Life cycles, molecular phylogeny and historical biogeography of the pygmaeus microphallids (Digenea: Microphallidae): Widespread parasites of marine and coastal birds in the Holarc…

Article *in* Parasitology · May 2012

DOI: 10.1017/S0031182012000583 · Source: PubMed

CITATIONS
29

READS
168

3 authors:

Kirill V. Galaktionov
Russian Academy of Sciences
198 PUBLICATIONS **883** CITATIONS
SEE PROFILE

Isabel Blasco-Costa
Natural History Museum of Geneva
58 PUBLICATIONS **910** CITATIONS
SEE PROFILE

Peter D Olson
Natural History Museum, London
409 PUBLICATIONS **4,210** CITATIONS
SEE PROFILE

Life cycles, molecular phylogeny and historical biogeography of the '*pygmaeus*' microphallids (Digenea: Microphallidae): widespread parasites of marine and coastal birds in the Holarctic

KIRILL V. GALAKTIONOV[1,2*], ISABEL BLASCO-COSTA[3,4] *and* PETER D. OLSON[5]

[1] *Zoological Institute of the Russian Academy of Sciences, St Petersburg, Russia*
[2] *Department of Invertebrate Zoology, St Petersburg State University, St Petersburg, Russia*
[3] *Department of Zoology, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand*
[4] *Institute of Parasitology, Biology Centre, Academy of Sciences of the Czech Republic, Branišovská 31, 370 05 České Budějovice, Czech Republic*
[5] *Department of Zoology, The Natural History Museum, London SW7 5BD, UK*

(*Received 5 October 2011; revised 5 January 2012; accepted 7 March 2012; first published online 1 May 2012*)

SUMMARY

The '*pygmaeus*' microphallids (MPG) are a closely related group of 6 digenean (Platyhelminthes: Trematoda) *Microphallus* species that share a derived 2-host life cycle in which metacercariae develop inside daughter sporocysts in the intermediate host (intertidal and subtidal gastropods, mostly of the genus *Littorina*) and are infective to marine birds (ducks, gulls and waders). Here we investigate MPG transmission patterns in coastal ecosystems and their diversification with respect to historical events, host switching and host-parasite co-evolution. Species phylogenies and phylogeographical reconstructions are estimated on the basis of 28S, ITS1 and ITS2 rDNA data and we use a combination of analyses to test the robustness and stability of the results, and the likelihood of alternative biogeographical scenarios. Results demonstrate that speciation within the MPG was not associated with co-speciation with either the first intermediate or final hosts, but rather by host-switching events coincident with glacial cycles in the Northern Hemisphere during the late Pliocene/Pleistocene. These resulted in the expansion of Pacific biota into the Arctic-North Atlantic and periodic isolation of Atlantic and Pacific populations. Thus we hypothesize that contemporary species of MPG and their host associations resulted from fragmentation of populations in regional refugia during stadials, and their subsequent range expansion from refugial centres during interstadials.

Key words: marine parasites, trematode, *Microphallus*, parasite speciation, parasite transmission, host-parasite co-evolution, host switching, host-parasite assemblages.

INTRODUCTION

The Digenea represents the most speciose group of platyhelminths and encompasses an extraordinarily diverse array of complex life cycles, the vast majority of which remain to be elucidated (Cribb *et al*. 2003). Molecular data have become essential to understand their inter- and intraspecific variability (for a review see Nolan and Cribb, 2005), especially for matching different stages of their life cycles (e.g. Jousson *et al*. 1998; Bartoli *et al*. 2000; Pina *et al*. 2009). However, studies attempting historical-biogeographical reconstructions of digeneans are few in number and primarily concern with taxa of medical and veterinary importance, such as *Schistosoma, Trichobilharzia*, *Fasciola*, and *Paragonimus* (e.g. Iwagami *et al*. 2000; Blair *et al*. 2001; Lockyer *et al*. 2003; Lotfy *et al*. 2008). This handful of genera poorly represents the tremendous breadth of digenean biodiversity, and for a better understanding it is necessary to study systems that provide a broader range of life histories, host associations and biogeographical patterns.

Here we reconstruct the evolutionary relationship of a highly specialized group of digeneans belonging to the Plagiorchiida: microphallids of the '*pygmaeus*' group (MPG). These intestinal parasites of coastal migrating birds (Anseriformes, Charadriiformes) are widespread in the Holarctic, with transmission occurring in coastal ecosystems of the boreal north and the Arctic seas. Until recently, there has been only 1 study that considered the phylogeny, host associations and biogeographical history of digeneans transmitted in Arctic ecosystems, and concerned the genus *Orthosplanchnus* (family Brachycladiidae, previously known as Campulidae), parasites of odontocete cetaceans (Hoberg and Adams, 2000). In a series of articles, Hoberg (1992, 1995) and Hoberg and Adams (1992) demonstrated that the history of

* Corresponding author: Zoological Institute of the Russian Academy of Sciences, St Petersburg, Russia. Fax: +78123282941. E-mail address: kirill.galaktionov@gmail.com

doi:10.1017/S0031182012000583

helminths of marine mammals and birds of the Arctic basin was deeply influenced by the glacial cycles during the late Pliocene/Quaternary that periodically provided favourable conditions for host-switching, co-speciation and geographical colonization (for a review see Hoberg and Brooks, 2008). Unlike the MPG studied here, the cestodes *Anophryocephalus* and *Alcataenia* studied by Hoberg (1992, 1995) and Hoberg and Adams (1992), use many species of common pelagic crustaceans and fishes as intermediate and paratenic hosts, and intermediate hosts for *Orthosplanchnus* species are yet unknown. Also unlike the MPG, these cestode and digenean species have a rather narrow specificity to their final hosts and rather high longevity as adults.

Microphallus species of the '*pygmaeus*' group are unusual in their lack of free-swimming cercariae, which develop to the metacercarial stage within daughter sporocysts (Galaktionov, 1993; Saville *et al*. 1997), facilitating transmission in the intertidal zone. For most MPG species, first intermediate hosts are intertidal periwinkles of the genus *Littorina*, and final hosts are infected by feeding on periwinkles containing sporocysts with invasive metacercariae (Fig. 1). Maturation in the final host is fast: eggs can be seen in the uterus as early as several hours following infection, and eggs containing fully-formed miracidia are seen after 3–4 days. The life span of the adults is also short, lasting 6–14 days. Thus the MPG, typical of most microphallids, is adapted to parasitism in migratory birds. A short stay at a marine coastal stretch is sufficient for the birds to become infected and for a new generation of parasites to be disseminated. MPG species are also characterized by a broad specificity to the final hosts, which further enhances transmission success.

The MPG is composed of six closely related species (Table 1) which, with the exception of the Far Eastern species *Microphallus calidris*, had long been considered as a single species, i.e. '*Microphallus pygmaeus*' Galaktionov (1983, 1984, 2009) has shown that this name has been applied to 4 independent species: *Microphallus pygmaeus, M. piriformes*, *M. triangulatus* and *M. pseudopygmaeus*. Recently sporocysts containing metacercariae of a previously unknown MPG species described as *M. kurilensis* were found in the periwinkle *Littorina sitkana* from the Sea of Okhotsk (Galaktionov *et al*. 2010). Final hosts of *M. kurilensis* remain unknown, and their discovery together with circumscription of all nominal MPG species using molecular data were among the aims of the present research. Here we use a combination of molecular loci to elucidate the host associations of MPG in coastal ecosystems and to specify the geographical range of these parasites. We explore the phylogeny and historical biogeography of MPG to test the role of co-evolution, host-switching and geographical colonization in diversification of these highly specialized digeneans.

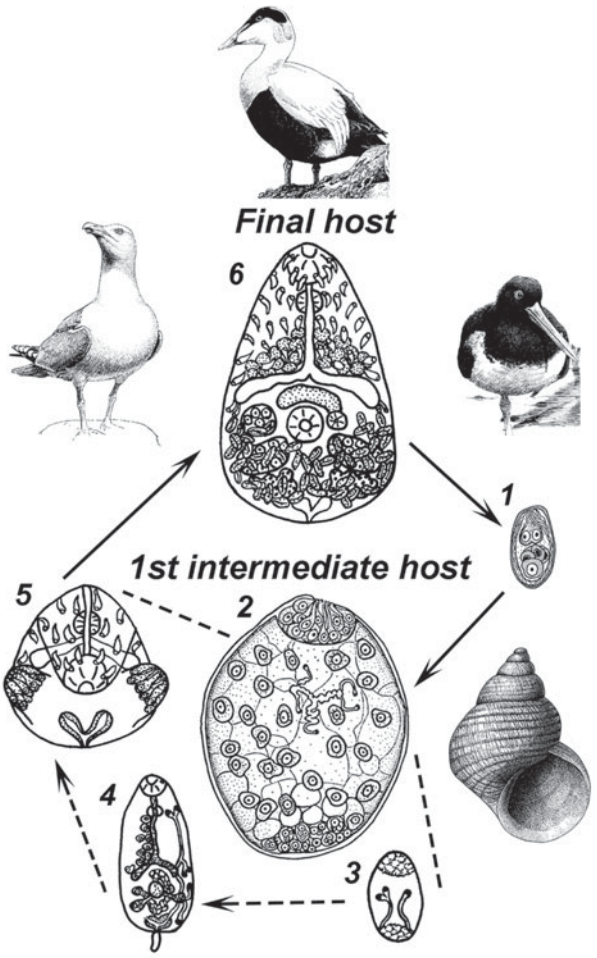

Fig. 1. Life cycle of the '*pygmaeus*' microphallids. (1) Eggs containing miracidia in the environment; (2) daughter sporocysts in the molluscan host; (3–5) successive stages of metacercarial development inside of the daughter sporocyst (3–4 – embryos, 5 – fully-formed metacercaria); (6) adults in the final host.

MATERIALS AND METHODS

Taxa sampled

Material for the present research was collected at the marine coasts of Northern Europe (Iceland; the Norwegian, Barents and White Seas) and Northern Asia (Sea of Okhotsk and the Bering Sea) during 2003–2008 (Fig. 2). The majority of samples consisted of sporocysts containing metacercariae collected from intertidal periwinkles of the genus *Littorina* spp. and, in the case of *M. pseudopygmaeus*, also from the species *Onoba aculeus* and *Falsicingula athera* (Table 2). Molluscs were dissected under a stereomicroscope to identify those with fully formed metacercariae. Several sporocysts from each infected mollusc were dissected to extract metacercariae that were identified according to the criteria of Galaktionov (1983, 1984) and Saville *et al*. (1997). Other sporocysts were separated from the molluscan host tissues, washed repeatedly in seawater, filtered though a bacterial filter (0·22 μm, Millipore) and

Table 1. Host range and geographical distribution of *Microphallus similis* and microphallids of the '*pygmaeus*' group

(Data stem from our literature and field surveys (see Supplementary File, online version only).)

Microphallus species	First intermediate (molluscan) host	Final host (main)	Distribution
M. similis Jägerskiöld, 1900	*Littorina (Neritrema) saxatilis* (Olivi, 1792), *L.* (*N.*) *obtusata* (Linnaeus, 1758), *L.* (*N.*) *fabalis* (W.Turton, 1825), *L.* (*N.*) *arcana* Hannaford Ellis, 1978, *L.* (*N.*) *compressa* Jeffreys, 1865, *L. (N.) sitkana* Philippi, 1846, *Littorina* (*Littorina*) *littorea* (Linnaeus, 1758),	Gulls, waders	Holarctic
M. piriformes (Odhner, 1905)	*L. (N.) saxatilis, L. (N.) obtusata, L.(N.) fabalis, L.(N.) arcana, L.(N.) compressa*	Gulls, waders	North Atlantic (Europe, probably North American Atlantic coast)
M. calidris Belopolskaia & Ryjikov, 1963	*L. (N.) sitkana, L.(N.) subrotundata* (Carpenter, 1864) (?)*, *L. (N.) kasatka* (Reid, Zaslavskaya & Sergievsky, 1991) (?)*,	Waders, herring gull chicks (exp.)	North Pacific (Sea of Okhotsk)
M. pseudopygmaeus Galaktionov, 2009	*L. (N.) saxatilis, L.(N.) obtusata, L.(N.) fabalis, L. (N.) arcana, L. (N.) compressa, L.(N.) sitkana, Littorina (Littorina) scutulata* Gould, 1849, *Epheria vincta* (Montagu, 1803), *Onoba aculeus* (Gould, 1841), *Hydrobia ventrosa* (Montagu, 1803), *Falsicingula kurilensis* (Pilsbry, 1905), *Falsicingula athera* Bartsch, 1936, *Boreocingula martyni* (Dall, 1886), *Cryptonatica affinis* (Gmelin, 1791), *Maragrites helicinus* (Phipps, 1774), *M. groenlandicus* (Gmelin, 1791), *Solariella varicosa* (Mighels and Adams, 1842)	Common eider, benthos-feeding seaducks	Holarctic (North Pacific, Europe, probably North American Atlantic coast)
M. triangulatus Galaktionov, 1984	*L. (N.) saxatilis, L. (N.) obtusata, L. (N.) fabalis, L.(N.) arcana, L.(N.) compressa, L. (N.) sitkana* (?)	Common eider	Holarctic (North Asiatic Pacific coast, Europe, probably North American Atlantic coast)
M. pygmaeus (Levinsen, 1881)	*L. (N.) saxatilis, L. (N.) obtusata, L. (N.) fabalis, L.(N.) arcana, L.(N.) compressa, L.(L.) littorea*	Common eider, benthos-feeding seaducks	North Atlantic (Europe, probably North American Atlantic coast)
M. kurilensis Galaktionov, Regel & Atrashkevich, 2010	*L. (N.) sitkana, L.(N.) natica, L. (N.) aleutica, L. (N.)* sp. 3 Zaslavskaya, 2006	Common eider, most probably other benthos-feeding seaducks	North Pacific (North Asiatic Pacific coast, probably North American Pacific coast)

* Tsimbaljuk *et al*. (1978) found sporocysts containing *M. calidris* metacercariae in *Littorina kurila* Middendorf, 1848 at the Iturup Island (the Kurile Islands). Reid (1996) showed that the species *L. kurila* was a composite, and in accordance with Zaslavskaya (2006) is presented at the Iturup Island by *L. sitkana, L. subrotundata* (Carpenter, 1864) and *L. kasatka* (Reid, Zaslavskaya and Sergievsky, 1991).

fixed live in 100% ethanol for subsequent DNA extraction.

In addition to intramolluscan stages, adult microphallids were obtained from the slaty-backed gull (*Larus schistisagus*) and the Pacific eider (*Somateria mollissima v-nigrum*) collected during an expedition to the north of the Sea of Okhotsk in August 2008 (Table 2). Birds were dissected immediately after being shot, and the parasites extracted from their small intestine. Live worms were sorted according to morphotypes under a stereomicroscope. Some of the worms thus sorted were studied live under a field microscope in order to check the morphological homogeneity of the samples. As it is difficult to reliably identify species of living adult worms, samples of similar morphotypes were referred to as 'Mkur' with a corresponding number. Adults of each morphotype were fixed in pure ethanol for subsequent DNA extraction. Before extraction, worms were cleaned from intestinal chyme by repeated washing in filtered seawater. Adults were also fixed in 70% ethanol, stained with alum carmine, cleared in isobutyl alcohol and xylene, and mounted in Canada balsam. This material was subsequently used for species identification on the basis of morphology. Voucher specimens of each morphotype are

Fig. 2. Geographical range of *Neritrema* species (based on Reid (1996) and our data) (dotted lines) and microphallids of the '*pygmaeus*' group. Sample sites are indicated as black circles.

deposited in the trematode collection of the Zoological Institute RAS (St Petersburg, Russia) (mount numbers: 3225–3234).

Molecular data

Specimens fixed live in 100% ethanol were stored at −20 °C. Specimens were transferred into 300 μl of 1M Tris-EDTA (pH 8) and genomic DNA (gDNA) was extracted using a Qiagen® DNeasy™ tissue kit following the manufacturer's protocol, except for the proteinase-K incubation which was extended to an overnight incubation, and the gDNA was concentrated to a volume of ∼30 μl using Millipore Microcon® columns. Partial 28S rDNA (domains D1-D3; ∼1400 bps) sequences were amplified using primers LSU-5 (5′-TAG GTC GAC CCG CTG AAY TTA AGC A-3′) and LSU-1500R (5′-GCT ATC CTG AGG GAA ACT TCG-3′) (Olson *et al.* 2003). Partial ITS1 rDNA sequences were amplified using primers M1780F (5′-ACACCGCCCGTCGC TACTA-3′; (Bulat and Alekhina, *unpublished observations*) and M5·8R (5′-GGCTGC GCTCTTCAT CGACA-3′; (Bulat and Alekhina, *unpublished observations*). Complete ITS2 rDNA sequences were amplified using 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′) (Anderson and Barker, 1993). Gene fragments were amplified using illustra PuReTaq Ready-To-Go™ PCR beads (GE Healthcare), with 20–70 ng of template DNA and 10 mM of each PCR primer. The following thermocycling profile was used for 28S rDNA amplification: denaturation (95 °C for 5 min); 35 cycles of amplification (94 °C for 50 sec, 58 °C for 50 sec and 72 °C for 1 min 20 sec); and 4 min extension holds at 72 °C. The same profile but with an annealing temperature of 54 °C was used for

Table 2. Taxa sequenced, isolate codes, localities, hosts and GenBank Accession numbers for each sequence

Isolate code	Species	Stage	Locality and Host	28S	ITS1	ITS2
Maa	*Maritrema arenaria* Hadley & Castle, 1940	Metacercariae	Belfast Lough, Northern Ireland; *Semibalanus balanoides* (Linnaeus, 1767) – acorn barnacle		HM584144	HM584171
Moo	*Maritrema oocysta* Lebour, 1907	Sporocyst containing encysted metacercariae	Belfast Lough, Northern Ireland; *Hydrobia ulvae* (Pennant, 1777) – laver spire shell		HM584143	HM584170
Msub	*Maritrema subdolum* Jägerskiöld, 1909	Sporocysts containing cercariae	Kandalaksha Bay, White Sea, Russia; *Hydrobia ulvae* – laver spire shell	HM584135	HM584145	HM584172
Msub2	*Maritrema subdolum*	Sporocysts containing cercariae	Kandalaksha Bay, White Sea, Russia; *Hydrobia ulvae* – laver spire shell		HM584146	
Mabo	*Microphallus abortivus* Deblock, 1974	Sporocysts containing encysted metacercariae	Belfast Lough, Northern Ireland; *Hydrobia ulvae* – laver spire shell		HM584159	HM584173
Mab	*Microphallus abortivus*	Sporocysts containing encysted metacercariae	Belfast Lough, Northern Ireland; *Hydrobia ulvae* – laver spire shell			HM584174
Mcal2	*Microphallus calidris* Belopolskaia & Ryjikov, 1963	Sporocysts containing metacercariae	Sea of Okhotsk, Sakhalin, Russia; *Littorina sitkana* Philippi, 1846 – Sitka periwinkle	HM584124	HM584151	HM584183
Mcal4	*Microphallus calidris*	Sporocysts containing metacercariae	Kunashir, Kuril Islands, Russia; *Littorina sitkana* – Sitka periwinkle	HM584125		
Mcal_Gal4/5	*Microphallus calidris*	Sporocysts containing metacercariae	Sea of Okhotsk, Sakhalin, Russia; *Littorina sitkana* – Sitka periwinkle		HM584150	HM584184
Mchul2	*Microphallus kurilensis* Galaktionov, Regel & Atrashkevich, 2010	Sporocysts containing metacercariae	Egvekinot Inlet, Bering Sea, Chukotka, Russia; *Littorina natica* Reid, 1996 – periwinkle	HM584129	HM584165	HM584185
Mchul3	*Microphallus kurilensis*	Sporocysts containing metacercariae	Egvekinot Inlet, Bering Sea, Chukotka, Russia; *Littorina natica* – periwinkle	HM584130		
Mchuk	*Microphallus kurilensis*	Sporocysts containing metacercariae	Lawrence Bay, Bering Sea, Chukotka, Russia; *Littorina aleutica* Dall, 1872 – periwinkle			HM584186
Mchul_Gal7	*Microphallus kurilensis*	Sporocysts containing metacercariae	Kunashir, Kuril Islands, Russia; *Littorina sitkana* – Sitka periwinkle		HM584164	
Mkur	*Microphallus kurilensis*	Sporocysts containing metacercariae	Kunashir, Kuril Islands, Russia; *Littorina sitkana* – Sitka periwinkle	HM584132		HM584189
Mkur3	*Microphallus kurilensis*	Sporocysts containing metacercariae	Kunashir, Kuril Islands, Russia; *Littorina sitkana* – Sitka periwinkle	HM584131	HM584166	
Mkur8A	*Microphallus kurilensis*	Adult	Yamskaya Bay, N Sea of Okhotsk, Russia; *Somateria mollissima v-nigrum* Bonaparte, 1855 – Pacific common eider (juv.)	HM584140	HM584169	
Mkur8B	*Microphallus kurilensis*	Adult	Yamskaya Bay, N Sea of Okhotsk, Russia; *Somateria mollissima v-nigrum* – Pacific common eider (juv.)	HM584141	HM584168	HM584187
Mkur8C	*Microphallus kurilensis*	Adult	Yamskaya Bay, N Sea of Okhotsk, Russia; *Somateria mollissima v-nigrum* – Pacific common eider (juv.)		HM584167	HM584188
Mkur5A	*Microphallus* sp. (unidentified)	Adult	Cape Taygonos, N Sea of Okhotsk, Russia; *Somateria mollissima v-nigrum* – Pacific common eider	HM584142	HM584160	
Mkur5C	*Microphallus* sp. (unidentified)	Adult	Cape Taygonos, N Sea of Okhotsk, Russia; *Somateria mollissima v-nigrum* – Pacific common eider		HM584161	HM584175

Table 2. (*Cont.*)

Isolate code	Species	Stage	Locality and Host	28S	ITS1	ITS2
Mpir	*Microphallus piriformes* (Odhner, 1905)	Sporocysts containing metacercariae	Vaygatch Island, SE Barents Sea, Russia; *Littorina saxatilis* (Olivi, 1792) – rough periwinkle	HM584123		
Mpir2	*Microphallus piriformes*	Sporocysts containing metacercariae	Grindavik, SW Iceland; *Littorina saxatilis* – rough periwinkle	HM584122	HM584154	HM584181
Mpir_Gal9	*Microphallus piriformes*	Sporocysts containing metacercariae	Balsfjord, Norwegian Sea, Norway; *Littorina obtusata* (Linnaeus, 1758) – flat periwinkle			HM584182
Mpse	*Microphallus pseudopygmaeus* Galaktionov, 2009	Sporocysts containing metacercariae	Kandalaksha Bay, White Sea, Russia; *Onoba aculeus* (Gould, 1841) – pointed cingula	HM584126		HM584198
Mpse2	*Microphallus pseudopygmaeus*	Sporocysts containing metacercariae	Kandalaksha Bay, White Sea, Russia; *Littorina saxatilis* – rough periwinkle	HM584127	HM584147	
Mpse_Gal2	*Microphallus pseudopygmaeus*	Sporocysts containing metacercariae	Kunashir, Kuril Islands, Russia; *Falsicingula athera* Bartsch, 1936			HM584199
Mpyg	*Microphallus pygmaeus* (Levinsen, 1881)	Sporocysts containing metacercariae	Grindavik, SW Iceland; *Littorina saxatilis* – rough periwinkle	HM584133	HM584153	HM584190
Mpyg2	*Microphallus pygmaeus*	Sporocysts containing metacercariae	Kandalaksha Bay, White Sea, Russia; *Littorina saxatilis* – rough periwinkle	HM584134		HM584191
Mpyg_Gal 8	*Microphallus pygmaeus*	Sporocysts containing metacercariae	Balsfjord, Norwegian Sea, Norway; *Littorina saxatilis* – rough periwinkle		HM584152	HM584192
Mis	*Microphallus similis* Jägerskiöld, 1900	Metacercaria	Belfast Lough, Northern Ireland; *Carcinus maenas* (Linnaeus, 1758) – shore crab		HM584155	HM584179
Msimilis	*Microphallus similis*	Metacercaria	Belfast Lough, Northern Ireland; *Carcinus maenas* – shore crab			HM584180
Mkur6A	*Microphallus similis*	Adult	Impoveem, Sea of Okhotsk, Russia; *Larus schistisagus* Stejneger, 1884 – slaty-backed gull (juv.)	HM584137	HM584156	HM584176
Mkur6B	*Microphallus similis*	Adult	Impoveem, Sea of Okhotsk, Russia; *Larus schistisagus* – slaty-backed gull (juv.)	HM584136	HM584157	HM584178
Mkur6C	*Microphallus similis*	Adult	Impoveem, Sea of Okhotsk, Russia; *Larus schistisagus* – slaty-backed gull (juv.)	HM584138	HM584158	HM584177
Mtri	*Microphallus triangulatus* Galaktionov, 1984	Sporocysts containing metacercariae	Kandalaksha Bay, White Sea, Russia; *Littorina saxatilis* – rough periwinkle	HM584128		HM584196
Mtri_Gal3	*Microphallus triangulatus*	Sporocysts containing metacercariae	Vaygach Island, SE Barents Sea, Russia; *Littorina saxatilis* – rough periwinkle		HM584148	HM584197
Mkur7A	*Microphallus triangulatus*	Adult	Yamskaya Bay, Sea of Okhotsk, Russia; *Somateria mollissima v-nigrum* – Pacific common eider (juv.)		HM584162	HM584195
Mkur7B	*Microphallus triangulatus*	Adult	Yamskaya Bay, Sea of Okhotsk, Russia; *Somateria mollissima v-nigrum* – Pacific common eider (juv.)		HM584149	HM584194
Mkur7C	*Microphallus triangulatus*	Adult	Yamskaya Bay, Sea of Okhotsk, Russia; *Somateria mollissima v-nigrum* – Pacific common eider (juv.)	HM584139	HM584163	

amplification of both ITS regions. PCR amplicons were either gel-excised or purified directly using a Qiagen QIAquick™ PCR Purification Kit and cycle-sequenced from both strands using ABI BigDye™ chemistry and sequenced on an ABI 3730 automated sequencer. PCR primers and internal primers 300F (5′-CAA GTA CCG TGA GGG AAA GTT G-3′), ECD2 (5′-CTT GGT CCG TGT TTC AAG ACG GG-3′) and LSU1200R (5′-GCA TAG TTC ACC ATC TTT CGG-3′) (Olson *et al*. 2003) in the case of 28S, were used for cycle sequencing. Contiguous sequences were assembled and edited using Sequencher™ (GeneCodes Corp., ver. 3.1.1) and submitted to GenBank (see Table 2 for sequence Accession numbers).

Alignment and phylogenetic analyses

Newly generated 28S, ITS1 and ITS2 rDNA sequences were aligned using ClustalW as implemented in MEGA 4.0 (Tamura *et al*. 2007) together with representatives of the Microphalloidea and Plagiorchioidea, and adjustments made by eye using MacClade 4.08 (Maddison and Maddison, 2005). After trimming ends to account for missing data and excluding regions of ambiguous alignment or containing indels in more than half the taxa, the final alignments were 943 (28S), 405 (ITS1), and 238 (ITS2) bps in length. Pairwise distances are reported as absolute pairwise differences and p-distances (gaps treated as missing data).

Data partitions were analysed independently using the methods of maximum parsimony (MP) and Bayesian inference (BI). MP analyses were performed with PAUP* 4.0b10 (Swofford, 2002) using a heuristic search strategy with 1000 search replicates, random-addition taxa sampling, tree-bisection-reconnection branch-swapping, with all characters run unordered with equal weights and gaps treated as missing data. Nodal support was estimated by bootstrap analysis (fast-heuristic search strategy with 10000 pseudoreplicates). BI analyses were conducted using MrBayes version 3.1.2 (Ronquist and Huelsenbeck, 2003). Prior to the analyses, nucleotide substitution models were estimated independently for each data partition using ModelTest 3.06 (Posada and Crandall, 1998). The models GTR + I + Γ (general-time-reversible model including estimates of invariant sites and gamma distributed among-site rate variation) and GTR + I were estimated as best fitting the 28S and ITS data sets, respectively. Analyses were run over 1 million generations with a sampling frequency of 100. Consensus trees with mean branch lengths were constructed from topologies saved after log-likelihood values and substitution parameters plateaued (i.e. 'burn-in'). Nodal support was estimated as posterior probabilities (Huelsenbeck *et al*. 2001).

In order to provide further means of examining support for the placement of taxa and clades, as well as for comparing the veracity of the individual gene regions, taxa were ranked according to their 'leaf stabilities' (Thorley and Wilkinson, 1999). The leaf stability metric provides a ranked measure of how labile the position of a taxon or clade is within a given set of input trees, on a scale of zero to 1, with 1 indicating that its position is the same in all trees. Stability values were calculated according to Thorley and Wilkinson (1999) using a Python script written by T. Hill (Natural History Museum, London; *unpublished data*) using the sets of trees generated by BI (without topologies estimated during burn-in).

Historical biogeographical analyses

Biogeographical history of the MPG species was investigated by Statistical Dispersal-Vicariance Analysis (S-DIVA) (Yu *et al*. 2010). This analysis provides ancestral reconstructions as given by Ronquist (1997, 2001) and accounts for phylogenetic uncertainty using a Bayesian approach (Nylander *et al*. 2008; Harris and Xiang, 2009). In addition, it determines statistical support (S-DIVA value) for the ancestral range reconstructions. S-DIVA 1.9 calculates the frequencies of an ancestral distribution range at a node that are averaged over all trees in ancestral reconstructions, so that each alternative ancestral range at a node is weighted by the frequency of the node occurring (Yu *et al*. 2010). In order to implement S-DIVA, we used phylogenetic hypotheses of the 6 MPG species and *Microphallus similis* Jägerskiöld, 1900 as an outgroup for the 28S and ITS1 rDNA regions independently, estimated by BI as described above. The ITS2 region was not considered for reasons detailed below. We allocated species to distribution ranges, North Atlantic (NA) or North Pacific (NP) or both, including the outgroup. We performed the analysis on 6000 randomly sampled trees from those evaluated by BI.

RESULTS

Molecular taxonomy and matching of life-cycle stages

There was a total of 404, 237 and 942 included (i.e. alignable) characters in the ITS1, ITS2 and 28S datasets respectively. Of these, 237 (59%), 154 (65%) and 525 (57%) were invariant, and 134 (33%), 64 (27%) and 339 (36%) informative under the principles of parsimony in the ITS1, ITS2 and 28S respectively. When considering the MPG taxa only ($N = 10$) for the 28S dataset, 1083 characters were alignable, of which 962 (89%) were invariant, 89 were uninformative (i.e. autapomorphic; 8%) and 32 were parsimony-informative (3%). Divergence within the genus *Microphallus* ranged from 0·01 to 0·16 in ITS1 and 0 to 0·12 in ITS2, and within the '*pygmaeus*'

group interspecific sequence variability was much more reduced, ranging from 0 to 0·02 respectively. Intergeneric divergences ranged from 0·17 to 0·22 in ITS1 and 0·12 to 0·19 in ITS2, showing small overlap with the interspecific range for the latter.

Intramolluscan stages of all isolates of each of the 6 MPG species identified on the basis of morphological criteria were found to be genetically identical, regardless of the geographical provenance of the material. Thus, molecular data of *M. kurilensis* isolates from *Littorina sitkana* sampled at the Kunashir Island turned out to be identical to those of the Chukotka isolates from *L. natica* and *L. aleutica*. This forced us to reject an initial proposition that the Chukotka isolates could represent a species other than *M. kurilensis*.

Molecular characterization of adult microphallids from the common eider and slaty-backed gull revealed the presence of 4 species, 3 of which could be readily identified according to species groups represented by larval sequences (Fig. 3). Moreover, all 3 molecular markers supported the same species-level clades that included both adult and metacercarial-derived sequences. Of these, Mkur8 and Mkur7 specimens represented isolates of *M. kurilensis* and *M. triangulatus*, respectively and, in most cases, exact matches between larval and adult sequences were obtained. Moreover, the identity of *M. triangulatus* was further corroborated by the presence of posterior-lateral glands, which are species-specific characters as previously defined by Galaktionov (1984). Mkur6, isolated from the slaty-backed gull, was identified as *Microphallus similis*, albeit the adult isolates differed by 3 transitions in ITS1 and by a 4 bp insertion (i.e. GTTT) in Msim that was not found among the adult isolates. Morphological characters of Mkur6 were consistent with the type descriptions of *M. similis* provided by Belopolskaya (1963) and Deblock (1971).

The fourth adult specimens sequence (Mkur5) did not correspond to any of the molecularly characterized microphallid species in the present or previous (e.g. Tkach *et al*. 2003) studies, and appeared outside of the MPG clade. The poor condition of the few specimens of Mkur5 at our disposal prevented species identification on the basis of morphology, and consequently we cannot determine whether the entity is new to science or simply a previously described species of *Microphallus* not represented by available molecular data.

Phylogenetic analyses

Figure 3A presents a species-level phylogram of the 28S rDNA dataset for the Microphallidae and closely related families according to Olson *et al*. (2003). The strict consensus of 4 equally parsimonious trees was congruent with the BI solution, thus we present the BI topology with MP bootstrap values and BI posterior probabilities (PP). Representatives of the Allocreadioidea and Plagiorchioidea were selected as outgroups. Three strongly supported clades were found in the ingroup that corresponded to the Lecithodendriidae, Pleurogenidae+Prosthogonimidae and the Microphallidae. The Microphallidae formed 2 clades with high PP, *Microphallus* and *Maritrema* + Microphallidae gen. sp. Within the latter clade, strong support was depicted for all relationships except between *Maritrema neomi* Tkach, 1998 and *M. heardi* (Kinsella and Deblock, 1994). *Microphallus* spp. were divided into 2 major clades, 1 including *M. similis* occupying a basal position, sister to the '*pygmaeus*' group. The topology depicted by the analyses of the ITS1 dataset was in agreement with the solution of the 28S dataset with only 2 departures, (i) the lack of a sister relationship between Mkur5C and *Microphallus abortivus* Deblock, 1974, and (ii) the position of *M. calidris* as sister to *M. piriformes* (see Fig. 3B). However, the hypothesis obtained from the analyses of the ITS2 region (Fig. 3C) was different to that previously described for the ITS1 and 28S datasets and most relationships found were characterized by low nodal support most likely due to the low number of parsimony-informative characters (64). Moreover, the ITS2 hypothesis showed the lowest relative average leaf stability values (see Table 3), indicating that it has the weakest phylogenetic signal of the 3 genes. With regard to individual taxa, *M. calidris* exhibited the lowest leaf stability among all datasets, reflecting a lack of support for its position among the other taxa: appearing as sister taxon to the clade [[*M. pseudopygmaeus* + *M. triangulatus*] + [*M. pygmaeus* + *M. kurilensis*]] (28S, see Fig. 3A) or to *M. piriformes* (see Fig. 3B, ITS1 dataset) or to [*M. pygmaeus* + *M. kurilensis*] (see Fig. 3C, ITS2 dataset).

Historical biogeographical analysis

The biogeographical reconstructions are shown in Fig. 4. Pie charts at nodes indicate the relative frequencies of ancestral region optimizations across the entire tree sample. Two alternative, equally optimal reconstructions were found for both data sets shown in Fig. 4C–F. The ancestral distribution range of *M. similis* + MPG was depicted as NA by 3 optimal reconstructions (node VI in Fig. 4C–E). An NA ancestral distribution range of the MPG was found to be slightly more likely (53% in Fig. 4A). A remarkable finding was a range expansion from NA to NP or NA + NP (50% each) for the ancestor of *M. calidris* + remaining MPG (Fig. 4A). The position of *M. calidris* and *M. piriformes* differed slightly in the phylogenetic trees obtained for 28S and ITS1 as mentioned above. Due to this departure, a range expansion from NA or NP to NA + NP was shown for

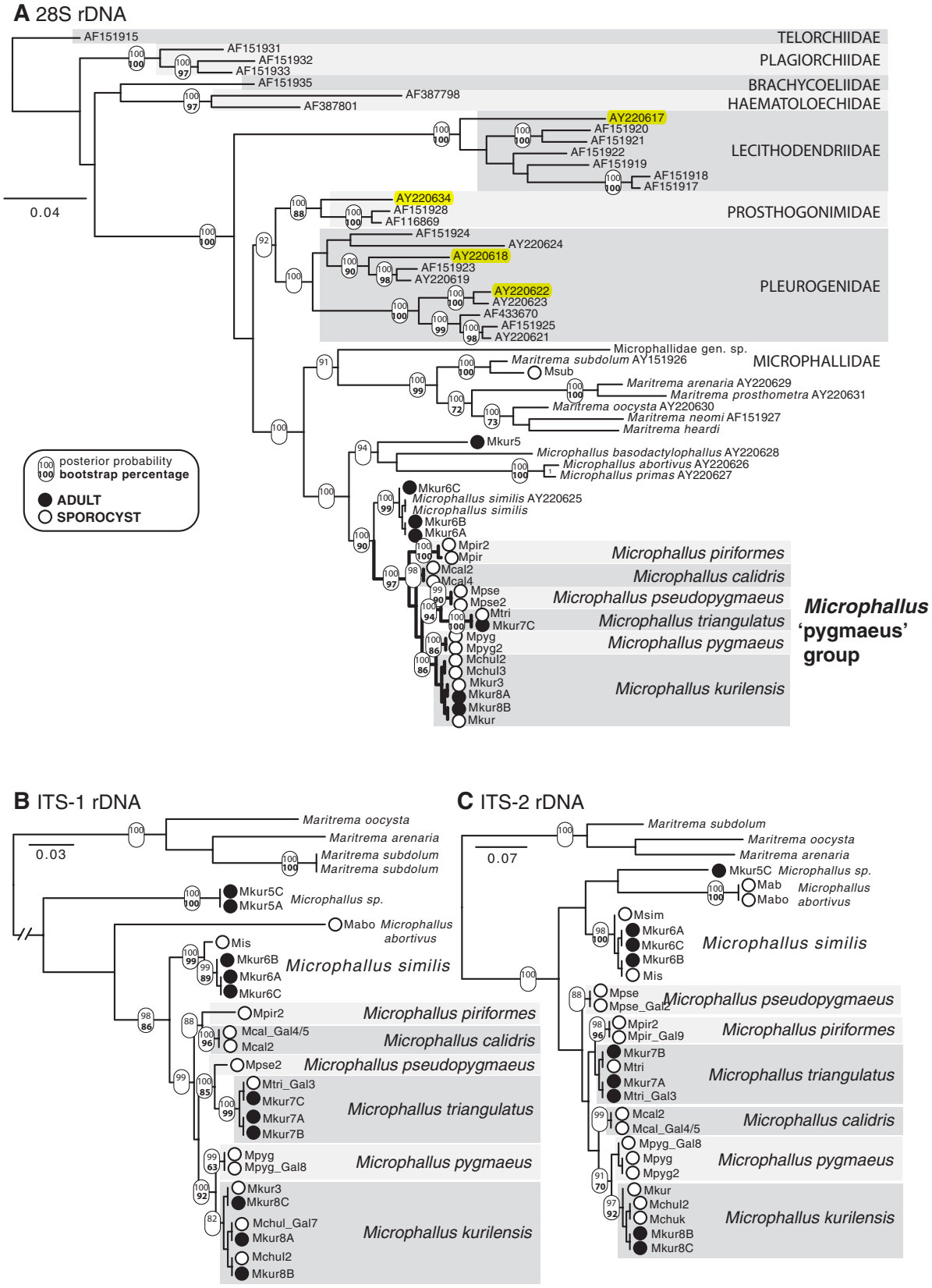

Fig. 3. Bayesian inference phylograms derived from 28S, ITS1 and ITS2 rRNA gene sequences with posterior probability and bootstrap percentage values above and below, respectively, within ellipses. Black dots indicate adult specimens sequenced whereas white dots indicate sporocysts or metacercariae stages sequenced. Species names are indicated in full.

Table 3. Relative Leaf Stability Values of each dataset and *Microphallus* spp.*

Microphallus species	28S	ITS1	ITS2
M. pygmaeus	0·924	0·893	0·708
M. kurilensis	0·909	0·893	0·748
M. similis	0·886		0·499
M. abortivus	0·886		0·451
M. pseudopygmaeus	0·885	0·893	0·491
M. triangulatus	0·885	0·893	0·534
M. piriformes	0·825	0·877	0·531
M. calidris	0·764	0·792	0·491
AVERAGE	0·880	0·874	0·591

* Except for *M. primas* Jägerskiöld, 1908 and *M. basodactylophallus* (Bridgman, 1969) that are only present in 1 dataset.

the ancestor of [*M. piriformes* + *M. calidris*] (100% Fig. 4B) and a slight increase of NP frequency for the ancestor of [[*M. pseudopygmaeus* + *M. triangulatus*] + [*M. pygmaeus* + *M. kurilensis*]].

DISCUSSION

Life cycles and transmission patterns

Pairing the larval and adult sequences of MPG taxa has permitted us to clarify host associations and thus the means by which these species are transmitted in their coastal ecosystems. Identical ITS1 and ITS2 sequences from *M. kurilensis* metacercariae isolated from the periwinkles *L. sitkana, L. natica* and *L. aleutica* and from adult digeneans collected from a common eider duckling shot in the Sea of Okhotsk (Yamskaya Bay) showed the samples to be conspecific, and thus Pacific eiders can be considered as the natural final host of *M. kurilensis*.

The finding of *M. triangulatus* adults in an eider from the northern part of the Sea of Okhotsk was unexpected. This species has been known before only from the NA (Galaktionov, 1984; Galaktionov *et al.* 2004). Now *M. triangulatus* should be considered as a species with amphiboreal (circum-polar) distribution. Its final host, both in NA and in NP, is the common eider, whereas the role of the first intermediate host is played by periwinkles *Littorina* (*Neritrema*).

It is of considerable interest that the worms found in the material from *Larus schistisagus* gulls from the Pacific have to be attributed according to ITS1 and ITS2 sequences to the species *M. similis*. The first intermediate host of *M. similis* at the Northern European coast and the Atlantic coast of America are periwinkles mostly from the subgenus *Neritrema*. In NP, sporocysts and cercariae are found in *L. sitkana* of the Sea of Okhotsk (Galaktionov, *unpublished observations*). At the American NP coast, *M. similis* metacercariae have only been reported from the crab *Cancer magister* Dana, 1852 (Ching, 1991). The final hosts of *M. similis* are many species of Charadriiformes, mostly, gulls (Belopolskaya, 1963; Deblock, 1971). The level of differences between the Okhotsk and the European isolates, according to the molecular markers revealed in our study, is not high and may reflect intraspecific geographical variability. Further corroboration is the morphological correspondence to the descriptions of this species and complete similarity between *M. similis* cercariae from *Neritrema* periwinkles in Europe and in the Sea of Okhotsk (Galaktionov, *unpublished observations*). These results support the idea about the amphiboreal distribution of *M. similis*, though the existence of sibling species or at least geographical races in NA and NP cannot be discounted.

Phylogeny and host associations

The MPG taxa were found to be monophyletic in all phylogenetic estimates. A universal synapomorphy supporting this clade is the possession of a 2-host life cycle. Typical of all digeneans, MPG have a rather narrow specificity to their first intermediate hosts, being restricted not only to periwinkles of the genus *Littorina*, but with only 1 exception, to the subgenus *Neritrema*. Even *M. pseudopygmaeus*, which colonized many species of marine prosobranchs, can still develop in *Neritrema*, suggesting that it may be the ancestral intermediate host species of the group. At the same time, no MPG taxon is closely associated with a particular *Neritrema* species; that is, both Atlantic and Pacific MPG species use as first intermediate hosts those *Neritrema* periwinkles that are available in the region.

A different picture is observed when the distribution of the final hosts across different phylogenetic branches of MPG is analysed. *Microphallus piriformes* and *M. calidris*, which are basal in relation to the other MPG species, use as their final hosts mostly charadriiforms (i.e. gulls and waders), whereas the more derived species parasitize anseriform birds (primarily seaducks and especially eiders). In all phylogenetic estimates, the sister taxon of the MPG is *M. similis*. Although the analyses involved only a few (and mostly Atlantic) *Microphallus* species, consistency in the position of *M. similis* as the sister taxa to MPG suggests that they shared a common ancestor.

A hypothetical scenario of MPG speciation consistent with these results could be as follows. Transmission of the common ancestor of *M. similis* and MPG could be confined to the same host range as that of the recent *M. similis*. When the immediate ancestor of MPG developed a 2-host life cycle, the composition of the first intermediate and the final hosts did not change. The same hosts were then retained by *M. piriformes* and *M. calidris*, although

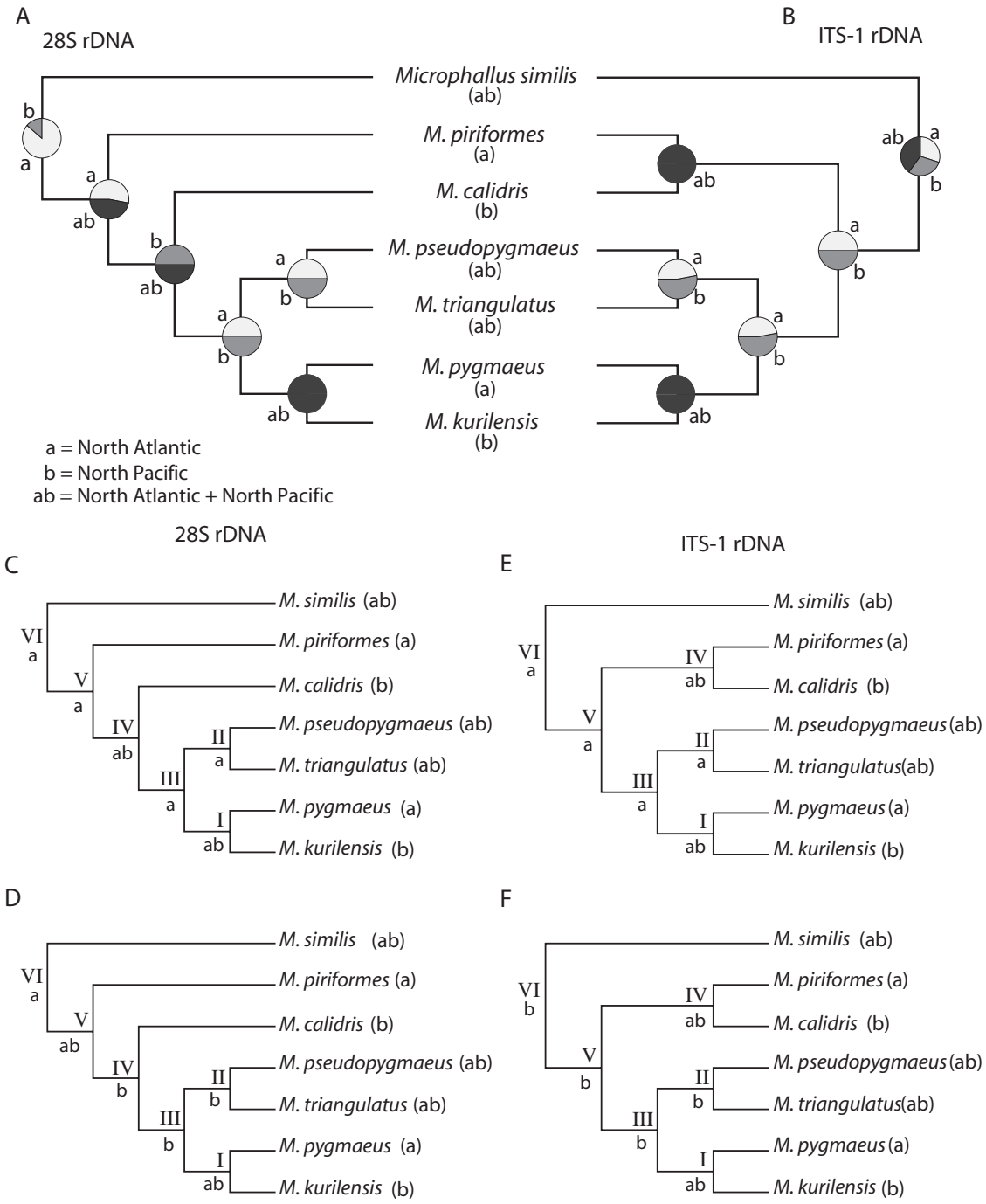

Fig. 4. Graphical output from S-DIVA for the '*pygmaeus*' microphallids. Two geographical areas were used: North Atlantic (a), North Pacific (b) and a combined distribution North Atlantic + North Pacific (ab) for the extant species. MPG ancestral range distributions based on 28S (A) and ITS1 (B) phylogenies with pie charts at nodes showing probabilities of alternative ancestral ranges (North Atlantic: a, white in pie chart, North Pacific: b, light grey, and a combined distribution North Atlantic + North Pacific: ab, dark grey); (C–F) 4 optimal reconstructions resulting in probabilities in A and B. Roman number at nodes correspond to those nodes in A and B and are referred to in the text.

in the course of their subsequent evolution, the former began to colonize seaducks and the latter specialized to become a specific parasite of waders (albeit without losing the capacity to also develop in gulls).

The subsequent colonization of benthos-feeding seaducks by the ancestors of these parasites appears to have been due to their diet, which included many coastal molluscs such as periwinkles. Moreover, periwinkles form a considerable part of the common eider's diet (Bianki *et al*. 1979; Krechmar and Kondratyev, 2006). Thus, the MPGs are found most often and in greatest numbers in this bird (Galaktionov, 1993). Transition from gulls and waders to seaducks (i.e. Mergini) appears to have played an important role in subsequent MPG

radiation, resulting in the formation of 4 new species: *M. pseudopygmaeus, M. triangulatus, M. pygmaeus* and *M. kurilensis*.

Another host-switching event in MPG evolution appears to be the colonization of a new intermediate host by *M. pseudopygmaeus*. Its broad specificity to the first intermediate hosts, among which there are molluscs not only from different species and genera but also from different families and orders of the prosobranchs, is exceptional for trematodes (for reviews see Cribb *et al.* 2003). In fact, *M. pseudopygmaeus* must be considered a generalist for its molluscan hosts in comparison with most digeneans.

In phylogenetic estimates based on ITS2, the basal position among the MPG taxa is occupied by *M. pseudopygmaeus*. As well as showing the weakest phylogenetic signal, the estimate based on ITS2 does not fit the sequence of host-switching events suggested by the ITS1 and 28S-based hypotheses. Previous analysis via UP-PCR (Universally Primed PCR is a PCR fingerprinting technique similar to RAPD) product cross-hybridization showed that *M. pseudopygmaeus* and *M. triangulatus* are the most genetically similar species among the European isolates (Galaktionov *et al.* 2004) and this is also supported by their sister-group relationship in the present analyses. In contrast, *M. pygmaeus* and *M. piriformes* are genetically well separated from each other, being also distinct from the *M. pseudopygmaeus* and *M. triangulatus* clade (Galaktionov *et al.* 2004). To sum up, the topologies obtained from the ITS1 and 28S analyses show the most consistency and support, and it is thus on this scenario that we base our discussions on their evolution and biogeography.

Historical biogeography

Hierarchical relationships for multiple species pairs of MPG (*M. piriformes* and *M. calidris, M. pygmaeus* and *M. kurilensis*) in NA and NP in our trees testify that their modern geographical distribution is not a consequence of simply terminal, post-Pleistocene expansion. They indicate instead a succession of episodes of geographical colonization, isolation and diversification. A distinct co-evolutionary association between the MPG and *Neritrema* in both the NA and NP calls for an examination of MPG evolution in the light of the speciation and geographical colonization of these molluscs.

It is thought that the subgenus *Neritrema* originated in NP, and their colonization of NA became possible starting from ca. 3·5 Myr BP, which is associated with the first Pliocene opening of the Bering Strait (Reid, 1996). At that time, there were intensive species interchanges between the Pacific and Atlantic (Golikov and Scarlato, 1989; Vermeij, 1991; Marincovich, 2000; Briggs, 2003), named by J. C. Briggs as the Great Trans-Arctic Biotic Interchange. The Arctic climate, which was then milder than ever after, was no barrier to dispersal (Reid, 1996).

Glaciation, which occurred in the end of the Pliocene ca. 2·9 – 2·4 Myr BP, was accompanied by marine regression and the emergence of the Bering Landbridge, often called 'Beringia'. Communication between the NP and Arctic-Atlantic closed again, which resulted in episodes of diversification and allopatric (vicariant) speciation in the Pacific species of marine organisms, including the periwinkles that had spread into the Atlantic (Reid, 1996; Reid *et al.* 1996; for review see Briggs, 2003). The late Pliocene and Pleistocene in the Northern Hemisphere was characterized by successive stadial-interstadial cycles, numbering ca. 20 events, which were accompanied by the appearance and disappearance of the Bering Landbridge (Hopkins, 1959; Gladenkov, 1978; Sher, 1999). However, colonization of the Atlantic by the Pacific periwinkles and colonization of the Pacific by the Atlantic periwinkles that were formed by that time was apparently precluded by a colder climate even during the warmest interglacials. Since the late Pliocene and up to now the distribution areas of the Atlantic and the Pacific *Neritrema* were separated from the Eurasian side by the Siberian seas, and from the American side by the Arctic coast of Alaska and the Canadian Arctic Archipelago.

Taking into account a relatively recent history of periwinkles in NA, it is most likely that the microphallid taxa ancestral to the clade [*M. similis* + MPG] formed in the Pacific and were associated with endemic periwinkle species. We may conjecture that under conditions of mild climate during the Great Trans-Arctic Biotic Interchange, birds could perform trans-Arctic flights along the North American coast, dispersing the parasites. In this way, the microphallid taxa ancestral to [*M. similis* + MPG] could follow the Pacific periwinkles into NA (node VI in Fig. 4C–E).

In accordance with the optimal S-DIVA reconstructions an NA range of the common ancestor of the MPG seems to be more probable (node V in Fig. 4C, E). Therefore, its diversification may have taken place only after the colonization of NA by *Neritrema* in the late Pliocene. The first intermediate hosts of the MPG ancestor were the Atlantic *Neritrema* species, which were forming at that time, while its final hosts were members of the Charadriiformes, mainly of the family Laridae. At the same time, S-DIVA reconstruction also points with a rather high probability to a broader, trans-Arctic distribution of the common ancestor of the MPG. This could only occur under interglacial conditions by means of trans-Arctic flights of birds.

Diversification of the common ancestor of the MPG in the NP appears less probable. However, if this did take place, it could also happen before the Pliocene colonization of NA by periwinkles. In such a case, it could be that the initial first intermediate host of the MPG was the NP *Neritrema*. However, even if the common ancestor of the MPG was distributed in NP, the main events in the formation of this microphallid group took place already after the expansion of *Neritrema* into NA, since the common ancestor of the [*M. piriformes* + *M. calidris*], the basal MPG branch, had trans-Arctic distribution. Its colonization of NA could proceed concurrently with the northeastern advent of the Pacific *Neritrema* along the North America coasts during the Great Trans-Arctic Biotic Interchange.

Ambiguity of the geographical distribution of the common ancestor of the MPG does not allow an exact reconstruction of the sequence of range expansion during their diversification. This is especially true for the species *M. piriformes* and *M. calidris*, whose formation might have been triggered by the dispersion of ancestral MPG from NA into NP or by fragmentation of its originally trans-Arctic distribution area into NA and NP parts. It seems most plausible that the formation of *M. piriformes* and *M. calidris* was driven by the vicariance event (glaciation) during the late Pliocene/Pleistocene, which interrupted the gene flow between NA and NP populations of the MPG ancestors associated with gulls and waders.

Isolation of the common ancestor of the 'duck' branch of the MPG in NA or in NP seems equally possible (node III in Fig. 4). Diversification within this clade of MPG appears to fit well with the Arctic Refugium Hypothesis suggested by Hoberg (1992, 1995) and Hoberg and Adams (1992, 2000) to explain speciation in the cestodes of marine birds and mammals. It is possible that the ancestral MPG circulated in glacial refugia that formed in the Atlantic and Pacific coastal waters of the continents and islands during stadials. In such refugia gulls, waders and seaducks concentrated in large numbers, and periwinkles could also be preserved. These conditions would facilitate colonization of new hosts, seaducks (host-switching event), while vicariance of host-parasite populations in different refugia and micro-refugia could serve as the basis for diversification and speciation due to allopatry.

The modern amphiboreal distribution of *M. pseudopygmaeus* and *M. triangulatus* can be associated with their trans-Arctic dispersion by birds (possibly more than once) during some Pleistocene interglacials/interstadials. However, isolation of their Atlantic and Pacific populations during stadials was not accompanied by speciation (similar to the case of *M. similis*, the sister species of MPG). In the case of *M. pseudopygmaeus*, the obstacle to vicariant speciation could be continuous gene flow between the Pacific and the Atlantic populations of this species. This gene flow seems to be possible now and could have also been possible during Pleistocene interstadials. This is associated with the range of first intermediate hosts of *M. pseudopygmaeus* that includes subtidal arctic and arcto-boreal molluscs. Some of them, including a number of species from the genera *Margarites, Solariella* and *Cryptonatica*, are widespread at the shelf of the Arctic seas and are known there since the early Pleistocene (Golikov and Scarlato, 1989). With the presence of suitable molluscan hosts, *M. pseudopygmaeus* could also colonize these regions, transferred by seaducks. Some of them, such as the Steller's eider and the king eider, make long migrations along the Arctic coast of Eurasia and North America (Dau *et al.* 2000; Webster *et al.* 2002; Petersen *et al.* 2006; Alerstam *et al.* 2007; Bustnes *et al.* 2010). As for *M. triangulatus*, its divergence could be hindered by the narrow morphological-functional specialization to a microhabitat in the final host.

All the variants of the S-DIVA reconstructions point to the trans-Arctic distribution of the common ancestor of *M. pygmaeus* and *M. kurilensis* (node I in Fig. 4). It can be supposed that species formation in this clade of the 'duck' branch is associated with vicariance events of the Quaternary, possibly, with the last Ice Age. Ancestral trans-Arctic populations of the common ancestor of *M. pygmaeus* and *M. kurilensis* in the course of glaciation could be fragmented into parts associated with Atlantic and Pacific refugia. This was the case with the initially unified population of common eider, the main species of the final hosts of *M. pygmaeus* and *M. kurilensis*. During the last glacial maximum, it was restricted to 4 glacial refugia, where its modern subspecies were formed (Sonsthagen *et al.* 2011). In particular, the Pacific eider (*Somateria mollissima v-nigrum*) originated in the Beringian refugium, and it is very probable that the formation of *M. kurilensis*, whose main species of the final hosts is Pacific eider, also proceeded there. As for the formation of *M. pygmaeus*, it is unequivocally associated with NA and the NA subspecies of common eider.

At present, colonization of the Pacific MPG species, with the exception of *M. pseudopygmaeus*, to the Atlantic and vice versa seems to be an implausible event despite the trans-Arctic migrations of some seaducks and shorebirds. The obstacles are the short lifespan of the adult phase of the parasite's life cycle and the absence of appropriate intermediate hosts at the coasts of the Arctic seas. Some shorebirds fly across the North American continent to their overwintering grounds in the Gulf of Mexico and South America, where periwinkles are absent. At the same time, trans-continental flights of birds in NA and NP within the distribution areas of periwinkles provide the background for amphi-Pacific and

amphi-Atlantic distribution of the Pacific and Atlantic MPG species, respectively.

ACKNOWLEDGEMENTS

We thank especially E. P. Hoberg for insightful discussions and very important comments to the early version of the manuscript. We also thank S. A. Bulat and I. A. Alekhina for design of primers for amplification of ITS1 rDNA sequences of MGP. Special thanks are due to G. I. Atrashkevich, J. O. Bustnes, K. V. Regel, D. H. Saville and K. Skirnisson for their valuable help during the fieldwork. We thank the anonymous reviewers for their well-considered comments on an earlier draft of the manuscript.

FINANCIAL SUPPORT

The project was financed by the RFBR (K.V.G., grant numbers 10-04-00430, 11-04-98545), INTAS (K.V.G., grant number 05-1000008-8056), St Petersburg State University (K.V.G., grant number 1.37.80.2011) and *Marie Curie Outgoing International Fellowship for Career Development* (I.B.-C., grant number PIOF-GA-2009-252124) within the 7th Framework Programme (FP7/2007–2013) of the European Commission.

REFERENCES

Alerstam, T., Bäckman, J., Gudmundsson, G. A., Hedenström, A., Henningsson, S. S., Håkan, K., Rosén, M. and Strandberg, R. (2007). A polar system of intercontinental bird migration. *Proceedings of the Royal Society of London, B* **274**, 2523–2530.

Anderson, G. R. and Barker, S. C. (1993). Species differentiation in the Didymozoidae (Digenea): Restriction fragment length differences in internal transcribed spacer and 5·8S ribosomal DNA. *International Journal for Parasitology* **23**, 133–136.

Bartoli, P., Jousson, O. and Russell-Pinto, F. (2000). The life cycle of *Monorchis parvus* (Digenea: Monorchiidae) demonstrated by developmental and molecular data. *The Journal of Parasitology* **86**, 479–489.

Belopolskaya, M. M. (1963). Family Microphallidae Travassos, 1920. In *Trematodes of Animals and Man. Vol. 21* (ed. Skrjabin, K. I.), pp. 259–502. USSR Academy of Sciences Press, Moscow-Leningrad, USSR (in Russian).

Bianki, V. V., Boyko, N. S., Ninburg, E. A. and Shklyarevich, G. A. (1979). Feeding of the common eider of the White Sea. In *Ecology and Morphology of Eiders in the USSR* (ed. Kishchinskiy, A. A.), pp. 126–170. Nauka, Moscow, USSR (in Russian).

Blair, D., Davis, G. M. and Wu, B. (2001). Evolutionary relationships between trematodes and snails emphasizing schistosomes and paragonimids. *Parasitology* **123**, S229–S243.

Briggs, J. C. (2003). Marine centers of origin as evolutionary engines. *Journal of Biogeography* **30**, 1–18.

Bustnes, J. O., Mosbech, A., Sonne, Ch. and Systad, G. H. (2010). Migration patterns, breeding and moulting locations of king eiders wintering in north-eastern Norway. *Polar Biology* **33**, 1379–1385.

Ching, H. L. (1991). List of larval worms from marine invertebrates of the Pacific coast of North America. *Journal of the Helminthological Society of Washington* **58**, 57–68.

Cribb, T. H., Bray, R. A., Olson, P. D. and Littlewood, D. T. J. (2003). Life cycle evolution in the Digenea: a new perspective from phylogeny. *Advances in Parasitology* **54**, 197–254.

Dau, C. P., Flint, P. L. and Petersen, M. R. (2000). Distribution of recoveries of Steller's eiders banded on the lower Alaska Peninsula, Alaska. *Journal of Field Ornithology* **71**, 541–548.

Deblock, S. (1971). Contribution à l'étude des Microphallidae Travassos, 1920. XXIV. Tentative de phylogénie et de taxonomie. *Bulletin de Muséum National d'Histoire Naturelle 3e sér, 7, Zoologie* **7**, 353–469.

Galaktionov, K. V. (1983). Microphallids of the "*pygmaeus*" group. I. Description of species *Microphallus pygmaeus* (Levinsen, 1881) nec Odhner, 1905 and *M. piriformes* (Odhner, 1905) nom. nov. (Trematoda: Microphallidae). *Vestnik Leningradskogo Universiteta (Bulletin of the Leningrad University)* **15**, 20–30 (in Russian).

Galaktionov, K. V. (1984). Microphallids of the "*pygmaeus*" group. II. *Microphallus triangulatus* sp. nov. (Trematoda: Microphallidae). *Vestnik Leningradskogo Universiteta (Bulletin of the Leningrad University)* **3**, 5–11 (in Russian).

Galaktionov, K. V. (1993). *Life Cycles of Trematodes as Components of Ecosystems*. Kola Scientific Centre of the Russian Academy of Sciences Press, Apatity (in Russian).

Galaktionov, K. V. (2009). Description of the maritae and determination of the species status of *Microphallus pseudopygmaeus* sp. nov. (Trematoda: Microphallidae). *Parazitologiya* **43**, 289–299 (in Russian).

Galaktionov, K. V., Bulat, S. A., Alekhina, I. A., Saville, D. H., Fitzpatrick, S. M. and Irwin, S. W. B. (2004). An investigation of evolutionary relationships within "*pygmaeus*" group microphallids (Trematoda: Microphallidae) using genetic analysis and scanning electron microscopy. *Journal of Helminthology* **78**, 231–236.

Galaktionov, K. V., Regel, K. V. and Atrashkevich, G. I. (2010). *Microphallus kurilensis* sp. nov., a new species of microphallids from the "*pygmaeus*" species group (Trematoda, Microphallidae) from the coastal areas of Okhotsk and Bering Seas. *Parazitologiya* **44**, 496–507 (in Russian).

Gladenkov, Yu. B. (1978). *Marine Upper Cenozoic of the Northern Regions*. Nauka, Moscow, USSR (in Russian).

Golikov, A. N. and Scarlato, O. A. (1989). Evolution of the Arctic ecosystems during the Neogene period. In *The Arctic Seas: Climatology, Oceanography, Geology, and Biology* (ed. Herman, Y.), pp. 257–279. Van Nostrand Reinhold, New York, USA.

Harris, A. J. and Xiang, Q.-Y. (2009). Estimating ancestral distributions of lineages with uncertain sister groups: a statistical approach to Dispersal-Vicariance Analysis and a case using *Aesculus* L. *(Sapindaceae) including fossils*. *Journal of Systematics and Evolution* **47**, 349–368.

Hoberg, E. P. (1992). Congruent and synchronic patterns in biogeography and speciation among seabirds, pinnipeds and cestodes. *Journal of Parasitology* **78**, 601–615.

Hoberg, E. P. (1995). Historical biogeography and modes of speciation across high-latitude seas of the Holarctic: concepts for host–parasite co-evolution among the Phocini (Phocidae) and Tetrabothriidae. *Canadian Journal of Zoology* **73**, 45–57.

Hoberg, E. P. and Adams, A. (1992). Phylogeny, historical biogeography, and ecology of *Anophryocephalus* spp. (Eucestoda: Tetrabothriidae) among pinnipeds of the Holarctic during the late Tertiary and Pleistocene. *Canadian Journal of Zoology* **70**, 703–719.

Hoberg, E. P. and Adams, A. (2000). Phylogeny, history and biodiversity: understanding faunal structure and biogeography in the marine realm. *Bulletin of the Scandinavian Society of Parasitology* **10**, 19–37.

Hoberg, E. P. and Brooks, D. R. (2008). A macroevolutionary mosaic: episodic host-switching, geographical colonization and diversification in complex host–parasite systems. *Journal of Biogeography* **35**, 1533–1550.

Hopkins, D. M. (1959). Cenozoic history of the Bering land bridge. *Science* **129**, 1519–1528.

Huelsenbeck, J. P., Ronquist, F., Nielsen, R. and Bollback, J. P. (2001). Bayesian inference of phylogeny and its impact on evolutionary biology. *Science* **294**, 2310–2314.

Iwagami, M., Ho, L. Y., Su, K., Lai, P. F., Fukushima, M., Nakano, M., Blair, D., Kawashima, K. and Agatsuma, T. (2000). Molecular phylogeographic studies on *Paragonimus westermani* in Asia. *Journal of Helminthology* **74**, 315–322.

Jousson, O., Bartoli, P., Zaninetti, L. and Pawlowski, J. (1998). Use of the ITS rDNA for elucidation of some life-cycles of Mesometridae (Trematoda, Digenea). *International Journal for Parasitology* **28**, 1403–1411.

Krechmar, A. V. and Kondratyev, A. V. (2006). *Waterfowl Birds of North-East Asia*. NESC FEB RAS, Magadan, Russia (in Russian).

Lockyer, A. E., Olson, P. D., Ostergaard, P., Rollinson, D., Johnston, D. A., Attwood, S. W., Southgate, V. R., Horak, P., Snyder, S. D., Le, T. H., Agatsuma, T., McManus, D. P., Carmichael, A. C., Naem, S. and Littlewood, D. T. J. (2003). The phylogeny of the Schistosomatidae based on three genes with emphasis on the interrelationships of *Schistosoma* (Weinland, 1858). *Parasitology* **126**, 203–224.

Lotfy, W. M., Brant, S. V., DeJong, R. J., Le, T. H., Demiaszkiewicz, A., Rajapakse, J. R. P. V., Perera, V. B. V. P., Laursen, J. R. and Loker, E. S. (2008). Evolutionary origins, diversification, and biogeography of liver flukes (Digenea, Fasciolidae). *The American Journal of Tropical Medicine and Hygiene* **79**, 248–255.

Maddison, D. R. and Maddison, W. P. (2005). *MacClade 4: Analysis of Phylogeny and Character Evolution*. Sinauer Associates, Inc. Sunderland, MA, USA.

Marincovich, L. (2000). Central American paleogeography controlled Pliocene Arctic Ocean molluscan migrations. *Geology* **28**, 551–554.

Nolan, M. J. and Cribb, T. H. (2005). The use and implications of ribosomal DNA sequencing for the discrimination of digenean species. *Advances in Parasitology* **60**, 102–163.

Nylander, J. A. A., Olsson, U., Alström, P. and Sanmartín, I. (2008). Accounting for phylogenetic uncertainty in biogeography: a Bayesian approach to Dispersal–Vicariance Analysis of the thrushes (Aves: Turdus). *Systematic Biology* **57**, 257–268.

Olson, P. D., Cribb, T. H., Tkach, V. V., Bray, R. A. and Littlewood, D. T. J. (2003). Phylogeny and classification of the Digenea (Platyhelminthes: Trematoda). *International Journal for Parasitology* **33**, 733–755.

Petersen, M. R., Bustnes, J. O. and Systad, G. H. (2006). Breeding and moulting locations and migration patterns of the Atlantic population of Steller's eiders *Polysticta stelleri* as determined from satellite telemetry. *Journal of Avian Biology* **37**, 58–68.

Pina, S., Tajdari, J., Russell-Pinto, F. and Rodrigues, P. (2009). Morphological and molecular studies on life cycle stages of *Diphtherostomum brusinae* (Digenea: Zoogonidae) from northern Portugal. *Journal of Helminthology* **83**, 321–331.

Posada, D. and Crandall, K. A. (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.

Reid, D. G. (1996). *Systematics and Evolution of Littorina*. The Ray Society, London, UK.

Reid, D. G., Rumbak, E. and Thomas, R. H. (1996). DNA, morphology and fossils: phylogeny and evolutionary rates of the gastropod genus *Littorina*. *Philosophical Transactions of the Royal Society of London, B* **351**, 877–895.

Ronquist, F. (1997). Dispersal-vicariance analysis: a new approach to the quantification of historical biogeography. *Systematic Biology* **46**, 195–203.

Ronquist, F. (2001). *DIVA Version 1.2. Computer Program for MacOS and Win32*. Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden.

Ronquist, F. and Huelsenbeck, J. (2003). MRBAYES 3: Bayesian inference under mixed models. *Bioinformatics* **19**, 1572–1574.

Saville, D. H., Galaktionov, K. V., Irwin, S. W. B. and Malkova, I. I. (1997). Morphological comparison and identification of metacercariae in the "*pygmaeus*" group of microphallids, parasites of seabirds in western palearctic regions. *Journal of Helminthology* **71**, 167–174.

Sher, A. (1999). Traffic lights at the Beringian crossroads. *Nature, London* **397**, 103–104.

Sonsthagen, S. A., Talbot, S. L., Scribner, K. T. and McCracken, K. G. (2011). Multilocus phylogeography and population structure of common eiders breeding in North America and Scandinavia. *Journal of Biogeography*, **38**, 1368–1380. doi: 10.1111/j.1365-2699.2011.02492.x.

Swofford, D. L. (2002). *PAUP*. Phylogenetic Analysis using Parsimony (*and other Methods)*. Sinauer Associates, Sunderland, MA, USA.

Tamura, K., Dudley, J., Nei, M. and Kumar, S. (2007). MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24**, 1596–1599.

Thorley, J. L. and Wilkinson, M. (1999). Testing the phylogenetic stability of early tetrapods. *Journal of Theoretical Biology* **200**, 343–344.

Tkach, V. V., Littlewood, D. T. J., Olson, P. D., Kinsella, J. M. and Swiderski, Z. (2003). Molecular phylogenetic analysis of the Microphalloidea Ward, 1901 (Trematoda: Digenea). *Systematic Parasitology* **56**, 1–15.

Vermeij, G. J. (1991). Anatomy of an invasion: the trans-Arctic interchange. *Paleobiology* **17**, 281–307.

Webster, M. S., Marra, P. P., Haig, S. M., Bensch, S. and Holmes, R. T. (2002). Links between worlds–unraveling migratory connectivity. *Trends in Ecology and Evolution* **17**, 76–83.

Yu, Y., Harris, A. J. and He, X. (2010). S-DIVA (Statistical Dispersal-Vicariance Analysis): A tool for inferring biogeographic histories. *Molecular Phylogenetics and Evolution* **56**, 848–850.