Supplementary Materials

Molecular Diversity of the Genus *Plagiorchis* Lühe, 1899 in Snail Hosts of Central Europe with Evidence of New Lineages

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Supplementary Table S1. *Plagiorchis* species reported from European freshwater snails. Records from Russia include only the European part. The reference numbering does not correspond to that in the main text.

***Plagiorchis* species**	**Snail host**	**Country**	**Reference**
Plagiorchis elegans (Rudolphi, 1802)	*Galba truncatula*	Spain	[1]
	Lymnaea stagnalis	Austria, Belarus, Czech Republic, Denmark, Finland, Germany, Ireland, Poland, Russia, Slovakia, Ukraine	[2], [3], [4–9,10[1]], [11[2]], [12–14, 15[1]], [16–20], [15[1]], [2, 21–24], [25], [2, 10[1]], [26,27]
	Radix auricularia	Czech Republic, Germany, Ukraine	[4], [19,28], [29]
	Radix peregra[3]	Czech Republic, Finland, Germany	[4], [12,13], [28]
	Radix spp.	Belarus	[3]
	Stagnicola corvus	Ukraine	[27]
	Stagnicola fuscus	Ireland	[15[1]]
	Stagnicola palustris	Belarus, Czech Republic, Denmark, Germany, Ukraine	[3], [4], [11[2]], [17,28], [27]
Plagiorchis koreanus Ogata, 1938	*Radix auricularia*	Czech Republic	[10[1]]
Plagiorchis laricola Skrjabin, 1924	*Lymnaea stagnalis*	Czech Republic	[30–32]
Plagiorchis maculosus (Rudolphi, 1802)	*Lymnaea stagnalis*	Bulgaria, Czech Republic, Denmark, Finland, Ireland, Poland	[33], [4,5,10[1],32,34], [11[2]], [15[1]], [15[1]], [24]
	Radix auricularia	Czech Republic	[4]
	Radix balthica[3]	Denmark	[11[2]]
Plagiorchis megalorchis Rees, 1952	*Lymnaea stagnalis*	Great Britain	[35]
Plagiorchis muelleri Tkach and Sharpilo, 1990	*Radix balthica*[3]	Ireland	[15[1]]
Plagiorchis multiglandularis Semenov, 1927	*Lymnaea stagnalis*	Belarus, Russia, Ukraine	[3], [36], [27]
	Myxas glutinosa	Russia	[36]
	Radix ovata[3]	Russia	[36]
	Radix spp.	Belarus	[3]
	Stagnicola palustris	Russia	[36]
Plagiorchis mutationis Panova, 1927	*Lymnaea stagnalis*	Ukraine	[37,38]
Plagiorchis nanus (Rudolphi, 1802)	*Lymnaea stagnalis*	Belarus	[3]
	Myxas glutinosa	Russia	[36]
	Radix ovata[3]	Russia	[36]
Plagiorchis neomidis Brendow, 1970	*Ampullaceana balthica*	Germany	[20]
	Lymnaea stagnalis	Slovakia	[10[1]]
	Radix ampla[3]	Germany	[18]
	Radix auricularia	Czech Republic	[4,32]
	Radix ovata[3]	Czech Republic, Germany	[4], [18]
	Radix peregra[3]	Czech Republic, Germany	[4,39], [18,40]
	Radix spp.	Belarus	[3]
Plagiorchis vespertilionis (Müller, 1780)	*Lymnaea stagnalis*	Belarus	[3]
Plagiorchis sp. 1 *sensu* Soldánová et al. [41]	*Radix balthica*[3]	Iceland, Norway	[15[1]], [41[2]]
Plagiorchis sp. 2 *sensu* Soldánová et al. [41]	*Radix balthica*[3]	Finland, Iceland, Norway	[15[1]], [15[1]], [41[2]]
Plagiorchis sp. 3 *sensu* Soldánová et al. [41]	*Radix balthica*[3]	Iceland, Ireland, Norway	[15[1]], [15[1]], [41[2]]
Plagiorchis sp. 4 *sensu* Soldánová et al. [41]	*Radix balthica*[3]	Norway	[41[2]]
Plagiorchis sp. 5 *sensu* Soldánová et al. [41]	*Radix balthica*[3]	Finland, Ireland, Norway	[15[1]], [15[1]], [41[2]]

Plagiorchis sp. 6 *sensu* Soldánová et al. [41]	*Radix balthica*[3]	Ireland, Norway	[15[1]], [41[2]]
Plagiorchis sp. 7 *sensu* Soldánová et al. [41]	*Myxas glutinosa*	Finland	[15[1]]
	Radix balthica[3]	Iceland, Ireland, Norway	[15[1]], [15[1]], [41[2]]
Plagiorchis sp. 8 *sensu* Kudlai et al. [15]	*Radix balthica*[3]	Finland, Iceland, Ireland	[15[1]]
Plagiorchis sp. 9 *sensu* Kudlai et al. [15]	*Stagnicola fuscus*	Ireland	[15[1]]
Plagiorchis sp. CR *sensu* Zikmundová et al. [10]	*Lymnaea stagnalis*	Czech Republic	[10[1]]
Plagiorchis sp. *sensu* Duan et al. [11]	*Lymnaea stagnalis*	Denmark	[11[2]]
	Radix balthica[3]	Denmark	[11[2]]
	Stagnicola palustris	Denmark	[11[2]]
Plagiorchis sp. *sensu* Huguenin et al. [42]	*Lymnaea stagnalis*	France	[42[2]]
Plagiorchis sp. 1	*Lymnaea stagnalis*	Slovakia, Germany, Serbia	[2], [2,43], [43,44]
Plagiorchis sp. 2	*Lymnaea stagnalis*	Slovakia	[2]
Plagiorchis sp. II	*Radix ovata*[3]	Russia	[36]
Plagiorchis sp.	*Galba truncatula*	Bulgaria	[45]
	Lymnaea stagnalis	Germany	[18]
	Radix auricularia	Germany, Switzerland	[46], [47]
	Radix euphratica	Georgia	[48]
	Stagnicola corvus	Ukraine	[38]

[1] Molecular (DNA sequences available) and morphological identification; [2] Molecular identification only;
[3] *Peregriana peregra* (former synonym *Radix peregra* and *R. labiata*); *Ampullaceana ampla* (former synonym *Radix ampla*), *A. balthica* (former synonym *Radix balthica* and *R. ovata*) according to Aksenova et al. [49] and Horsák et al. [50]

Supplementary Table S2. The composition of birds in Lakes Medard and Most based on literature data and online databases [51–61]. Definitive bird hosts of *Plagiorchis* spp. (see footnote) are provided based on records in the Host–parasite Database of the Natural History Museum, London [62] and additional literature data [63–69]. The reference numbering does not correspond to that in the main text.

Family	Lake Medard	Lake Most
	Species	
Accipitridae	*Buteo buteo, Circus aeruginosus*[1], *Milvus milvus*	*Accipiter nisus*[1,3], *A. gentilis*[1,2], *B. buteo, B. lagopus*[4], *C. aeruginosus*[1], *C. cyaneus*[1,3], *C. pygargus, Haliaeetus albicilla*[3], *M. milvus, Milvus migrans, Pernis apivorus*
Acrocephalidae	*Acrocephalus scirpaceus*[1], *A. arundinaceus*[1]	*A. scirpaceus*[1], *A. schoenobaenus*[1,2], *A. arundinaceus*[1], *A. palustris, Hippolais icterina*[1]
Aegithalidae	*Aegithalos caudatus*	*A. caudatus*
Alaudidae	*Alauda arvensis*[1,2]	*A. arvensis*[1,2], *Lullula arborea*
Alcedinidae	*Alcedo atthis*	*A. atthis*
Anatidae	*Alopochen aegyptiacus, Anas clypeata*[1,3], *A. crecca*[2], *A. penelope*[4], *A. platyrhynchos*[1,4], *A. strepera, Anser albifrons*[1,4], *An. anser, An. fabalis*[3], *Aythya ferina*[1,2], *Ay. fuligula*[1,2], *Ay. marila*[4], *Bucephala clangula*[3], *Clangula hyemalis*[1,4], *Cygnus olor, Melanitta fusca*[3], *M. nigra*[4], *Mergellus albellus*[3], *Mergus merganser*[1,3], *Netta rufina*	*Al. aegyptiacus, A. acuta*[1,3], *A. clypeata*[1,3], *A. crecca*[2], *A. penelope*[4], *A. platyrhynchos*[1,4], *A. querquedula*[2], *A. strepera, An. albifrons*[1,4], *An. anser, An. fabalis*[3], *An. indicus, Ay. ferina*[1,2], *Ay. fuligula*[1,2], *Ay. marila*[4], *Branta leucopsis*[4], *B. clangula*[3], *C. hyemalis*[1,4], *Cy. olor, M. fusca*[3], *M. nigra*[4], *Me. albellus*[3], *Me. merganser*[1,3], *Mergus serrator*[3], *Tadorna tadorna, T. ferruginea, N. rufina*
Apodidae	*Apus apus*[1,2]	*A. apus*[1,2]
Ardeidae	*Ardea cinerea, Botaurus stellaris, Egretta alba*	*A. cinerea, A. purpurea, Ardeola ralloides, Botaurus stellaris, Casmerodius albus, E. alba, Ixobrychus minutus*
Bombycillidae	*Bombycilla garrulus*[3]	
Calcariidae	*Plectrophenax nivalis*[4]	*P. nivalis*[4]
Charadriidae		*Charadrius dubius*[1], *Vanellus vanellus*[1,2]
Ciconiidae		*Ciconia ciconia, Ciconia nigra*
Columbidae	*Columba livia* f. *domestica, Columba palumbus*	*Columba oenas, C. livia* f. *domestica, C. palumbus, Streptopelia turtur, Streptopelia decaocto*
Corvidae	*Corvus corax*[1,2], *Garrulus glandarius*[1], *Pica pica*[1,2]	*C. corax*[1,2], *C. corone*[1,2], *C. cornix*[1,2], *C. monedula, G. glandarius*[1], *P. pica*[1,2]
Cuculidae		*Cuculus canorus*[1,2]
Emberizidae	*Emberiza schoeniclus*[1,2], *Emberiza citrinella*[1,2]	*E. schoeniclus*[1,2], *E. hortulana*[1], *E. citrinella*[1,2], *Miliaria calandra*
Falconidae	*Falco tinnunculus*[1]	*Falco peregrinus*[3], *F. tinnunculus*[1], *F. subuteo*[1,2]
Fringillidae	*Carduelis carduelis*[1], *C. chloris*[2], *C. flammea flammea*[2], *C. spinus*[2]	*C. cannabina, C. carduelis*[1], *C. chloris*[2], *C. flammea flammea*[2], *C. spinus*[2], *Coccothraustes coccothraustes, Fringilla coelebs*[1]
Gaviidae	*Gavia arctica*[3], *Gavia immer*[3]	*G. arctica*[3], *G. stellata*[4]
Gruidae		*Grus grus*[3]
Hirundinidae	*Delichon urbicum*[1,2], *Hirundo rustica*[1,2], *Picus viridis, P. canus*	*D. urbicum*[1,2], *Dendrocopos minor, Hirundo daurica*[1], *H. rustica*[1,2], *Jynx torquilla, P. viridis, Riparia riparia*[1,2]
Laniidae	*Lanius excubitor*[1,2]	*Lanius collurio*[1,2], *L. excubitor*[1,2]
Laridae	*Chlidonias niger*[1], *Larus argentatus*[1,4],	*C. niger*[1], *Chroicocephalus ridibundus*[2], *L.*

	L. cachinnans, L. canus[1,3], *L. fuscus*[1,4], *L. ridibundus*[1,3], *L. minutus*[1], *Sterna sandvicensis*	*argentatus*[1,4], *L. cachinnans, L. canus*[1,3], *L. fuscus*[1,4], *L. ridibundus*[1,3], *L. michahellis, L. melanocephalus*[1]
Locustellidae		*Locustella naevia*
Meropidae		*Merops apiaster*[1]
Motacillidae	*Anthus trivialis*[1,2], *A.pratensis*[1,3], *A. spinoletta, Motacilla alba*[1,2]	*A. campestris, A. trivialis*[1,2], *A. pratensis*[1,3], *A. spinoletta, M. alba*[1,2], *M. flava*[1,2]
Muscicapidae	*Erithacus rubecula*[1], *Oenanthe oenanthe*[1,2], *Saxicola torquatus*[1,3], *S. rubetra*[1,2]	*E. rubecula*[1], *Luscinia svecica cyanecula*[3], *L. megarhynchos, O. oenanthe*[1,2], *Phoenicurus ochruros, S. torquatus*[1,3], *S. rubetra*[1,2], *Muscicapa striata*[1,2]
Oriolidae		*Oriolus oriolus*[1]
Pandionidae		*Pandion haliaetus*[3]
Panuridae		*Panurus biarmicus*
Paridae	*Parus major*[1,2], *P. caeruleus*	*P. major*[1,2], *P. caeruleus*
Passeridae	*Passer domesticus*[1,2], *P. montanus*[1,2]	*P. domesticus*[1,2], *P. montanus*[1,2]
Phalacrocoracidae	*Phalacrocorax carbo*	*P. carbo*
Phasianidae		*Phasianus colchicus, Coturnix coturnix*[1]
Phylloscopidae	*Phylloscopus collybita*[1], *P. trochilus*[1,2]	*P. collybita*[1], *P. trochilus*[1,2]
Picidae	*Dendrocopos major*[1,2], *Picus viridis*[1], *P. canus*	*D. major*[1,2], *D. minor*[1,2], *Jynx torquilla*[2], *P. viridis*[1]
Podicipedidae	*Podiceps auritus, P. cristatus*[1], *P. grisegena*[1], *P. nigricollis*[1], *Tachybaptus ruficollis*[1]	*P. auritus, P. cristatus*[1], *P. grisegena*[1], *P. nigricollis*[1], *T. ruficollis*[1]
Prunellidae		*Prunella modularis*[1,2]
Rallidae	*Fulica atra, Gallinula chloropus*	*F. atra, G. chloropus, Porzana porzana*[2], *Rallus aquaticus*
Remizidae	*Remiz pendulinus*	*R. pendulinus*
Scolopacidae	*Actitis hypoleucos*[1,2], *Numenius arquata*[1,2], *Tringa nebularia*[3]	*A. hypoleucos*[1,2], *Calidris alpina*[1,4], *Gallinago gallinago*[2], *N. arquata*[1,2], *Philomachus pugnax*[1,4], *Tringa glareola*[1,2], *T. nebularia*[3], *T. ochropus*[1,3], *T. totanus*[1,2], *T. stagnatilis*[1], *T. erythropus*[4]
Sittidae		*Sitta europaea*[1,2]
Strigidae		*Bubo bubo*[2]
Sturnidae	*Sturnus vulgaris*[1,2]	*S. vulgaris*[1,2]
Sylviidae	*Sylvia atricapilla*[1], *S. communis*	*S. atricapilla*[1], *S. borin*[1,2], *S. communis, S. curruca*[2], *S. nisoria*[1]
Troglodytidae	*Troglodytes troglodytes*	*T. troglodytes*
Turdidae	*Turdus pilaris*[1,2], *T. merula*[1]	*T. pilaris*[1], *T. philomelos*[1,2], *T. merula*[1]
Upupidae		*Upupa epops*[1]

[1] Possible definitive host of *Plagiorchis* spp.
[2] Native or breeding birds in Central Europe
[3] Migratory birds (occur in Central Europe during a relatively short period of the year on migration between breeding and non–breeding areas)
[4] Native or breeding birds in the sub–Arctic

Supplementary Table S3. Nucleotide comparison of *cox*1 mtDNA sequences of *Plagiorchis* spp. (compatible with data from Europe) based on 324 nt long alignment. The percentage of *p*-distance is presented below the diagonal, while the count of variable nucleotides is displayed above the diagonal. Sequences generated in this study are indicated in bold. Provided as a separate file in the Excel format.

Supplementary Table S4. Nucleotide comparison of *cox*1 mtDNA sequences of *Plagiorchis* spp. (compatible with data from North America) based on 371 nt long alignment. The percentage of *p*-distance is presented below the diagonal, while the count of variable nucleotides is displayed above the diagonal. Sequences generated in this study are indicated in bold. Provided as a separate file in the Excel format.

Supplementary Table S5. Nucleotide comparison of the partial 28S rDNA sequences of *Plagiorchis* spp. based on 1119 nt long alignment. The percentage of *p*-distance is presented below the diagonal, while the count of variable nucleotides is displayed above the diagonal. Sequences generated in this study are indicated in bold. Provided as a separate file in the Excel format.

Supplementary Table S6. Overview of newly generated sequences of snail hosts infected with *Plagiorchis* spp.

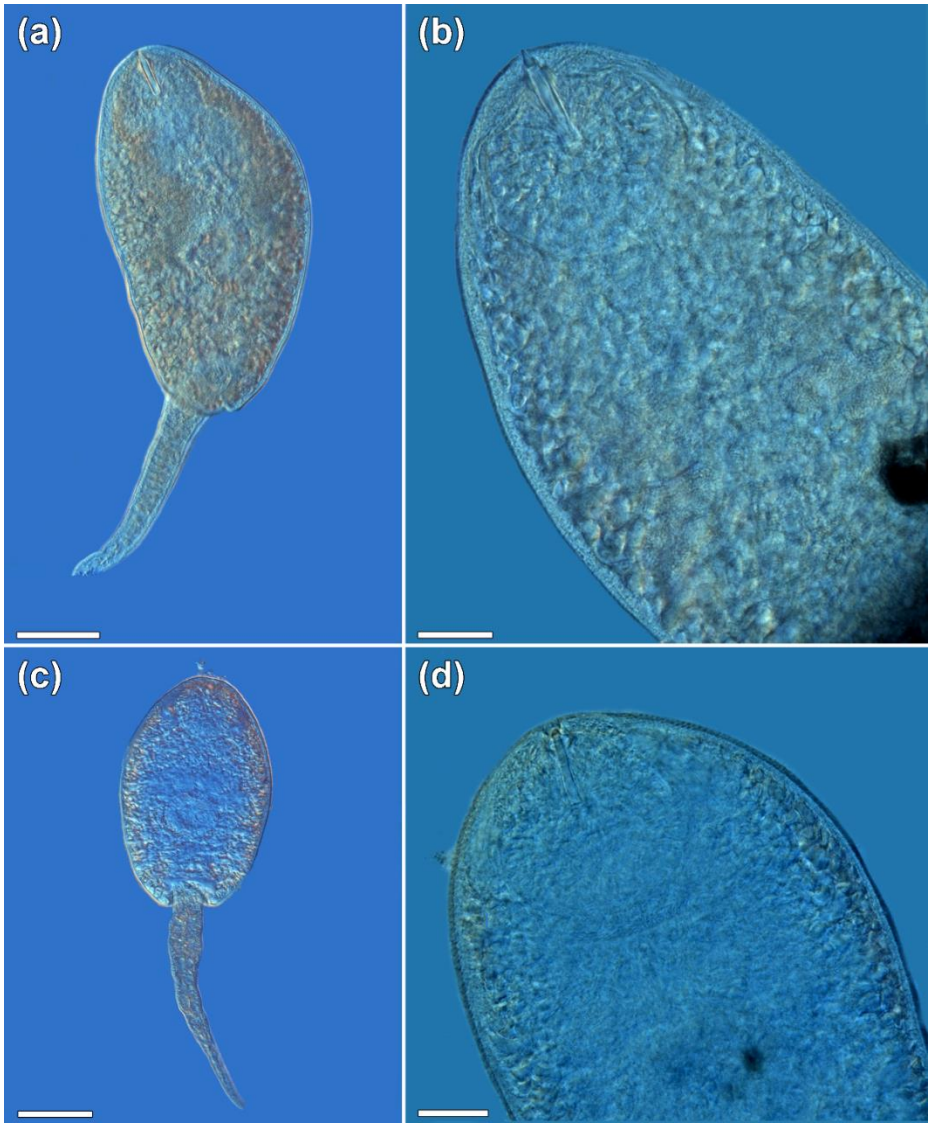
Locality	Snail host	Isolate	Genetic marker	GenBank accesion numbers
Barbora	*Radix auricularia*	RA21	ITS2	PP391333
	Radix auricularia	RA22	ITS2	PP391334
Medard	*Ampullaceana balthica*	RA15	ITS2	PP391329
	Ampullaceana balthica	RA16	ITS2	PP391330
Milada	*Ampullaceana lagotis*	MIL1	ITS2	PP391341
	Ampullaceana lagotis	MIL2	ITS2	PP391342
Most	*Ampullaceana lagotis*	RA17	ITS2	PP391340
	Ampullaceana lagotis	RA18	ITS2	PP391339
Otakar	*Radix auricularia*	RA23	ITS2	PP391335
	Radix auricularia	RA24	ITS2	PP391336
Písník Dubina	*Radix auricularia*	RA19	ITS2	PP391331
	Radix auricularia	RA20	ITS2	PP391332
Spůle	*Radix auricularia*	RA27	ITS2	PP391337
	Radix auricularia	RA28	ITS2	PP391338

Supplementary Table S7. Comparative metrical data for cercariae of *Plagiorchis vespertilionis* isolated from different snail hosts (grouped as (1) – *Ampullaceana balthica*, (2) – *A. lagotis*, (3) – *Radix auricularia*). Data are presented in micrometers as minimum to maximum values, mean in parenthesis, and as a mathematical difference between the two respective mean values (symbol **Δ)**. See Materials and Methods for abbreviations of cercarial morphological parameters.

Snail host	*A. balthica* (1)	*A. lagotis* (2)	**Δ (1–2)**	*A. balthica* (1)	*A. lagotis* (2)	*R. auricularia* (3)	**Δ (1–2)**	**Δ (1–3)**	**Δ (2–3)**	*A. lagotis* (2)
N snails	3	6	3–6	5	2	5	5–2	5–5	2–5	2
N cercariae	4	7	4–7	33	56	24	33–56	33–56	56–24	29
Fixation	Live	Live	Live	Ethanol	Ethanol	Ethanol	Ethanol	Ethanol	Ethanol	Formalin
ToL	332–425 (363)	337–420 (388)	-25	298–446 (371)	233–437 (358)	251–407 (349)	13	22	9	263–387 (328)
BL	190–320 (242)	226–303 (261)	-19	160–290 (215)	153–263 (212)	155–245 (206)	3	9	6	171–217 (190)
BW	162–202 (178)	124–178 (149)	29	91–141 (118)	70–138 (111)	96–145 (121)	7	-3	-10	103–135 (119)
TL	99–142 (121)	110–156 (128)	-7	99–200 (156)	102–174 (148)	96–185 (143)	8	13	5	77–195 (138)
TW	25–57 (39)	29–45 (37)	2	21–38 (30)	23–36 (28)	19–35 (28)	2	2	0	22–48 (32)
OSL	57–80 (72)	48–74 (65)	7	39–60 (50)	40–58 (48)	41–64 (51)	2	-1	-3	39–57 (48)
OSW	62–73 (69)	50–69 (62)	7	44–67 (55)	41–62 (52)	49–62 (55)	3	0	-3	48–63 (57)
VSL	35–57 (47)	38–51 (45)	2	28–49 (38)	28–44 (35)	30–47 (38)	3	0	-3	32–43 (38)
VSW	44–57 (51)	37–52 (46)	5	36–53 (45)	34–54 (42)	36–58 (44)	3	1	-2	40–52 (45)
OSW/VSW	1.3–1.4 (1.4)	1.3–1.4 (1.4)	0	0.9–1.5 (1.2)	0.9–1.7 (1.2)	1.0–1.5 (1.2)	0	0	0	1.1–1.6 (1.3)
TL/BL (%)	33.0–74.9 (53.8)	38.2–58.8 (49.4)	4.4	47.8–95.7 (73.3)	58.7–85.5 (70.3)	56.3–86.4 (69.5)	3	3.8	0.8	41.4–110.9 (73.5)
SL	30.0–31.5 (30.9)	30.2–31.8 (31.0)	-0.1	28–33 (30)	27–32 (30)	27–31 (29)	0	1	1	–
SWantt	7.1–9.0 (7.8)	7.2–8.2 (7.8)	0	–	–	–	–	–	–	–
SWabt	4.5–5.3 (5.0)	4.2–5.0 (4.8)	-0.2	–	–	–	–	–	–	–
SWbt	4.7–5.2 (5.2)	4.2–5.6 (4.8)	-0.4	–	–	–	–	–	–	–
SWantt/SL (%)	22.8–28.7 (25.3)	23.8–26.3 (25.2)	0.1	–	–	–	–	–	–	–

Supplementary Table S8. Comparative metrical data for cercariae of *Plagiorchis* sp. 10 isolated from different snail hosts (grouped as (1) – *Ampullaceana balthica*, (2) – *A. lagotis*). Data are presented in micrometers as minimum to maximum values, mean in parenthesis, and as a mathematical difference between the two respective mean values (symbol **Δ)**. See Materials and Methods for abbreviations of cercarial morphological parameters.

Snail host	*A. balthica* (1)	*A. lagotis* (2)	**Δ (1–2)**	*A. balthica* (1)	*A. lagotis* (2)	**Δ (1–2)**	*A. lagotis* (2)
N snails	1	1	1–1	2	1	2–1	1
N cercariae	1	4	1–4	30	19	30–19	19
Fixation	Live	Live	Live	Ethanol	Ethanol	Ethanol	Formalin
ToL	418	353–513 (416)	2	244–353 (319)	287–356 (314)	5	225–316 (266)
BL	282	238–372 (302)	-20	145–227 (194)	171–203 (186)	8	124–165 (147)
BW	138	132–156 (150)	-12	71–100 (87)	75–102 (89)	-2	77–101 (92)
TL	137	87–141 (114)	23	99–150 (124)	104–153 (127)	-3	73–169 (119)
TW	39	39–53 (46)	-7	20–30 (25)	20–28 (25)	0	18–29 (23)
OSL	59	60–69 (65)	-6	35–52 (43)	35–47 (41)	2	36–42 (38)
OSW	66	57–69 (65)	1	36–55 (47)	42–53 (48)	-1	39–45 (42)
VSL	44	43–50 (47)	-3	27–45 (33)	27–34 (31)	2	24–33 (30)
VSW	45	50–58 (53)	-8	32–46 (38)	32–43 (38)	0	31–37 (35)
OSW/VSW	1.5	1.0–1.3 (1.2)	0.3	1.0–1.6 (1.3)	1.1–1.5 (1.3)	0	1.1–1.3 (1.2)
TL/BL (%)	48.6	30.3–48.7 (38.4)	10.2	52.8–92.1 (64.3)	53.6–88.9 (68.7)	-4.4	44.2–134.7 (82.5)
SL	–	28.8–30.3 (29.5)	–	26–29 (27)	27–29 (28)	-1	–
SWantt	–	6.7–6.8 (6.7)	–	–	–	–	–
SWabt	–	4.3–4.5 (4.4)	–	–	–	–	–
SWbt	–	5.1–5.2 (5.1)	–	–	–	–	–
SWantt/SL (%)	–	22.1–23.6 (22.8)	–	–	–	–	–



Supplementary Figure S1. Comparison of *Plagiorchis* cercariae fixed in ethanol and cold formalin used for the measurements. Ethanol-fixed cercariae, **(a)** body view; **(b)** anterior body with detail of stylet; formalin-fixed cercariae **(c)** body view; **(d)** anterior body with detail of stylet. Scale-bars in **(a)** and **(c)**: 50 µm; Scale-bars in **(b)** and **(d)**: 20 µm.

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