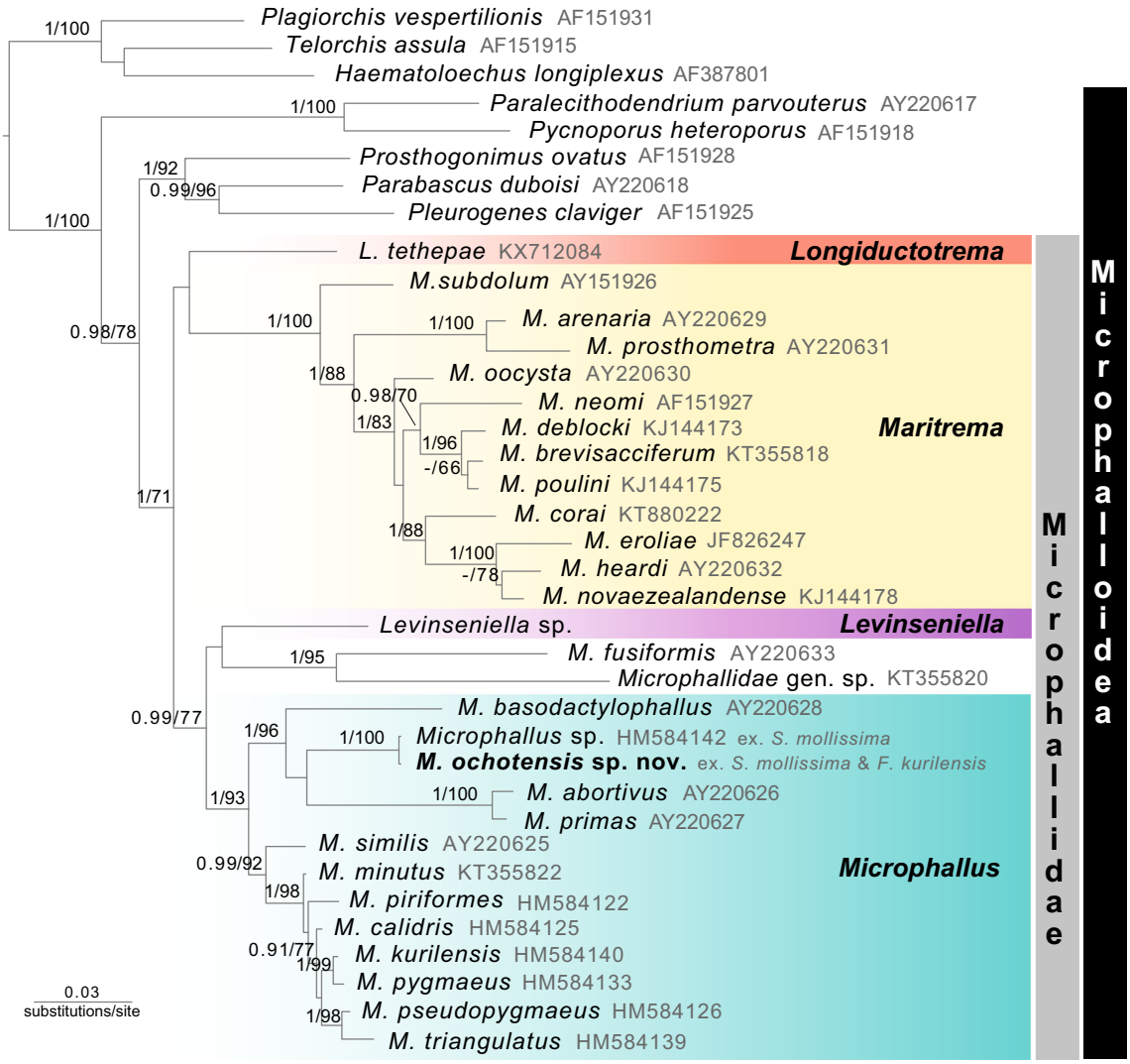


Fig. 7 Phylogenetic tree based on Bayesian inference and maximum likelihood analyses of the partial 28S rDNA dataset showing the relationships between the new species and other microphallids. Values above the branches represent Bayesian inference posterior probabilities (PP) followed by maximum likelihood bootstrap support (BS) percentages (PP < 90 and BS < 60 not shown). GenBank accession codes for sequences and host species names of the sequences of the new species in grey

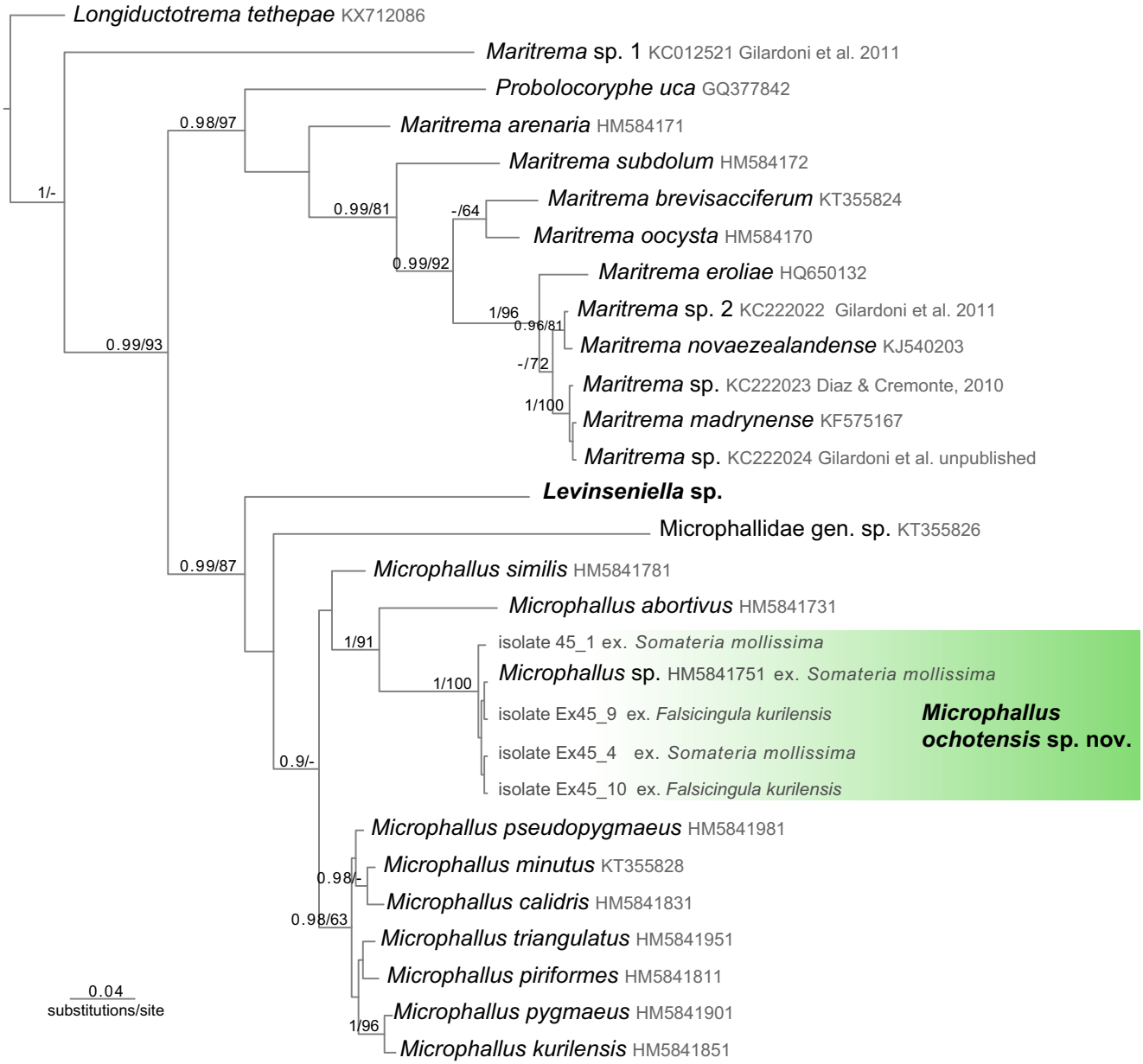


Fig. 8 Phylogenetic tree based on Bayesian inference and maximum likelihood analyses of the ITS2 dataset showing the relationships between the new species and other microphallid genera. Values above the branches represent Bayesian inference posterior probabilities (PP) followed by maximum likelihood bootstrap support (BS) percentages (PP < 90 and BS < 60 not shown). GenBank accession codes for sequences and host species names of the sequences of the new species in grey. Unidentified species names are followed by the name of the authors of the sequence after the GenBank code

Reimer, 1963 showed an average genetic divergence as high or higher than that observed between genera (on the average 10.5–12.7%). The genetic divergence between the unidentified microphallid and *Mi. fusiformis* was also high (10.5%).

ITS2 genetic distances varied within the range 1.0–10.1% between *Microphallus* spp. and 1.1–23.8% between *Maritrema* spp. Sequences of *Mi. ochotensis* sp. nov. diverged 8.5–10.0% from all other *Microphallus* spp. Two unidentified sequences of *Maritrema* (KC222024 Gilardoni et al. unpublished and KC222023 Diaz & Cremonte 2010) were found to be identical to *Maritrema madrynense* Deblock & Rausch, 1968. *Maritrema* sp. 1 Gilardoni et al., 2011 (KC012521) was the most divergent *Maritrema* species (20.6–23.8%). Average intergeneric genetic divergence between representative sequences of species of microphallid genera ranged from 13.8 to 21.0% in the ITS2 region. Thus, *Maritrema* sp. 1 mentioned above showed a divergence similar to that expected between genera within the Microphallidae.

The BI and ML phylogenies based on the 28S rDNA region were congruent and depicted the Microphallidae as monophyletic (Fig. 7), as well as the genera *Maritrema* and *Microphallus* (the sequence of *Mi. fusiformis* should be disregarded from *Microphallus*, see Kudlai et al. (2016) and the distance results above). However, support for the relationships amongst the genera within the family was weak and resulted in the labile placement of *Longiductotrema* as sister to *Maritrema* (BI tree) or sister to the *Microphallus* + (*Levinseniella* sp. + Microphallidae gen. sp. + *Mi. fusiformis*) clade (ML tree). *Mi. ochotensis* sp. nov. matched the sequence of *Microphallus* sp. (HM584142; ex. *So. mollissima*) in the phylogenetic tree. The new species fell within a strongly supported clade including *Microphallus basodactylophallus* (Bridgman, 1969), parasitic in raccoons and rats, *Microphallus primas* (Jägerskiöld, 1908) and *Mi. abortivus*, parasitic in birds. However, the relationships amongst the species within this clade were unsupported. The ITS2 phylogenies were also mostly congruent, except for the placement of *Probolocoryphe uca* (Sarkisian, 1957) that either diverged earlier from *Maritrema* or was found within the *Maritrema* clade as sister to *Maritrema gratiosum* Nicoll, 1907 (syn. *Maritrema arenaria* Hadley & Castle, 1940) (Fig. 8). The monophyly of *Maritrema* and *Microphallus* was weakly supported. *Maritrema* sp. 1 of Gilardoni et al. (2011) (KC012521) appeared as early divergent within the tree, not closely related to other *Maritrema* spp., as noted above on the basis of the high genetic divergence. The sequence of *Levinseniella* sp., a representative of the subfamily Levinseniellinae, appeared as early divergent from the Microphallidae gen. sp. and *Microphallus* clade. *Mi. ochotensis* sp. nov. was depicted sister to *Mi. abortivus* within the *Microphallus* clade. Thus, genetic data for both the 28S and the ITS2 regions of the rRNA gene corroborated the morphological distinctiveness of *Mi. ochotensis* sp. nov. and supported its distinct species status.

Remarks

Molecular data indicate clearly that *Mi. ochotensis* sp. nov. should be included in the genus *Microphallus* (Figs. 7 and 8). Its morphological description corresponds to the diagnostic characters of the Microphallini and of the genus *Microphallus* within this tribe (Deblock 1971, 2008). In particular, the adult and metacercaria described above possess a fleshly, muscular phallus of "microphalloid" type, morphologically simple, smaller than the ventral sucker; a simple genital atrium enveloping the phallus and the metraterm opening in the genital atrium is sinistral. The species described in this paper differs from other known species of the genus *Microphallus*, first of all, in the structure of the metraterm, which consists of two distinct parts: a sac with spicule-like structures and a muscular duct opening into the genital atrium. Although the metraterm is well developed in all adults of *Microphallus*, no such structure has ever been described before (Belopol'skaya 1952a, 1963; Deblock 1971, 2008). In particular, it is absent in the adults of *Mi. abortivus* and *Mi. primas* (syn. *Microphallus canchei* Biguet et al., 1958), the two species with which *Mi. ochotensis* forms a clade in the 28S tree. It is also absent in *Mi. basodactylophallus*, which is closely related to the aforementioned species in the 28S phylogeny (Biguet et al. 1958; Bridgman 1969; Deblock 1974). In other respects, the structure of adults of *Mi. ochotensis* corresponds to that of other *Microphallus* species. In *Mi. primas*, the metraterm is separated into two parts: a transitional (proximal) part lined with papillae and a muscular terminal (distal) part connected to the genital atrium (Biguet et al. 1958; Deblock 1971). However, these parts are developed much less strongly than in *Mi. ochotensis*, and neither spicule-like structures nor longitudinal cords of fibrous material are present in the proximal part.

A powerful metraterm is characteristic of some species of *Megalophallus* (Cable et al. 1960; Prévot and Deblock 1970; Prévot 1974; Overstreet and Heard 1995). In some respects, it is similar to the metraterm of *Mi. ochotensis*: it is also long, broad and muscular and it also passes around the genital atrium dorsally opening into the sinistral wall of the genital atrium. In contrast to *Mi. ochotensis*, *Megalophallus* spp. have no metratermal sac with spicule-like structures. However, in *Megalophallus deblocki* Kostadinova et al., 2006, the extended terminal portion of the metraterm forms two indistinct lobes covered with minute spines (Kostadinova et al. 2006). *Megalophallus* spp. differ from all species of the genus *Microphallus*, including *Mi. ochotensis*, in the structure of the phallus, which is ornamented by several prominent structures.

Amongst other microphallid genera, a structure superficially similar to that in *Mi. ochotensis* is found in *Spiculotrema littorale* Belopol'skaya, 1949 (subfamily Levinseniellinae Stiles & Hassal, 1901, tribe Ascorhytini Deblock, 1971) (Belopol'skaya 1949, 1952a; Deblock 2008). This large (by the standards of microphallids) worm, 0.552–0.951 × 0.337–0.410, parasitises shorebirds in Primorye (Russian Far East) (Belopol'skaya 1949, 1954; Tsimbalyuk et al. 1968b, 1978). *Sp. littorale* has an accessory sac connected to the genital atrium (type IV atrial sac according to Deblock (2008)). The sac contains a large sclerotised plate, pyriform in dorsoventral view and long spicule-like in profile (Deblock 2008). According to Belopol'skaya (1949), this structure serves as an organ of stimulation during copulation. The metraterm in *Sp. littorale* opens into the genital atrium independently. The modification of the metraterm described in *Mi. ochotensis* is probably also involved in copulation. Besides stimulation, it may serve for retention of the partner's phallus in the metraterm, which serves as vagina in trematodes.

One character of the new species seems to be at variance with the classical diagnosis of the genus *Microphallus* and Microphallini on the whole (Belopol'skaya 1963; Deblock 1971, 2008): the seminal vesicle and the prostatic part do not lie freely in the parenchyma but are enclosed in a fine membranous envelope. This structure, which was also found in other representatives of *Microphallus*, is a reduced male genital pouch (see "Discussion" for details).

In conclusion, we would like to note that, in contrast to many other microphallids, metacercariae and adults of *Mi. ochotensis* are easy to identify. In live encysted metacercariae, the distended distal parts of the ducts of the prostatic glands are clearly visible. They are arranged in a dense bundle and open into the prostatic chamber (Fig. 4a). The distended part of the metraterm (the metratermal sac) can be easily seen in live metacercariae and adults (Fig. 4b) expanded under slight pressure of a coverslip in a drop of water as well as in mounted specimens; in fully formed adults, the spicule-like structures are also clearly visible (Fig. 2). These distinct diagnostic characters allow a trouble-free identification of metacercariae and adults even in the field, so that whole mounts in Canada balsam are unnecessary.

Discussion

We described a new microphallid species, *Mi. ochotensis*, with a unique structure of the metraterm. No such metraterm structure has been described in this family before. In general, the structure of the copulatory organ and the genital atrium in microphallids is highly variable, and their organisation forms the basis of microphallid taxonomy (Belopol'skaya 1963; Deblock 1971, 2008). However, the greatest modifications concern the male copulatory organ and the genital atrium, which can develop diverticula and accessory sac(s). Variations of the metraterm are mostly represented by the degree of its differentiation and the development of the wall musculature. Yet amongst the known species of *Microphallus*, the differentiation of the metraterm into two parts (the muscular part opening in the genital atrium and the transitional part neighbouring the uterus and lined with papillae) has been described in *Mi. primas* (see "Remarks"). These parts are much more weakly differentiated in *Mi. primas* than in *Mi. ochotensis*, but the very presence of the metraterm consisting of two parts indicates that a tendency towards this kind of morphological and functional specialisation is present in this genus.

Taking into account the differential ability of the genital atrium and the metraterm to form structural modifications (see above), it is logical to assume that the structures that we described as parts of the metraterm in *Mi. ochotensis* (or, at least, the muscular one) are also modifications of the genital atrium. If so, they should be considered as type IV atrial sac, and then we will have to attribute the new species to the genus *Spiculotrema*. However, the resemblance of the complex metraterm of *Mi. ochotensis* to type IV atrial sac is purely superficial. These structures cannot correspond to each other for three reasons: (1) the connection of the metratermal sac to the uterus, (2) its position in the worm's body and (3) the position of the new species on the molecular tree. The connection of the uterus with the metratermal sac is clearly seen in live metacercariae and adult worms as well as in worms placed into a mixture of glycerine and lactic acid (Fig. 3). In whole mounts, the transition of the metratermal sac into the uterus proper is difficult to observe.

The large atrial sac in *Sp. littorale* is located sinistral to the genital atrium. In *Mi. ochotensis*, the metratermal sac bearing longitudinal cords of amorphous fibrous material (in metacercariae) or spicule-like structures (in fully formed adults) lies dorsally of the genital atrium, either almost perpendicularly or at an acute angle to the longitudinal body axis. Its distal part gradually becomes the muscular part, which bends ventrally and opens into the sinistral wall of genital atrium. This position of the metraterm in the worm's body is characteristic of Microphallini (see Deblock 2008).

It should be noted that amongst Microphallini, considerable structural modifications of the metraterm are recorded in *Megalophallus* spp. (see "Remarks"). However, Kostadinova et al. (2006) in a detailed study of *Me. deblocki* have shown that the metraterm in this species is short and tubular rather than enlarged and muscular, as in other species of the genus. At the same time, they found a large folded structure considered as a modification of the genital atrium, not of the metraterm. It is unclear whether this observation is also true of other species of *Megalophallus*, and further studies are necessary to elucidate this. Regarding the structures described as metratermal sac and the muscular part of the metraterm in *Mi. ochotensis*, their origin can only be ascertained in a detailed study of morphogenesis of female terminal genitalia and the genital atrium in the course of metacercarial development.

The genetic divergence of *Mi. ochotensis* from other *Microphallus* spp. falls within the range of interspecific genetic divergences between species of this genus. In the 28S rDNA phylogenetic tree (Fig. 7), *Mi. ochotensis* forms a clade with morphologically typical species of *Microphallus*, but not with *Levinseniella* sp., which belongs to the same subfamily Levinseniellinae Stiles & Hassall, 1901 as *Spiculotrema* (see Deblock 2008). Though molecular data of other representatives of the Microphallidae are scarce, the phylogenetic position of the newly described species clearly indicates that it belongs to *Microphallus*. At the moment, it would be unreasonable to erect a new genus for the new species on the basis of morphological characters (a complex structure of the metraterm). This would have made *Microphallus* paraphyletic. We cannot rule out that with the accumulation of genetic data for other genera of the tribe Microphallini may

promote taxonomic changes within this diverse group and a revision of the validity of some morphological characters considered as diagnostic to date.

The representative sequence of *Mi. fusiformis* (GenBank accession number AY220633; Olson et al. 2003) appeared as highly divergent from other representatives of *Microphallus* spp. (divergence comparable to that found between species belonging to different genera) in the current and previous molecular studies (Olson et al. 2003; Galaktionov et al. 2012; Kudlai et al. 2016). Given the molecular divergence from other *Microphallus* and that *Mi. fusiformis* was not originally found in the mudsnail *Hydrobia ulvae* in the Belfast Lough (see Field and Irwin 1999), the identification of the specimen used for generating this sequence (AY220633) is likely to be erroneous.

We shall discuss another morphological detail of *Mi. ochotensis*, which conflicts with the diagnoses of *Microphallus* and Microphallidi in general, according to which the seminal vesicle and the prostatic part are embedded in the parenchyma (Belopol'skaya 1963; Deblock 1971, 2008). This detail is a fine membranous structure enveloping the seminal vesicle and the prostatic part. This structure has been described before in other species of *Microphallus* as a reduced male genital pouch (Galaktionov 1983, 1984, 1991a; Galaktionov et al. 2010). The study of morphogenesis of microphallid metacercariae (*Mi. pygmaeus* (Levinsen, 1881), *Microphallus pirum* (Lebour, 1907) and *Maritrema subdolum* (Jägerskiöld, 1909) has shown that in all of them the primordium of the male genital pouch differentiates as a part of the common genital primordium. However, in the studied species of *Microphallus*, its development slows down considerably, so that in fully developed metacercariae the male genital pouch is a sac devoid of musculature and consists of two or three layers of flattened cells (Galaktionov 1991a; Galaktionov and Dobrovolskij 2003). The structure described by Biguet et al. (1958) in *Mi. primas* (syn. *Mi. canchei*) and *Microphallus debuni* Biguet et al., 1958 as a fine but distinct membrane separating the prostatic glands from the parenchyma was probably a reduced genital pouch. To note, a reduced genital pouch is easy to observe in live metacercariae and adults as well as in histological sections. However, it is very difficult to see on whole mounts, which seems to explain why this structure has been so rarely described in *Microphallus* species before.

The use of molecular markers made it possible to elucidate the life cycle of *Mi. ochotensis*. It involves only two hosts, the first intermediate and the final host. In the molluscan first intermediate host, *F. kurilensis*, the larvae develop inside daughter sporocysts until the stage of encysted invasive metacercariae. The final host is the Pacific common eider, which becomes infected as it consumes infected molluscs.

Microphallids have a rather low specificity to the final host (Belopol'skaya 1963). Therefore, it can be expected that *Mi. ochotensis* parasitises not only eiders but also other marine benthos-feeding ducks and shorebirds such as the oystercatcher (*Haematopus ostralegus*), the red-necked stint (*Calidris ruficollis*), the red knot (*Calidris canutus*) and the great knot (*Calidris tenuirostris*). On autumn migration, these birds gather in great numbers in the nearshore areas in the north of the Sea of Okhotsk. During this time, shorebirds feed intensively on intertidal invertebrates including molluscs. For the great knot, which has been studied in detail in this respect, molluscs, including *F. kurilensis*, are the basis of the diet (Andreev 2010). Incidentally, one of the largest accumulations of migrating great knots and red knots is formed in the area of Vnutrenn'aya Bay, where we found *F. kurilensis* infected with *Mi. ochotensis*.

Mi. ochotensis has not, however, been recorded in *Falsicingula* spp. on the south coast of Sakhalin and the South Kuril Islands (Paramushir, Iturup and Kunashir) (Kulikov et al. 1970; Tsimbalyuk et al. 1978; our data). The common eider is absent in these areas but migratory corridors of shorebirds pass across them (Andreev 2005). *Mi. ochotensis* has not been recorded in shorebirds in Primorye either (Belopol'skaya 1956; Deblock 1975). The area in the Sea of Okhotsk where *Mi. ochotensis* was found coincides with the distribution of the Okhotsk population of the Pacific eider, which is limited by the north-western part of this basin (Babushkin Bay and Shelikhov Bay) (Krechmar and Kondratyev 2006). The north-eastern border of the range lies near the Taygonos Peninsula, where the male of Pacific eider infected with *Mi. ochotensis* was obtained in 2008. The Okhotsk population of Pacific eider is rather small (6000–7000 individuals). It is isolated from the neighbour population of eiders of the Bering Sea, not mixing with it even at wintering sites. Eiders of the Okhotsk population spend the winter in ice-clear areas southwards of the Shelikhov Bay (Andreev 2005; Krechmar and Kondratyev 2006). No infection with *Mi. ochotensis* was found in the *F. kurilensis* molluscs collected in nearshore areas of the north-eastern Sea of Okhotsk (Srednyaya Bay, Seglan, Ol'skaya Bay), where the Pacific common eider does not occur but shorebirds gather in large numbers during seasonal migrations (unpublished original data). All this may indicate that the range of final hosts of this species includes only the common eider and, possibly, some other benthos-eating ducks.

The distribution of this species seems to be limited to the coast of the northern Sea of Okhotsk, where the common eider and molluscs *F. kurilensis* co-occur. *Falsicingula aleutica*, which is similar to this species, is common in the coastal areas of the Aleutian Islands and in the Gulf of Alaska (Baxter 1987; Foster and Feder 2002), where, it would seem, the circulation of *Mi. ochotensis* is possible. Transcontinental flights of sea ducks, including the Pacific eider, and shorebirds in the north of the Pacific Ocean are well-documented (Petersen and Flint 2002; Webster et al. 2002;

Andreev 2005; Petersen et al. 2006; Alerstam et al. 2007). This provides the background for an amphi-Pacific distribution of *Mi. ochotensis*.

As noted in the "Introduction", the elimination of the second intermediate host from the life cycle and the transition to dixenous life cycles, which is characteristic of *Mi. ochotensis*, are common amongst microphallids. Two-host life cycles occur in various genera of these trematodes but are especially common amongst species of *Microphallus* (Belopol'skaya 1963; Deblock 1977; Galaktionov and Skírnisson 2007). According to the classification of Galaktionov and Skírnisson 2007, *Mi. ochotensis* falls into the second, the most species-rich category of dixenous life cycles of microphallids. Morphogenesis of the larvae of the hermaphroditic generation (cercaria, metacercaria, adult) of these species is completed within daughter sporocysts, with the cercarial development being suppressed. The tail is reduced to become a short, poorly differentiated appendage; the stylet and the penetration glands are lost. The only cercarial provisional structures left are tegumental glands, whose secretions are used for cyst construction (Galaktionov 1991a, b; Galaktionov and Skírnisson 2007). *Mi. ochotensis* is so far the only known species with such a life cycle in the North Pacific. All other such species (14 described to date) are known from the Atlantic. The only exception is *Atriophallophorus coxiellae* Smith, 1974, whose metacercariae encysted in sporocysts have been recorded from the brackish-water mollusc *Coxiella striata* in Tasmania (Smith 1974). At the same time, microphallids of the "pygmaeus" group (*Mi. calidris*, *Mi. kurilensis*, *Mi. pseudopygmaeus* and *Microphallus triangulatus*), whose non-encysted metacercariae develop inside daughter sporocysts in the molluscan host, are common at the coast of the Sea of Okhotsk. In their dixenous life cycles, metacercariae develop inside daughter sporocysts without encysting (Galaktionov et al. 2012).

Truncation of trematode life cycles entails several setbacks, which have been analysed in detail by Poulin and Cribb (2002). In the case of microphallids, which lose the second intermediate host, the most important setbacks are reduced possibilities of transmission and a possible decrease in genetic diversity in the final host. The former is due to the limitation of spatial dispersion determined by the activity of cercariae and the second intermediate host and to the narrowing of the range of final hosts to the rather few mollusc-feeding vertebrates. The latter is due to the accumulation of metacercariae with different genotypes in the second intermediate host, which increases the genetic diversity of adults in the final host (Rauch et al. 2005). This, in turn, entails the production of offspring with a high genetic variability and, potentially, a higher viability (Rauch et al. 2005; Keeney et al. 2007; Leung et al. 2009). On the contrary, in a mollusc infected with a single miracidium, all parthenitae, cercariae and metacercariae, if they develop in the mother organism, have the same genotype, that is, belong to one and the same clone. However, due to mitotic recombination in the course of sporocysts' reproduction, small intraclonal genetic variability is possible (Grevelding 1999; Bayne and Grevelding 2003; Korsunenko et al. 2012; Galaktionov et al. 2016).

When the final host eats the mollusc, adults of the same clone develop in it. Crossing between them is basically the same as self-fertilisation. This can lead to the production of offspring with a low genetic heterogeneity. Such a situation was noted for *Fascioloides magna* Bassi, 1875, which has no second intermediate host in the life cycle, with a deficiency of heterozygous genotypes in local populations of flukes relative to that expected for a randomly mating population (Mulvey et al. 1991). This begs the question: why are truncated life cycles so common amongst microphallids despite their evident disadvantages? The answer may be associated with the behavioural features of the final hosts of most microphallids, i.e. birds migrating along sea coasts. Belopol'skaya (1956, 1983) attributed microphallids to the group of "migratory" parasites of birds. Transmission of many microphallid species is possible only at the sites where birds stop for a short time during their seasonal migrations. As noted in the "Introduction", microphallids are adapted to such hosts as they have a short maturation period (hours) and a short reproduction period of adults in the final host (days). This increases the chances that the birds would disperse eggs of these parasites in the same areas where they had been infected, that is, in the areas where completion of the life cycle is possible.

We should also remember that when a bird eats a single mollusc infected with a dixenous microphallid species, it gets from several hundred to several thousand metacercariae at once (up to 7600 in the case of *Mi. pygmaeus* from infected periwinkle *Littorina saxatilis* according to Belopol'skaya (1952b)). This ensures high numbers of the infrapopulation of adults in the final host (dozens to hundreds of thousand individuals—Galaktionov (1996)), and, thus, a mass production of eggs. It is not very likely that migrating birds would stop at the sites where suitable first and second intermediate hosts occur. The situation is aggravated by the fact that microphallids have a narrow specificity not only to the first intermediate host, which is characteristic of all trematodes, but also to the second intermediate host (Belopol'skaya 1963; Galaktionov 1993). This is probably due to the intensive metamorphosis the larvae undergo in the second intermediate host (see "Introduction"). One way to increase the probability of transmission is to use abundant and broadly distributed crustacean species as second intermediate hosts, which is characteristic of microphallids. Another way is to eliminate the second intermediate host from the life cycle, i.e. to obtain a dixenous cycle. This is an especially profitable variant for the species whose final hosts are benthos-feeding ducks, primarily, the common eider, whose diet is rich in molluscs (Madsen 1954; Cantin et al. 1974; Bustnes and Erikstad

1988; Węsławski et al. 1994; Krechmar and Kondratyev 2006; Krasnov et al. 2009). *Mi. ochotensis* described in this paper is one such species.

Another possible advantage of dixenous life cycles in intertidal biotopes is the lack of the free-swimming cercaria. This stage is susceptible to adverse factors markedly expressed at the intertidal zone (increased turbulence, periodic exposure, dramatic fluctuations of temperature and salinity). This may to some extent preclude the transmission of trixenous microphallids, especially in biotopes with extreme environmental parameters (surf-beaten shore, the Arctic intertidal). Only dixenous microphallids of the "pygmaeus" group proved capable of implementing life cycles in the ecosystems of the Arctic intertidal (Galaktionov and Bustnes 1999; Galaktionov 2017).

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