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Trematode species diversity in the faucet snail, *Bithynia tentaculata* at the western edge of its native distribution, in Ireland

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Research Paper

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Abstract

Trematodes and their snail hosts have developed intimate parasite-host associations, with snails supporting a diverse and often species-specific trematode fauna. In the faucet snail, *Bithynia tentaculata* (Caenogastropoda, Littorinimorpha), a unique trematode fauna has been recorded recently. However, knowledge of the exact species identity, phylogenetic relationships, and geographical distribution remains limited as many of the species belong to groups with unclear or controversial taxonomical assignment. To contribute to our knowledge of the trematodes, we investigated the trematode fauna of *B. tentaculata* by examining a total of 556 snails from lakes in County Galway, Ireland. Using an integrative taxonomic approach including DNA sequence data analyses (28S rRNA gene, ITS1-5.8S-ITS2, ITS2, *cox*1, *nad*1) and morphological tools (taxonomical drawings and measurements), we identified nine trematode species of seven families, with seven species occurring as cercariae (*Cyathocotyle prussica*, *Lecithodendrium linstowi*, *Lecithodendrium* sp., *Asymphylodora progenetica*, *Sphaerostoma bramae*, *Metorchis xanthosomus*, and *Notocotylus* sp.) and three species occurring as metacercariae (*A. progenetica*, *Parasymphylodora parasquamosa*, and *Sphaeridiotrema* sp.). Except for *S. bramae*, all are new species records for Ireland and provide the most western distribution of these trematodes in Europe. The trematode species recorded are known to use a wide range of definitive hosts and have a wide geographical distribution; among them are species members of genera that are zoonotic (*Metorchis*) and pathogenic to wildlife (*Cyathocotyle*, *Sphaeridiotrema*, and *Notocotylus*). There remains an ongoing need for precise identification of the trematode species to ensure that wider ecological contexts are correctly understood and biodiversity and disease threats can be accurately evaluated.

Introduction

Molluscs, particularly snails (Gastropoda) are, with few exceptions, the exclusive first intermediate hosts for digenean trematode parasites (Trematoda, Digenea) (Cribb *et al.* 2003). These parasites and their snail hosts have developed intimate parasite–host associations, with snails supporting a diverse trematode fauna (Cribb *et al.* 2003; Poulin & Mouritsen 2003; Lockyer *et al.* 2004).

Trematodes are important components of ecosystems and can have ecosystem-wide impacts through their effects on snail biology (Wood *et al.* 2007; Bernot & Lamberti 2008). Although a wide range of studies have examined the biological and ecological effects of trematode parasites and snails (e.g., see reviews by Sorensen & Minchella 2001; Thieltges *et al.* 2013), the trematode fauna of many snail taxa remains poorly characterised. In freshwater snails, the pulmonate snail families Lymnaediae and Planorbidae (Heterobranchia) have been extensively studied for trematode parasites (Bargues *et al.* 2001; Faltýnková et al. 2007, 2008; Vázquez *et al.* 2018). However, data are scarce for non-pulmonate freshwater snails (formerly called 'prosobranch snails', now in subclass Caenogastropoda), as was also highlighted by Schwelm *et al.* (2020), who provided the first comprehensive integrative taxonomic study of trematode parasites in *Bithynia tentaculata* (Linnaeus, 1758), a caenogastropod snail of the family Bithyniidae.

The faucet snail, *B. tentaculata*, inhabits both lentic and lotic water bodies; it is highly resistant and adaptable in its feeding strategy and survival (Brendelberger 1995; Glöer 2002). It is widespread in the Palaearctic; it is one of the most common and abundant snail species in Ireland and Great Britain (Kerney 1999), as well as in other parts of Europe, from Scandinavia to the Mediterranean (Glöer 2002). *Bithynia tentaculata* is well known as an intermediate host of trematodes in Europe (Glöer 2002), and within the order Littorinimorpha, it is recorded as

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harbouring the most trematode species (see Cichy *et al.* 2011; Faltýnková et al. 2016; Schwelm *et al.* 2020). However, there has been a substantial gap in knowledge of the exact species identity of the trematode parasites of *B. tentaculata*. The trematode cercariae found in these snails belong to families with members that are particularly difficult to identify. Thus, classification has been vague, and authors often used traditional names for the cercariae that are not connected to the adult trematode parasites (see e.g. Morley *et al.* 2004). The first step to clarify this situation was recently completed by Schwelm *et al.* (2020) who were the first to link DNA sequences to morphological data, followed by Katokhin & Serbina (2023). Schwelm *et al.* (2020) found a diverse and unique spectrum of trematodes, revealing the need for more studies to further resolve the species identity, their phylogenetic relationships, and geographical distribution. A thorough knowledge of the trematode fauna hosted by *B. tentaculata* based on precise identification via integrative taxonomy is crucial for both conservation and epidemiology. Such information is even more important because *B. tentaculata* is an efficient coloniser and is invasive in North America; the trematode parasites it hosts (*Cyathocotyle bushiensis*, *Sphaeridiotrema globulus*, and notocotylids) have been reported from mass mortalities of American migratory birds (Sauer *et al.* 2007; Karatayev *et al.* 2012; Weeks *et al.* 2017).

Ireland is situated on the East Atlantic bird flyway and is a wintering ground for birds nesting further north in Iceland, Greenland, and/or Canada (Cramp & Simmons 1980). Consequently, a diverse assembly of trematode parasites of migratory birds can be expected to occur in snails in Ireland. Recently, an abundant fauna of trematode parasites from freshwater pulmonate snails (Lymnaeidae and Planorbidae) in Ireland was recorded, overlapping with trematode data reported from other parts of Europe including Finland, Iceland, and Norway (Kudlai *et al.* 2021; Pantoja *et al.* 2021; Faltýnková et al. 2022). In Ireland, *B. tentaculata* has so far not been examined for trematodes, although the species is occurring in most parts of the country (Kerney 1999).

In the present study, during a screening of snails for trematodes in lakes in Ireland (i.e. the surroundings of Galway) we found *B. tentaculata* harbouring trematode parasite infections. Our aim was to characterise these trematodes using molecular genetic tools and morphology to contribute to further knowledge of these groups, as genetic data are still scarce (see Schwelm *et al.* 2020). All but one trematode species found are new species records for Ireland and provide the most western distribution of these parasites in Europe.

Material and methods

Sample collection and morphological examination

Specimens of the faucet snail, *Bithynia tentaculata* (Caenogastropoda, Littorinimorpha, Bithyniidae) were collected by hand or with a strainer from stones, mud, or aquatic plants on the bottom of littoral areas of lakes in the surroundings of County Galway, Ireland. In July 2019, a total of 556 *B. tentaculata* were collected, i.e 22 snails from Hackett Pond (53°29’27.0”N 9°02’31.0”W), 125 snails from Lough Corrib (53°21’27.0”N, 9°04’36.0”W), 15 snails from Lough Mask (53° 37’41.0”N, 9°17’01.0”W), 320 snails from Loughrea (53°11’49.8”N, 8°34’51.6”W), and 74 snails from Killeeneen (53°13’30.0”N, 8°47’43.0”W).

The snails were identified based on their shell morphology, and identification was confirmed using Glöer (2002). Snails were placed individually into small plastic cups with dechlorinated tap water, and emergence of cercariae from snails was stimulated by light and heat from a lamp overnight for two consecutive days.

Emerged cercariae were examined under a light microscope, and for vital staining Neutral red and Nile blue were used; for primary identification, Yamaguti (1975) was used. Live or fixed cercariae from each snail were examined and photographed with a digital camera on Olympus BX41 and BX51 light microscopes to document morphology. Drawings of cercariae were based on microphotographs taken on live specimens. Afterwards, all snails were dissected under a stereomicroscope to detect trematode parasite stages, including non-emerged cercariae, sporocysts or rediae, and metacercariae. Cercariae and metacercariae (isogenophores, sensu Pleijel *et al.*, 2008) were fixed in molecular grade ethanol (96%) for subsequent molecular analyses and for morphological examination.

Measurements of live and fixed cercariae were taken from digital images with the aid of ImageJ image analysis software (Schneider *et al.* 2012). All measurements are given in micrometres as the range followed by the mean in parentheses. The following abbreviations of morphological features are used in the text: TSL/BL or TL/BL, body length (BL) to tail stem length (TSL)/tail length (TL) ratio; TSL/FL, furca length (FL) to tail stem length ratio; VSW/OSW, oral sucker width (OSW) to ventral sucker width (VSW) ratio.

Voucher material of cercariae fixed in molecular grade ethanol is deposited in the collection of the National Museum of Ireland Natural History (NMINH), Merrion Street, Dublin 2, D02 F627, Ireland.

DNA processing and phylogenetic analyses

Total genomic DNA was extracted from approximately 20–25 cercariae or one cercariaeum or metacercaria (Lissorchiidae and Psilostomidae) detected in a single snail using the Monarch Genomic DNA Purification Kit (New England Biolabs, Inc., Ipswich, MA, USA) following the manufacturer’s recommendations. Following the recommendations of Blasco-Costa *et al.* (2016), DNA sequence-based identification of trematode larval stages recorded in the present study was based on multiple molecular markers. Depending on the trematode family, the taxonomic identity and phylogenetic relationships of the larval stages were evaluated on DNA sequences of the partial 28S rRNA gene (D1–D3 region), partial mitochondrial cytochrome c oxidase subunit 1 (*cox*1), partial mitochondrial nicotinamide adenine dinucleotide dehydrogenase subunit 1 (*nad*1) genes, complete second internal transcribed spacer region (ITS2), and/or the entire internal transcribed spacer (ITS1-5.8S-ITS2) region. Amplification of the partial 28S rRNA gene was completed as described by Scholz *et al.* (2013) using the primers ZX-1 and 1500R. Amplification of the partial *cox*1 gene was completed as described by Van Steenkiste *et al.* (2015) using primers DICE1F and DICE14R and by Wee *et al.* (2017) using primers Dig_cox1Fa and Dig_cox1R. Amplification of the partial *nad*1 gene was completed as described by Laidemitt *et al.* (2019) using primers NDJ11 and NDJ2A. Amplification of the complete ITS2 region was completed as described by Cutmore *et al.* (2013) using primers 3S and ITS2.2. Finally, amplification of the entire ITS1-5.8S-ITS2 was completed as described by Galazzo *et al.* (2002) using primers D1 and D2. Amplified DNA was purified using ExoSAP-IT PCR Cleanup enzymatic kit from Thermo Fisher Scientific, Inc. (Waltham, MA, USA) and sequenced from both strands using the PCR primers and additional internal sequencing primers ECD2 (5′-CCT TGG TCC GTG TTT CAA GAC GGG-3′) (Littlewood *et al.* 1997) and 300F (5′-CAA GTA CCG TGA GGG AAA

GTT G-3′) (Littlewood *et al.* 2000) for 28S rDNA. Cycle sequencing of DNA was carried out applying ABI Big Dye™v.3.1 chemistry at the commercial company SEQme (Dobříš, Czech Republic, https://www.seqme.eu) with the use of an AB3730x1 capillary sequencer. Geneious Prime ver. 2023.2.1 (Biomatters, Auckland, New Zealand, https://geneious.com) was used to assemble and edit contiguous sequences. The newly generated sequences were deposited in GenBank with accession numbers PP840844–PP840849, PP844658–PP844660, PP849699–PP849722, and PP856383–PP856385.

Identification of novel sequences was checked using the Basic Local Alignment Search Tool (BLAST) (www.ncbi.nih.gov/BLAST/), and thereafter they were aligned with the selected representative sequences from GenBank using MUSCLE implemented in Geneious Prime (Table 1). Ten alignments were prepared for phylogenetic analyses. Muscle standard algorithm was applied. Alignment 1 – Cyathocotylidae (1,122 nt); Alignment 2 – Lecithodendriidae (1,055 nt); Alignment 3 – Lissorchiidae (1,226 nt); Alignment 4 – Opecoelidae (1,199 nt); Alignment 5 – Opisthorchiidae (1,071 nt); Alignment 6 – Notocotylidae (1,156 nt); and Alignment 7 – Psilostomidae (1,120 nt), which all consisted of the 28S rDNA sequences. Two alignments, Alignment 8 – Lecithodendriidae (339 nt) and Alignment 9 – Lissorchiidae (407 nt) consisted of ITS2 sequences, and Alignment 10 – Opisthorchiidae (775 nt) consisted of *cox*1 sequences. The *cox*1 alignment was aligned with reference to the amino acid translation, using the trematode mitochondrial code (translation table 21; https://www.ncbi.nlm.nih.gov/Taxonomy/Utils/wprintgc.cgi#SG21) (Garey & Wolstenholme 1989; Ohama *et al.* 1990).

To provide the phylogenetic hypotheses for all alignments, Bayesian inference and maximum likelihood analyses were applied. The best-fitting evolutionary model was estimated using the AIC criterion in jModelTest 2.1.2. (Darriba *et al.* 2012). These were the GTR + I + G (Alignments 1, 2, 4, 6, 7, and 10), GTR + G (Alignments 3, 5, and 9), and HKY + I +G (Alignment 8). The maximum likelihood analysis was conducted using PhyML ver. 3.0 (Guindon *et al.* 2010) run on the ATGC bioinformatic platform (http://www.atgc-montpellier.fr/

Table 1. Summary data for the sequences of trematodes used for phylogenetic analyses; novel sequences generated in the present study are in bold

			GenBank accession numbers			
Family/species	Host	Locality	28S	ITS2	*cox*1/*nad*1	Reference
Cyathocotylidae						
Braunina cordiformis	*Delphinus delphis*	Argentina	KM258670	–	–	Fraija–Fernández *et al.* (2015)
Braunina sp.	*Tursiops truncatus*	USA	MK650439	–	–	Achatz *et al.* (2019)
Cyathocotyle bushiensis	*Aythya affinis*	USA	MK650440	–	–	Achatz *et al.* (2019)
Cyathocotyle prussica	***Bithynia tentaculata***	**Ireland**	**PP849699**	**PP849714***	**PP840844**/–	**present study**
Cyathocotyle prussica	*Gasterosteus aculeatus*	Germany	MH521249	–	–	Locke *et al.* (2018)
Cyathocotyle sp. 1	*Bithynia tentaculata*	Germany	MN726941	–	–	Schwelm *et al.* (2020)
Cyathocotyle sp. 2	*Bithynia tentaculata*	Germany	MN726943	–	–	Schwelm *et al.* (2020)
Cyathocotylidae gen. sp.	*Clypeomorus batillariaeformis*	Australia	MH257776	–	–	Huston *et al.* (2018)
Gogatea mehri	*Acrochordus granulatus*	Vietnam	MK650441	–	–	Achatz *et al.* (2019)
Gogatea sp.	*Acrochordus javanicus*	Thailand	MK650442	–	–	Achatz *et al.* (2019)
Holostephanoides ictaluri	*Ameiurus* sp.	USA	MK650443	–	–	Achatz *et al.* (2019)
Holostephanus dubinini	*Phalacrocorax carbo*	Ukraine	MK650444	–	–	Achatz *et al.* (2019)
Mesostephanus cubaensis	*Morus bassanus*	USA	MK650445	–	–	Achatz *et al.* (2019)
Mesostephanus microbursa	*Sula nebouxii*	Mexico	MF398326	–	–	Hernández–Mena *et al.* (2017)
Mesostephanus microbursa	*Morus bassanus*	USA	MK650446	–	–	Achatz *et al.* (2019)
Neogogatea sp.	*Lophodytes cucullatus*	USA	MK650447	–	–	Achatz *et al.* (2019)
Suchocyathocotyle fraterna	*Crocodylus niloticus*	South Africa	MK650452	–	–	Achatz *et al.* (2019)
Lecithodendridae						
Lecithodendriidae sp.	*Melanoides tuberculata*	Brazil	MW414608	MW414609	–	Lopes *et al.* (2021)
Lecithodendriidae sp. A	*Semisulcospira libertina*	Japan	LC704418	–	–	Nakao & Sasaki (unpublished)
Lecithodendriidae sp. B	*Semisulcospira libertina*	Japan	LC599539	–	–	Nakao & Sasaki (2021)
Lecithodendriidae sp. C	*Semisulcospira libertina*	Japan	LC599541	–	–	Nakao & Sasaki (2021)
Lecithodendriidae sp. D	*Semisulcospira libertina*	Japan	LC704419	–	–	Nakao & Sasaki (unpublished)
Lecithodendriidae sp. E	*Semisulcospira libertina*	Japan	LC704421	–	–	Nakao & Sasaki (unpublished)
Lecithodendriidae sp. F	*Semisulcospira libertina*	Japan	LC704422	–	–	Nakao & Sasaki (unpublished)
Lecithodendrium linstowi	***Bithynia tentaculata***	**Ireland**	**PP849700**	**PP849715**	**PP844658/–**	**present study**
Lecithodendrium linstowi	***Bithynia tentaculata***	**Ireland**	**PP849701**	**PP849716**	**PP844659/–**	**present study**

(*Continued*)

Table 1. (*Continued*)

			GenBank accession numbers			
Family/species	Host	Locality	28S	ITS2	*cox*1/*nad*1	Reference
Lecithodendrium linstowi	*Bithynia tentaculata*	Germany	MN726965	MN726993	–	Schwelm *et al.* (2020)
Lecithodendrium linstowi	*Radix balthica*	UK	MF498821	–	–	Enabulele *et al.* (2018)
Lecithodendrium linstowi	*Pipistrellus pipistrellus*	UK	–	JF784190	–	Lord *et al.* (2012)
Lecithodendrium linstowi	*Nyctalus noctula*	Ukraine	–	KJ934792	–	Kudlai *et al.* (2015)
Lecithodendrium spathulatum	*Pipistrellus pipistrellus*	UK	–	JF784192	–	Lord *et al.* (2012)
***Lecithodendrium* sp.**	***Bithynia tentaculata***	**Ireland**	**PP849702**	**PP849717**	**PP844660/–**	**present study**
Lecithodendrium sp.	*Bithynia tentaculata*	Lithuania	KJ126726	KJ126724	–	Kudlai *et al.* (2015)
Lecithodendrium sp.	*Bithynia siamensis goniomphalos*	Vietnam	ON986398	–	–	Nguyen *et al.* (2022)
Lecithodendrium sp.**	*Stagnicola palustris*	Denmark	–	MW001039	–	Duan *et al.* (2021)
Lecithodendrium sp.	*Filopaludina sumatrensis polygramma*	Thailand	–	MN432606	–	Dunghungzin & Chontananarth (2020)
Lecithodendrium sp.	not provided	not provided	–	MW598491	–	Abuelmaged *et al.* (unpublished)
Ochoterenatrema diminutum	*Molossus molossus*	Brazil	OM574912	–	–	Fernandes *et al.* (2022)
Ochoterenatrema cf. *labda*	*Myotis* sp.	Brazil	OM574913	–	–	Fernandes *et al.* (2022)
Ophiosacculus mehelyi	*Eptesicus serotinus*	Ukraine	AF480167	–	–	Tkach (unpublished)
Paralecithodendrium chilostomum	*Viviparus viviparus*	Ukraine	KJ126725	KJ126723	–	Kudlai *et al.* (2015)
Paralecithodendrium chilostomum	*Nyctalus noctula*	Ukraine	–	KJ920281	–	Kudlai *et al.* (2015)
Paralecithodendrium hurkovaae	*Myotis daubentonii*	Ukraine	AF151922	–	–	Tkach *et al.* (2000)
Paralecithodendrium hurkovaae	*Myotis daubentonii*	Ukraine	–	KJ920282	–	Kudlai *et al.* (2015)
Paralecithodendrium longiforme	*Myotis daubentonii*	Ukraine	AF151921	–	–	Tkach *et al.* (2000)
Paralecithodendrium longiforme	*Myotis daubentonii*	Ukraine	–	KJ920283	–	Kudlai *et al.* (2015)
Paralecithodendrium parvouterus	*Miniopterus schreibersi*	Spain	AY220617	–	–	Tkach *et al.* (2003)
Paralecithodendrium sp.	*Pipistrellus pipistrellus*	UK	–	JF784195	–	Lord *et al.* (2012)
Paralecithodendrium sp. 1	not provided	not provided	–	MW685826	–	Abuelmaged *et al.* (unpublished)
Paralecithodendrium sp. 2	not provided	not provided	–	MW581581	–	Abuelmaged *et al.* (unpublished)
Paralecithodendrium sp. 4	not provided	not provided	–	MW581583	–	Abuelmaged *et al.* (unpublished)
Paralecithodendrium sp. 5	not provided	not provided	–	MW581582	–	Abuelmaged *et al.* (unpublished)
Prosthodendrium sp.	not provided	not provided	MW566603	MW566599	–	Abuelmaged *et al.* (unpublished)
Pycnoporus heteroporus	*Pipistrellus kuhlii*	Ukraine	AF151918	–	–	Tkach *et al.* (2000)
Pycnoporus heteroporus	*Pipistrellus kuhlii*	Ukraine	–	KJ920284	–	Kudlai *et al.* (2015)
Pycnoporus megacotyle	*Pipistrellus kuhlii*	Ukraine	AF151917	–	–	Tkach *et al.* (2000)
Pycnoporus megacotyle	*Pipistrellus kuhlii*	Ukraine	–	KJ920285	–	Kudlai *et al.* (2015)
Virgulate xiphidiocercaria 3	*Bithynia siamensis goniomphalos*	Thailand	–	ON312602	–	Pitaksakulrat *et al.* (2022)
Opecoelidae						
Neoplagioporus ayu	*Plecoglossus altivelis altivelis*	Japan	KX553947	–	–	Fayton & Andres (2016)
Neoplagioporus elongatus	*Sarcocheilichthys variegatus microoculus*	Japan	KX553948	–	–	Fayton & Andres (2016)
Neoplagioporus kajika	*Triplophysa fuxianensis*	China	MH277497	–	–	Ding (unpublished)
Neoplagioporus zacconis	*Opsariichthys platypus*	Japan	KX553949	–	–	Fayton & Andres (2016)

(*Continued*)

Table 1. (*Continued*)

			GenBank accession numbers			
Family/species	Host	Locality	28S	ITS2	*cox*1/*nad*1	Reference
Opecoelidae gen. sp.	*Bithynia tentaculata*	Germany	MN726964	–	–	Schwelm *et al.* (2020)
Plagiocirrus loboides	*Fundulus nottii*	USA	EF523477	–	–	Curran *et al.* (2007)
Plagioporus aliffi	*Etheostoma blennioides newmanni*	USA	KX905056	–	–	Fayton *et al.* (2017)
Plagioporus boleosomi	*Percina maculata*	USA	KX553953	–	–	Fayton & Andres (2016)
Plagioporus carolini	*Cottus carolinae*	USA	MG214680	–	–	Fayton *et al.* (2018)
Plagioporus chiliticorum	*Notropis chiliticus*	USA	KX553943	–		Fayton & Andres (2016)
Plagioporus fonti	*Percina nigrofasciata*	USA	KX905054	–	–	Fayton *et al.* (2017)
Plagioporus hageli	*Oncorhynchus mykiss*	USA	KX553950	–	–	Fayton & Andres (2016)
Plagioporus ictaluri	*Noturus lachneri*	USA	MG214679	–	–	Fayton *et al.* (2018)
Plagioporus kolipinskii	*Gasterosteus aculeatus*	USA	KX553952	–	–	Fayton & Andres (2016)
Plagioporus limus	*Etheostoma squamosum*	USA	KX905055	–	–	Fayton *et al.* (2017)
Plagioporus shawi	*Oncorhynchus tshawytscha*	USA	KX553951	–	–	Fayton & Andres (2016)
Plagioporus sinitsini	*Notemigonus crysoleucas*	Canada	KX553944	–	–	Fayton & Andres (2016)
Plagioporus wataugaensis	*Hypentelium nigricans*	USA	ON059353	–	–	Truong *et al.* (2022)
Sphaerostoma bramae	***Bithynia tentaculata***	**Ireland**	**PP849703**	**PP849718**	–	**present study**
Sphaerostoma bramae	***Bithynia tentaculata***	**Ireland**	**PP849704**	**PP849719**	–	**present study**
Sphaerostoma bramae	*Abramis brama*	Russia	MH161435	–	–	Sokolov *et al.* (2019)
Sphaerostoma sp.	*Bithynia tentaculata*	Germany	MN726960	–	–	Schwelm *et al.* (2020)
Urorchis acheilognathi	*Tanakia limbata*	Japan	KX553945	–	–	Fayton & Andres (2016)
Urorchis goro	*Semisulcospira libertina*	Japan	LC149879	–	–	Shimazu (2017)
Opisthorchiidae						
Amphimerus ovalis	*Trionyx muticus*	USA	AY116876	–	–	Olson *et al.* (2003)
Amphimerus sp.	*Homo sapiens*	Ecuador	–	–	MK238506/–	Ma *et al.* (2019)
Clonorchis sinensis	*Homo sapiens*	Vietnam	JF823989	–	–	Thaenkham *et al.* (2011)
Clonorchis sinensis	*Felis catus*	China	–	–	MT292143/–	Kinkar *et al.* (2020)
Metorchis bilis	*Milvus migrans*	Russia	OK358937	–	–	Sokolov *et al.* (2021)
Metorchis orientalis	*Anas platyrhynchos f. dom.*	China	MK482051	–	–	Qiu *et al.* (unpublished)
Metorchis xanthosomus	***Bithynia tentaculata***	**Ireland**	**PP849705**	–	**PP840845/–**	**present study**
Metorchis xanthosomus	***Bithynia tentaculata***	**Ireland**	**PP849706**	–	**PP840846/–**	**present study**
Metorchis xanthosomus	*Fulica atra*	Russia	OK358938	–	–	Sokolov *et al.* (2021)
Metorchis sp.	*Anas platyrhynchos f. dom.*	Russia	KY075777	–	KY075780/–	Shumenko *et al.* (unpublished)
Opisthorchiidae gen. sp.	*Bithynia tentaculata*	Germany	MN726966	–	–	Schwelm *et al.* 2020)
Opisthorchis felinus	*Felis catus f. dom.*	Russia	MF099790	–	–	Dao *et al.* (2017)
Opisthorchis noverca	*Sus scrofa f. dom.*	India	KC295443	–	–	Tandon *et al.* (unpublished)
Opisthorchis viverrini	*Mesocricetus auratus*	Thailand	HM004188	–	–	Thaenkham *et al.* (2010)
Opisthorchis sp.	*Anas platyrhynchos f. dom.*	Vietnam	MF110001	–	MF287764/–	Dao *et al.* (2017)
Metorchis bilis	*Mesocricetus auratus*	Russia	–	–	NC079698/–	Katokhin (unpublished)
Metorchis orientalis	*Anas platyrhynchos f. dom.*	China	–	–	NC028008/–	Na *et al.* (2016)
Metorchis ussuriensis	*Anas platyrhynchos f. dom.*	Russia	–	–	KP222507/–	Besprozvannykh et al. (2019)
Metorchis xanthosomus	*Gallus gallus*	Russia	–	–	NC079699/–	Katokhin (unpublished)
Opisthorchis felineus	*Felis catus*	Russia	–	–	NC011127/–	Shekhovtsov *et al.* (2010)
Opisthorchis sudarikovi	*Egretta garzetta*	Pakistan	–	–	MK033132/–	Suleman *et al.* (2019)
Opisthorchis viverrini	*Homo sapiens*	Thailand	–	–	MF287782/–	Dao *et al.* (2017)

(*Continued*)

Table 1. (*Continued*)

			GenBank accession numbers			
Family/species	Host	Locality	28S	ITS2	*cox*1/*nad*1	Reference
Psilostomidae						
Byrdtrema sponsae	*Aix sponsa*	USA	KT956955	–	–	Tkach *et al.* (2016)
Longisaccus elvirae	*Aix sponsa*	USA	KT956954	–	–	Tkach *et al.* (2016)
Macrocetabulum albeolae	*Bucephala albeola*	USA	KT956953	–	–	Tkach *et al.* (2016)
Neopsilotrema affine	*Aythya affinis*	USA	KT956952	–	–	Tkach *et al.* (2016)
Neopsilotrema lakotae	*Aythya americana*	USA	KU379696	–	–	Kudlai *et al.* (2016)
Neopsilotrema lisitsynae	*Anas crecca*	Ukraine	KT956951	–	–	Tkach *et al.* (2016)
Psilostomidae gen. sp. 1	*Bithynia tentaculata*	Germany	MN726950	–	–	Schwelm *et al.* (2020)
Psilostomidae gen. sp. 2	*Bithynia tentaculata*	Germany	MN726954	–	–	Schwelm *et al.* (2020)
Psilotrema cf. *simillimum*	*Bithynia tentaculata*	Russia	MW962239	–	–	Achatz *et al.* (2021)
Psilotrema limosum	*Gallus gallus f. dom.*	Russia	MT986042	–	–	Kalinina *et al.* (2022)
Sphaeridiotrema aziaticum	*Gallus gallus f. dom.*	Russia	MT986043	–	–	Kalinina *et al.* (2022)
Sphaeridiotrema globulus	*Anas platyrhynchos f. dom.*	USA	GQ890331	–	–	Bergmame *et al.* (2011)
Sphaeridiotrema monorchis	*Gallus gallus f. dom.*	Vietnam	JQ890544	–	–	Besprozvannykh *et al.* (2013)
Sphaeridiotrema pseudoglobulus	*Aythya affinis*	USA	KT956957	–	–	Tkach *et al.* (2016)
Sphaeridiotrema pyriforme	*Gallus gallus f. dom.*	Russia	MT986045	–	–	Kalinina *et al.* (2022)
Sphaeridiotrema ussuriensis	*Gallus gallus f. dom.*	Russia	MT986040	–	–	Kalinina *et al.* (2022)
***Sphaeridiotrema* sp.**	***Bithynia tentaculata***	**Ireland**	**PP849708**	–	**–/PP856384**	**present study**
***Sphaeridiotrema* sp.**	***Bithynia tentaculata***	**Ireland**	**PP849709**	–	**–/PP856385**	**present study**
***Sphaeridiotrema* sp.**	***Bithynia tentaculata***	**Ireland**	**PP849707**	–	**–/PP856383**	**present study**
Sphaeridiotrema sp.	*Bithynia tentaculata*	Lithuania	KT956958	–	–	Tkach *et al.* (2016)
Sphaeridiotrema sp.	*Bithynia tentaculata*	Germany	MN726949	–	–	Schwelm *et al.* (2020)
Lissorchiidae						
Asymphylodora percotti	*Perccottus glenii*	Russia	FR822731	–	–	Besprozvannykh *et al.* (2012)
Asymphylodora progenetica	***Bithynia tentaculata***	**Ireland**	**PP849710**	**PP849720**	**PP840847/–**	**present study**
Asymphylodora progenetica	***Bithynia tentaculata***	**Ireland**	**PP849711**	**PP849721**	**PP840848/–**	**present study**
Asymphylodora progenetica	*Bithynia tentaculata*	Lithuania	MT103403	MT103399	–	Petkevičiūtė *et al.* (2020)
Asymphylodora tincae	*Anisus vortex*	Lithuania	OP106446	–	–	Petkevičiūtė *et al.* (2022)
Asymphylodora tincae	*Tinca tinca*	Lithuania	–	OP106427	–	Petkevičiūtė *et al.* (2022)
Asymphylodora sp.	*Bithynia tentaculata*	Germany	MN726955	–	–	Schwelm *et al.* (2020)
Asymphylodora sp.	*Lithoglyphus naticoides*	Hungary	MT153917	MT153915	–	Petkevičiūtė *et al.* (2020)
Lissorchis cf. gullaris	*Ictiobus niger*	USA	MT928353	MT928353	–	Truong *et al.* (2021)
Lissorchis kritskyi	*Carpiodes velifer*	USA	MT928329	MT928329	–	Truong *et al.* (2021)
Lissorchis cf. nelsoni	*Minytrema melanops*	USA	MT928354	MT928354	–	Truong *et al.* (2021)
Palaeorchis incognitus	*Lithoglyphus naticoides*	Lithuania	MT103409	–	–	Petkevičiūtė *et al.* (2020)
Palaeorchis incognitus	*Rutilus rutilus*	Lithuania	–	MT103406	–	Petkevičiūtė *et al.* (2020)
Parasymphylodora markewitschi	*Bithynia tentaculata*	Lithuania	OP106447	OP106430	–	Petkevičiūtė *et al.* (2022)
Parasymphylodora parasquamosa	***Bithynia tentaculata***	**Ireland**	**PP849712**	**PP849722**	**PP840849/–**	**present study**
Parasymphylodora parasquamosa	*Bithynia tentaculata*	Lithuania	OP106442	OP106425	–	Petkevičiūtė *et al.* (2022)
Posthovitellinum psiloterminae	*Cyclocheilos enoplos*	Vietnam	MT928352	MT928348	–	Truong *et al.* (2021)

(*Continued*)

Table 1. (*Continued*)

			GenBank accession numbers			
Family/species	Host	Locality	28S	ITS2	*cox*1/*nad*1	Reference
Notocotylidae						
Catatropis indicus	*Cairina moschata*	Australia	AY222220	–	–	Olson *et al.* (2003)
Catatropis onobae	*Somateria mollissima*	Iceland	MN963032	–	–	Gonchar & Galaktionov (2020)
Catatropis vietnamensis	*Anas platyrhynchos*	Vietnam	MH750021	–	–	Izrailskaia *et al.* (2019)
Catatropis sp.	*Bithynia funiculata*	Vietnam	ON986399	–	–	Nguyen *et al.* (2022)
Hippocrepis hippocrepis	*Biomphalaria straminea*	Brazil	MN270932	–	–	Assis *et al.* (2019)
Notocotylidae gen. sp.	*Bithynia tentaculata*	Germany	MN726958	–	–	Schwelm *et al.* (2020)
Notocotylus atlanticus	*Anas platyrhynchos*	Russia	MH818008	–	–	Gonchar *et al.* (2019)
Notocotylus attenuatus	*Aythia ferina*	Ukraine	AF184259	–	–	Tkach *et al.* (2001)
Notocotylus chionis	*Calidris fuscicollis*	Argentina	MN877911	–	–	Capasso *et al.* (2020)
Notocotylus cygni	*Cygnus melancoryphus*	Argentina	OR501502	–	–	Flores *et al.* (2023)
Notocotylus ephemera	*Planorbis planorbis*	Russia	OP720893	–	–	Svinin *et al.* (2023)
Notocotylus fosteri	*Oryzomys palustris*	USA	MK614163	–	–	Díaz *et al.* (2020)
Notocotylus ikutai	*Radix auricularia*	Japan	LC596925	–	–	Sasaki *et al.* (2021)
Notocotylus intestinalis	*Gallus gallus f. dom.*	Vietnam	JQ890559	–	–	Besprozvannykh *et al.* (2013)
Notocotylus magniovatus	“Chicken”	Russia	MH750018	–	–	Izrailskaia *et al.* (2019)
Notocotylus malhamensis	*Myodes glareolus*	UK	JQ766939	–	–	Boyce *et al.* (2012)
***Notocotylus* sp.**	***Bithynia tentaculata***	**Ireland**	**PP849713**	–	–	**present study**
Notocotylus sp.	*Bithynia tentaculata*	Germany	MN726957	–	–	Schwelm *et al.* (2020)
Notocotylus sp.	*Bithynia tentaculata*	Germany	MN726956	–	–	Schwelm *et al.* (2020)
Notocotylus sp.	*Radix balthica*	Norway	KY513158	–	–	Soldánová *et al.* (2017)
Notocotylus sp.	*Stagnicola palustris*	UK	AY222219	–	–	Olson *et al.* (2003)
Notocotylus sp.	*Physa gyrina*	USA	EU712725	–	–	Hanelt (2009)
Notocotylus sp.	*Physella acuta*	USA	MW358653	–	–	Oliver *et al.* (unpublished)
Ogmogaster antarctica	*Balaenoptera borealis*	Argentina	KM258675	–	–	Fraija–Fernández et al. (2015)
Opisthotrema dujonis	*Dugong dugon*	Australia	AY222223	–	–	Olson *et al.* (2003)
Paramonostomum anatis	*Tringa erythropus*	Ukraine	AF184258	–	–	Tkach *et al.* (2001)
Pronocephaloidea sp. S5M1A5	*Potamopyrgus antipodarum*	USA	EU371602	–	–	Adema *et al.* (2009)
Pseudocatatropis dvoryadkini	*Anas platyrhynchos f. dom.*	Russia	MH750024	–	–	Izrailskaia *et al.* (2019)
Pseudocatatropis sp.	*Anas penelope*	Japan	LC596916	–	–	Sasaki *et al.* (2021)
Pseudocatatropis sp.	“snail”	China	OR509500	–	–	Li *et al.* (unpublished)
Tabrobanella bicaudata	*Dugong dugon*	Australia	AY222217	–	–	Olson *et al.* (2003)
Outgroups						
Apophallus zalophi	*Callorhinus ursinus*	USA	MG806918	–	–	Kuzmina *et al.* (2018)
Buticulotrema thermichthysi	*Thermichthys hollisi*	Pacific Ocean	KF733984	–	–	Bray *et al.* (2014)
Haplorchis taichui	*Homo sapiens*	Laos	–	KF214770	–	Lee *et al.* (2013)
Labicola cf. *elongata*	*Dugong dugon*	Australia	AY222221	–	–	Olson *et al.* (2003)
Monorchis lewisi	*Acanthopagrus australis*	Australia	MF503309	MF503313	–	Cribb *et al.* (2018)
Phaneropsolus praomidis	not provided	Egypt	MW988461	MW988538	–	El–Zeiny *et al.* (unpublished)
Posthodiplostomum brevicaudatum	*Perca fluviatilis*	Czech Republic	KX931426	–	–	Stoyanov *et al.* (2017)
Stephanoprora pseudoechinata	*Chroicocephalus genei*	Ukraine	KT956935	–	–	Tkach et al. (2016)

*Sequence of ITS1-5.8S-ITS2;
**Originally identified as *Lecithodendrium linstowi*, see Duan *et al.* (2021)

phyml/). Nodal support for the ML analysis was generated by performing 100 bootstrap pseudoreplicates. The Bayesian inference analysis was conducted using MrBayes software ver. 3.2.3 (Ronquist *et al.* 2012). Markov Chain Monte Carlo simulations were run for 3,000,000 generations, log likelihood scores were recorded to estimate burn-in, and only the last 75% of trees were used to build the consensus tree. FigTree ver. 1.4 software (Rambaut 2010) was used for the tree visualisation. Nucleotide differences between sequences were estimated using MEGA ver. 11 (Tamura *et al.* 2021) using the following conditions: "Variance Estimation Method = None", "Model/Method = No. of differences" or "p-distance", and "Substitutions to Include = d: Transitions + Transversions" and "Gaps/Missing Data Treatment = Pairwise deletion".

Results

In the present study, we found trematode infections in 38 (6.8%) snails of *Bithynia tentaculata* in two lakes, Loughrea (0.6%, one species recorded in two snails) and Lough Corrib (28.8%, eight species recorded in 36 snails); in the other three lakes, no infections were found (see Table 2). In total, we found nine species of trematodes in *B. tentaculata*; seven species were recorded as cercariae and three species as metacercariae (*Asymphylodora progenetica* Serkova & Bykhowskiy, 1940, *Parasymphylodora parasquamosa* Kulakova, 1972, and *Sphaeridiotrema* sp.), and they belong to seven families (Cyathocotylidae, Opisthorchiidae, Lecithodendriidae, Lissorchiidae, Opecoelidae, Psilostomidae). One species recorded as cercariae (i.e. a tailless cercariaeum), *A. progenetica*, was also observed as a progenetic metacercaria, but genetic analysis was only possible for its cercariae.

DNA sequence-based identification

Thirty-six novel sequences were generated for 15 trematode isolates; sequences of the partial 28S rRNA (15 sequences), *cox*1 (9 sequences), *nad*1 (3 sequences) genes as well as ITS1-5.8S-ITS2 (1 sequence) and ITS2 (8 sequences) regions (see Table 1).

The 28S rDNA phylogeny of available sequences of the Cyathocotylidae demonstrated that the newly sequenced isolate of *Cyathocotyle* (PP849699) clustered with sequences of *Cyathocotyle prussica* Mühling, 1896 ex *Gasterosteus aculeatus* L., and *Cyathocotyle* sp. 1 ex *B. tentaculata* in a well-supported clade (Figure 1). Our 28S rDNA sequence and that of *C. prussica* were identical and differed from the sequence of *Cyathocotyle* sp. 1 by 0.2–0.3% (2–3 nt). Comparison of ITS1-5.8S-ITS2 sequences of the same isolates demonstrated that our sequence was identical to the sequence of *Cyathocotyle* sp. 1 and slightly differed by 0.2% (3 nt) from the sequence of *C. prussica*. The comparison of the *cox*1 sequences demonstrated a low level of divergence between our sequence and two sequences of *C. prussica*; it ranged between 0.85 and 1.03% (5–6 nt). Based on these results we suggest that our species and the unidentified species of *Cyathocotyle*, *Cyathocotyle* sp. 1, are conspecific with *C. prussica*. Thus, we detected a single species of *Cyathocotyle* ex *B. tentaculata* in our study.

In the phylogenetic analysis for the Lecithodendriidae based on the 28 rDNA sequences, two of our isolates (PP849700 and PP849701) clustered with sequences of *Lecithodendrium linstowi* Dollfus, 1931 ex *B. tentaculata* and *Radix balthica* (L.), whereas the sequences of the third isolate (PP849702) clustered with sequences of an unidentified *Lecithodendrium* sp. ex *B. tentaculata* (Figure 2A). The interspecific divergence between *L. linstowi* and *Lecithodendrium* sp. was 0.5% (5 nt). Similar to these results, the phylogenetic analyses based on the ITS2 sequences demonstrated that the sequences of two of our isolates (PP849715 and PP849716) clustered with sequences of *L. linstowi*, and the sequence of the third isolate (PP849717) clustered with sequences of *Lecithodendrium* sp. and one isolate identified as *L. linstowi* (Figure 2B). The ITS2 sequences of *L. linstowi* were identical as well as the ITS2 sequences of *Lecithodendrium* sp. The interspecific divergence between two species was 1.2% (4 nt). Thus, two species of *Lecithodendrium* were detected.

The 28S rDNA phylogeny of the Opecoelidae parasitising freshwater fish demonstrated that the newly sequenced isolates clustered with the isolates of *Sphaerostoma bramae* (Müller, 1776) ex

Table 2. List of larval trematode species and their prevalence in % (No. of infected snails) in the faucet snail, *Bithynia tentaculata*, in Irish lakes in County Galway

Trematode species	Trematode stage*	Hackett Pond	Killeeneen	Lough Corrib	Lough Mask	Loughrea
Cyathocotyle prussica	C	–	–	–	–	0.6% (2)
Lecithodendrium linstowi	C	–	–	1.6% (2)	–	–
Lecithodendrium sp.	C	–	–	0.8% (1)	–	–
Sphaerostoma bramae	C	–	–	1.6% (2)	–	–
Metorchis xanthosomus	C	–	–	2.4% (3)	–	–
Sphaeridiotrema sp.	M	–	–	3.2% (4)	–	–
Asymphylodora progenetica	C	–	–	0.8% (1)	–	–
Asymphylodora progenetica	M	–	–	0.8% (1)	–	–
Parasymphylodora parasquamosa	M	–	–	0.8% (1)	–	–
Notocotylus sp.	C	–	–	0.8% (1)	–	–
Unidentified sporocysts		–	–	2.4% (3)	–	–
Unidentified metacercariae		–	–	13.6% (17)	–	–
Total No. of snails sampled		22	74	125	15	320
Total prevalence (No. infected)		–	–	28.8 % (36)	–	0.6% (2)

*C, cercaria; M, metacercaria

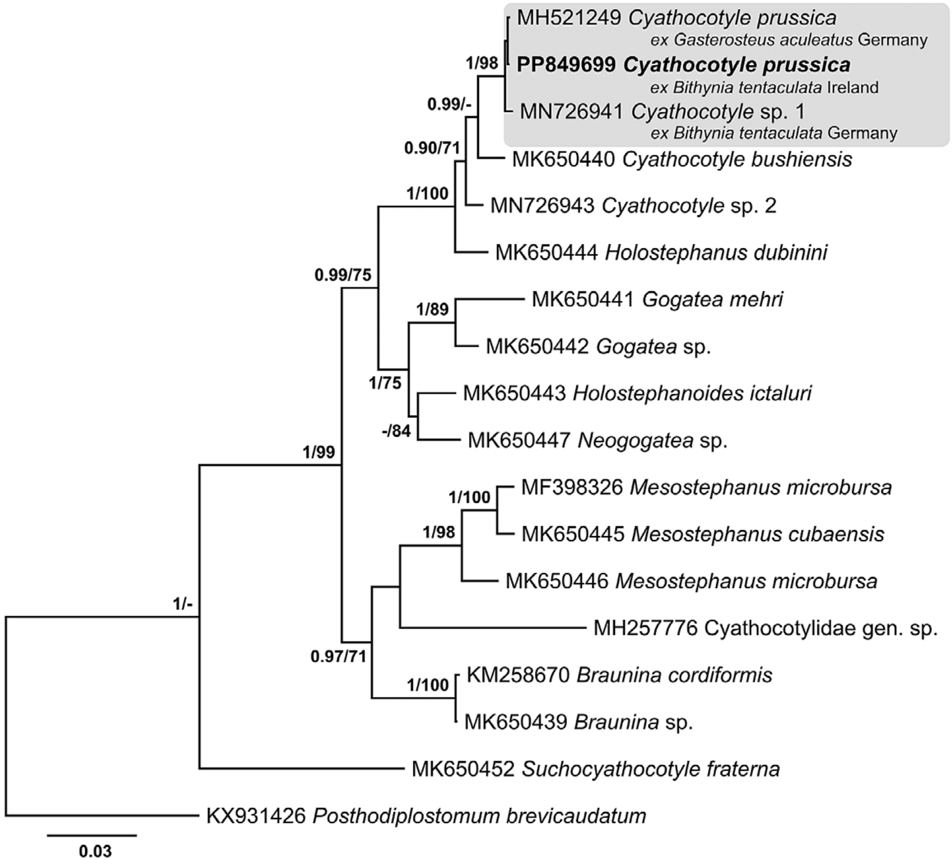

Figure 1. Phylogram resulting from Bayesian inference (BI) analysis based on the 28S rDNA sequences for species of *Cyathocotyle* and of the Cyathocotylidae with nodal support values shown at the node as BI/ML (maximum likelihood). Only values > 0.90 (BI) and > 70 (ML) are displayed. Scale bar indicates the expected number of substitutions per site. The sequence generated in this study is in bold.

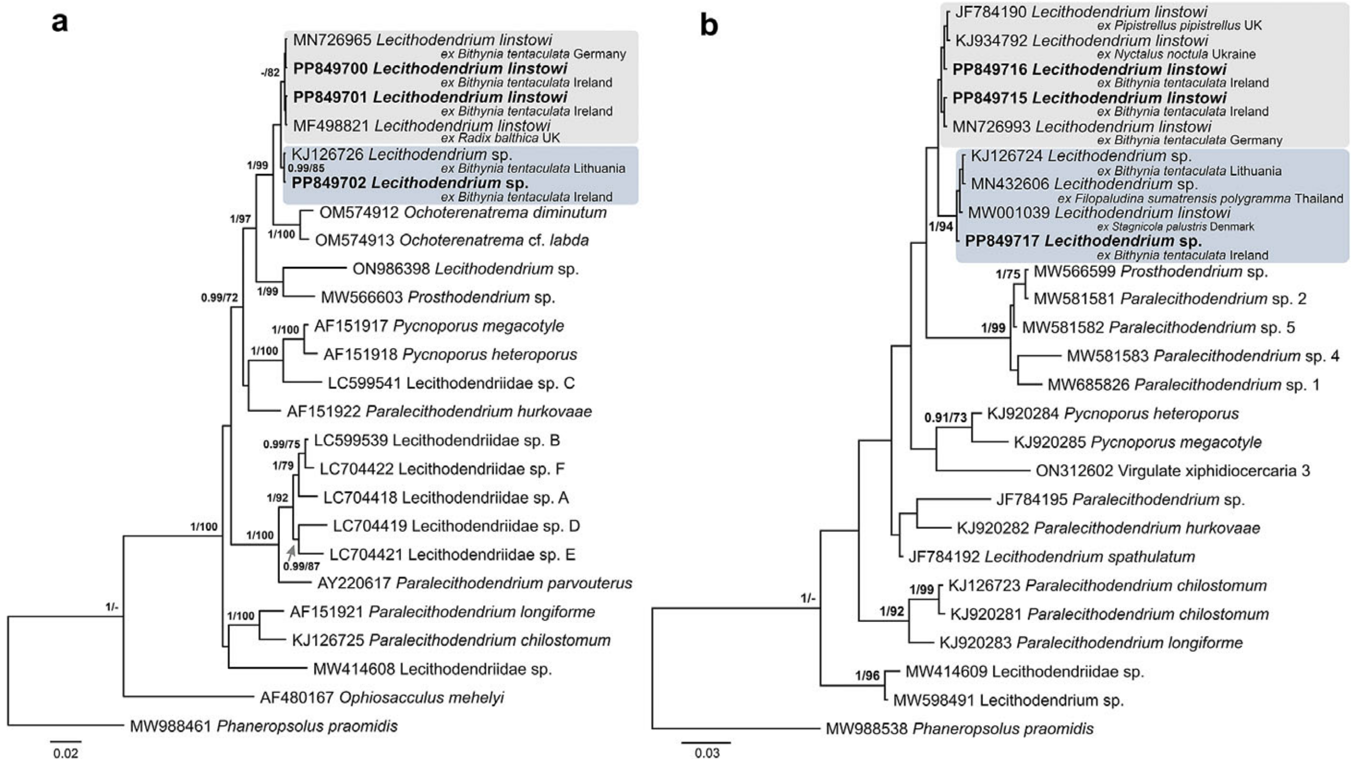

Figure 2. Phylogram resulting from Bayesian inference (BI) analysis based on the 28S rDNA sequences (A) and ITS2 sequences (B) for species of *Lecithodendrium* and of the Lecithodendriidae with nodal support values shown at the node as BI/ML (maximum likelihood). Only values > 0.90 (BI) and > 70 (ML) are displayed. Scale bar indicates the expected number of substitutions per site. Sequences generated in this study are in bold.

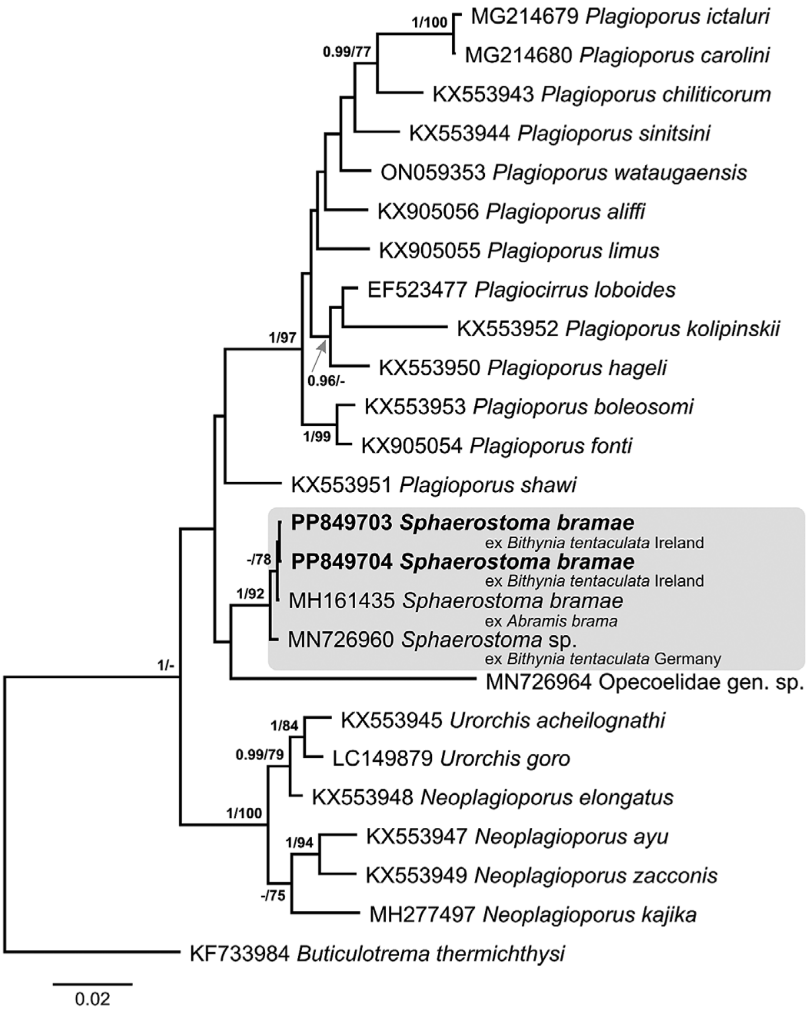

Figure 3. Phylogram resulting from Bayesian inference (BI) analysis based on the 28S rDNA sequences for species of *Sphaerostoma* and of the Opecoelidae with nodal support values shown at the node as BI/ML (maximum likelihood). Only values > 0.90 (BI) and > 70 (ML) are displayed. Scale bar indicates the expected number of substitutions per site. Sequences generated in this study are in bold.

Abramis brama (L.) and an unidentified isolate, *Sphaerostoma* sp. ex *B. tentaculata* in a well-supported clade (Figure 3). Sequences of our isolates were identical to the sequence of *S. bramae* ex *A.brama*, whereas the sequence of the unidentified isolate, *Sphaerostoma* sp. differed by 0.25% (3 nt). The ITS2 sequence comparison demonstrated that our sequences and the sequence of the same isolate of *Sphaerostoma* sp. (MN726988) available in GenBank were identical. Based on these results we suggest that our species and the unidentified isolate of *Sphaerostoma*, *Sphaerostoma* sp. are conspecific with *S. bramae*. Thus, a single species of *Sphaerostoma* was detected ex *B. tentaculata* in the present study.

Both 28S rDNA and *cox*1 phylogenetic analyses including representatives of the Opistorchiidae demonstrated that our isolates belong to one species of the genus *Metorchis* Looss, 1899 and were identified to the species level as *M. xanthosomus* (Creplin, 1846) (Figures 4A, B). The 28S rDNA sequences of our isolates and the isolate of *M. xanthosomus* ex *Fulica atra* L. were identical and differed from the sequence of an unidentified isolate, Opisthorchiidae gen. sp. by 0.1% (1 nt). The intraspecific divergence between *cox*1 sequences was low and ranged between 0.1 and 0.3% (1–3 nt).

Phylogenetic analyses of the Lissorchiidae based on both 28S rDNA and ITS2 sequences demonstrated that the isolates collected in the present study represented two genera and were identified to the species level (Figure 5A, B). Two cercariaeum isolates of *Asymphylodora* Looss, 1899 (PP849710 and PP849711) clustered with sequences of isolates of *A. progenetica* ex *B. tentaculata* and with a sequence of an unidentified isolate of *Asymphylodora* sp. ex *B. tentaculata* in a well-supported clade. The sequence divergence in this clade was low, 0–0.1% (0–1 nt). The 28S rDNA sequence of the metacercarial isolate of *Parasymphylodora* Szidat, 1943 clustered with a sequence of *P. parasquamosa* ex *B. tentaculata*; the sequences were identical. The ITS2 sequences of *A. progenetica* were identical as well as the ITS2 sequences of *P. parasquamosa*. Thus, two species of lissorchiids belonging to two genera were found in our study parasitising *B. tentaculata* in Ireland.

The 28S rDNA phylogeny of available sequences of the Psilostomidae demonstrated that the sequences of three isolates generated in the present study grouped with the representatives of *Sphaeridiotrema* Odhner, 1913 in a well-supported clade (Figure 6A). Within this clade, our isolates clustered with two unidentified isolates, *Sphaeridiotrema* sp. ex *B. tentaculata* and an isolate of *Sphaeridiotrema aziaticum* Kalinina, Tatonova & Besprozvannykh, 2022 ex *Gallus gallus* (L.) f. dom. The latter sequence differs from other identical sequences in the same clade by 0.1% (1 nt). Three *nad*1 sequences generated for our isolates of *Sphaeridiotrema* sp. were identical. There were no *nad*1 or any other mitochondrial

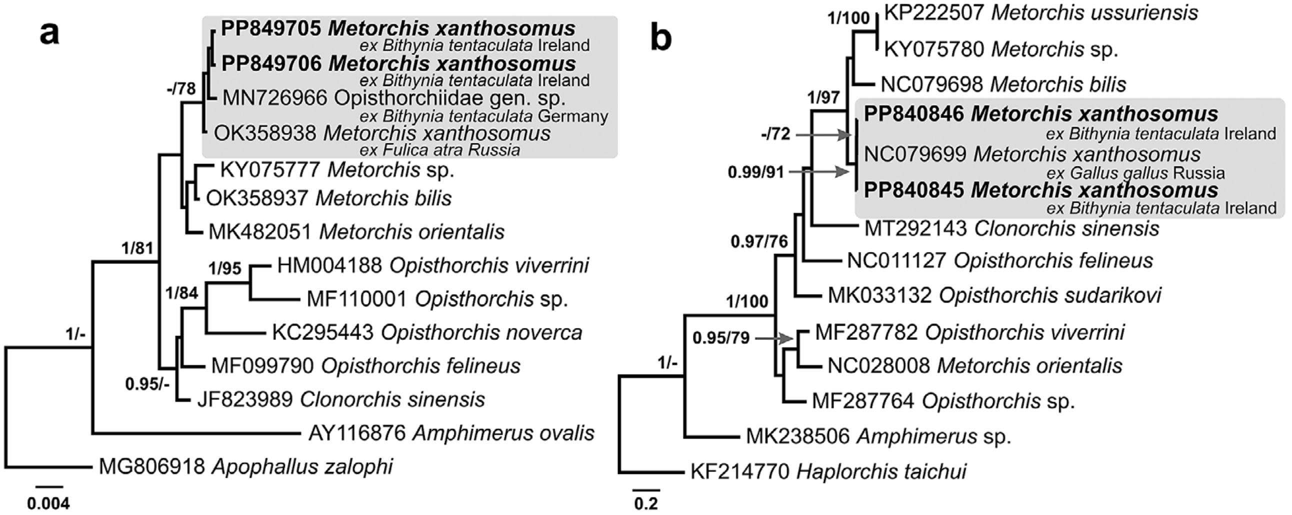

Figure 4. Phylogram resulting from Bayesian inference (BI) analysis based on the 28S rDNA sequences (A) and partial *cox*1 sequences (B) for species of *Metorchis* and the Opisthorchiidae with nodal support values shown at the node as BI/ML (maximum likelihood). Only values > 0.90 (BI) and > 70 (ML) are displayed. Scale bar indicates the expected number of substitutions per site. Sequences generated in this study are in bold.

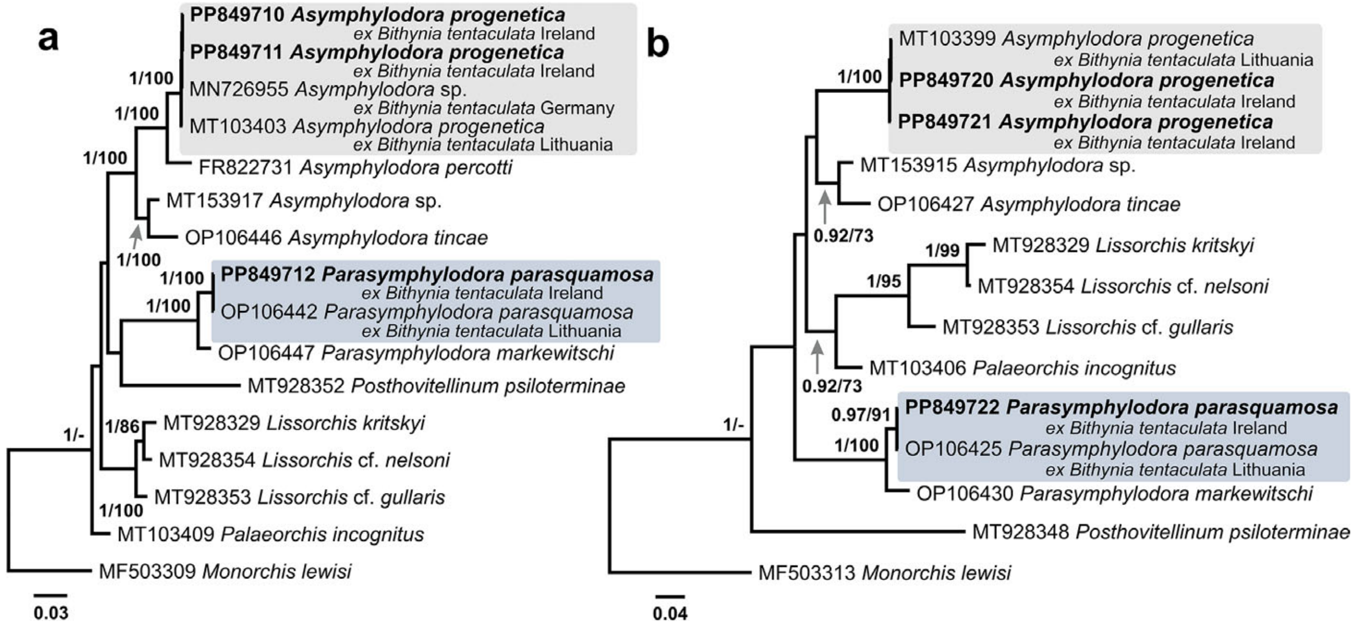

Figure 5. Phylogram resulting from Bayesian inference (BI) analysis based on the 28S rDNA sequences (A) and ITS2 sequences (B) for species of *Asymphylodora*, *Parasymphylodora*, and the Lissorchiidae with nodal support values shown at the node as BI/ML (maximum likelihood). Only values > 0.90 (BI) and > 70 (ML) are displayed. Scale bar indicates the expected number of substitutions per site. Sequences generated in this study are in bold.

sequences of *Sp. aziaticum* available for comparison; however, our sequences showed 0.2% (1 nt) difference from the *nad*1 sequence of an unidentified *Sphaeridiotrema* sp. ex *B. tentaculata* collected in Germany (MN720149) and 25.3% (110 nt) difference from sequences of *Sphaeridiotrema pseudoglobulus* McLaughlin, Scott & Huffman, 1993 ex *Aythya affinis* (Eyton, TC 1838) in the USA (MW963182). Thus, a single species of psilostomids was detected in our study.

In the phylogenetic analysis for the Notocotylidae based on the 28S rDNA sequences, our newly sequenced isolate (PP849713) clustered with two sequences of unidentified isolates, *Notocotylus* sp. ex *B. tentaculata* in a well-supported clade with the type species of the genus, *Notocotylus attenuatus* (Rudolphi, 1809) (Figure 6B). The three sequences were identical and differed from the closely related *N. attenuatus* by 0.3% (3 nt). A single species of notocotylids was detected from *B. tentaculata* in our study.

Thus, our DNA sequence data analyses identified nine species of trematodes, namely *C. prussica*, *L. linstowi*, *Lecithodendrium* sp., *A. progenetica*, *P. paraquamosa*, *S. bramae*, *M. xanthosomus*, *Sphaeridiotrema* sp., and *Notocotylus* sp. parasitising snails of *B. tentaculata* in Ireland.

Taxonomic summary and morphological descriptions

Cyathocotylidae Mühling, 1898

Cyathocotyle prussica Mühling, 1896

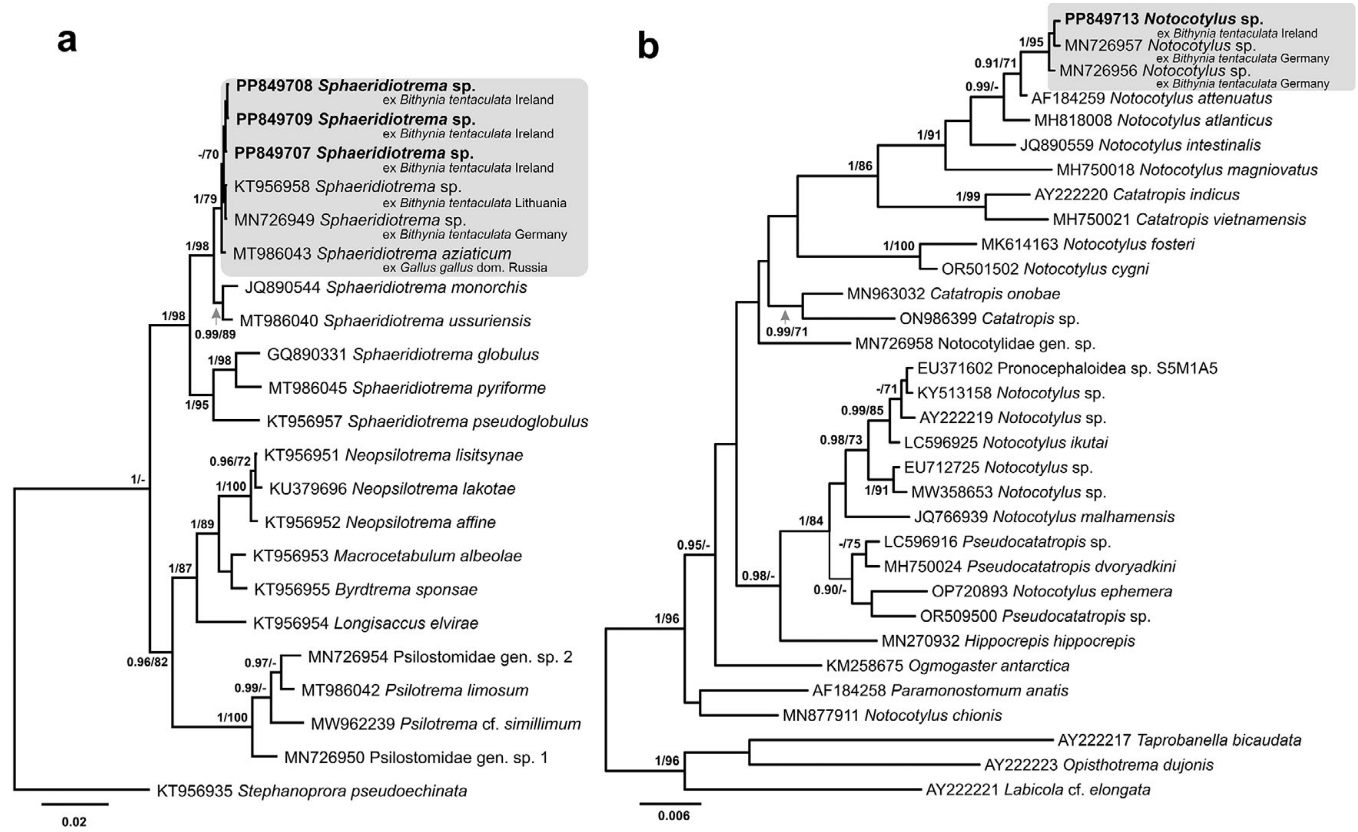

Figure 6. Phylogram resulting from Bayesian inference (BI) analysis based on the 28S rDNA sequences for species of *Sphaeridiotrema* and of the Psilostomidae (A) and for species of *Notocotylus* and of the Notocotylidae (B) with nodal support values shown at the node as BI/ML (maximum likelihood). Only values > 0.90 (BI) and > 70 (ML) are displayed. Scale bar indicates the expected number of substitutions per site. Sequences generated in this study are in bold.

First intermediate host: *Bithynia tentaculata* (L.) (Gastropoda, Littorinimorpha).

Site of infection: hepatopancreas.

Locality: Loughrea, Ireland.

Prevalence: 0.6% (2 of 320 snails).

Representative DNA sequences: PP849699 (28S), PP849714 (ITS1-5.8S-ITS2) and PP840844 (*cox*1).

Voucher material: cercariae (isogenophores) in ethanol in one vial (Coll. No. NMINH: 2024.5.1., coll. 27.vii. 2019).

Cercaria (Figures 7 A, B)

[Description and measurements based on four live specimens.] Body oval, with maximum width at its mid-level, 170–221 × 120–141 (185 × 131). Anterior organ oval, with sub-terminal mouth opening, 50–71 × 47–57 (61 × 51). Ventral sucker absent. Prepharynx short, distinct, of c. half-length of pharynx, 7–11 (10). Pharynx well-developed, oval to pear-shaped, 16–21 × 18–19 (19 × 19). Oesophagus very short, shorter than prepharynx. Intestinal bifurcation just posterior to pharynx. Caeca well-developed, conspicuous, with wide lumen and undulating edges, reaching back to junction of body with tail stem. Cystogenous gland cells in lateral part of body, posterior to anterior organ, extending to posterior extremity of body. Penetration gland cells of different size, with short ducts, of at least four pairs, with fine granular content and nucleus, surrounding anterior organ and reaching back to level of pharynx or slightly beyond; ducts opening anterolaterally to anterior organ. Genital anlagen just anterior to excretory vesicle, 22–27 × 22–31 (25 × 27). Excretory vesicle transversely oval, thin-walled. Main excretory vessels ascending from excretory vesicle in two pairs, median pair uniting at c. mid-body to a short channel that bifurcates and fuses with the ascending lateral vessels in the first half of body. Excretory pore with Cort's isle, running through tail stem and opening at tips of furca. Flame cell formula not observed. Tail bifurcate; tail stem, 285–354 × 40–55 (324 × 46), longer than body, TSL/BL ratio 1:1.49–1.90 (1.71). Furca with sharply pointed tips, without fin-folds, 254–295 × 21–29 (273 × 27); slightly shorter than tail stem, TSL/FL ratio 1:1.12–1.36 (1.21).

Body spination: Circumoral spines surrounding the first half of anterior organ, larger than tegumental spines, of c. 10 rows. Tegumental spines small, slender, with sharp tip, backwards oriented, longer in forebody, becoming shorter posteriorly, distributed on whole body, only ventral area delimited by median excretory vessels devoid of spines. Very fine, slender spines on tail stem and furca.

Measurements of cercariae fixed in ethanol (based on 11 specimens; not all specimens contributed a data point to all metrical variables): Body 162–218 × 78–106 (185 × 95). Oral sucker 31–53 × 35–46 (44 × 40). Tail stem 299–325 × 29–39 (312 × 35). Furca 202–261 × 24–25 (228 × 25). TSL/BL ratio 1:1.39–1.99 (1.66). TSL/FL ratio 1.28–1.57 (1.39).

Remarks

The present cercariae correspond well in morphology to *Cyathocotyle* Mühling, 1896 in the presence of a characteristic excretory

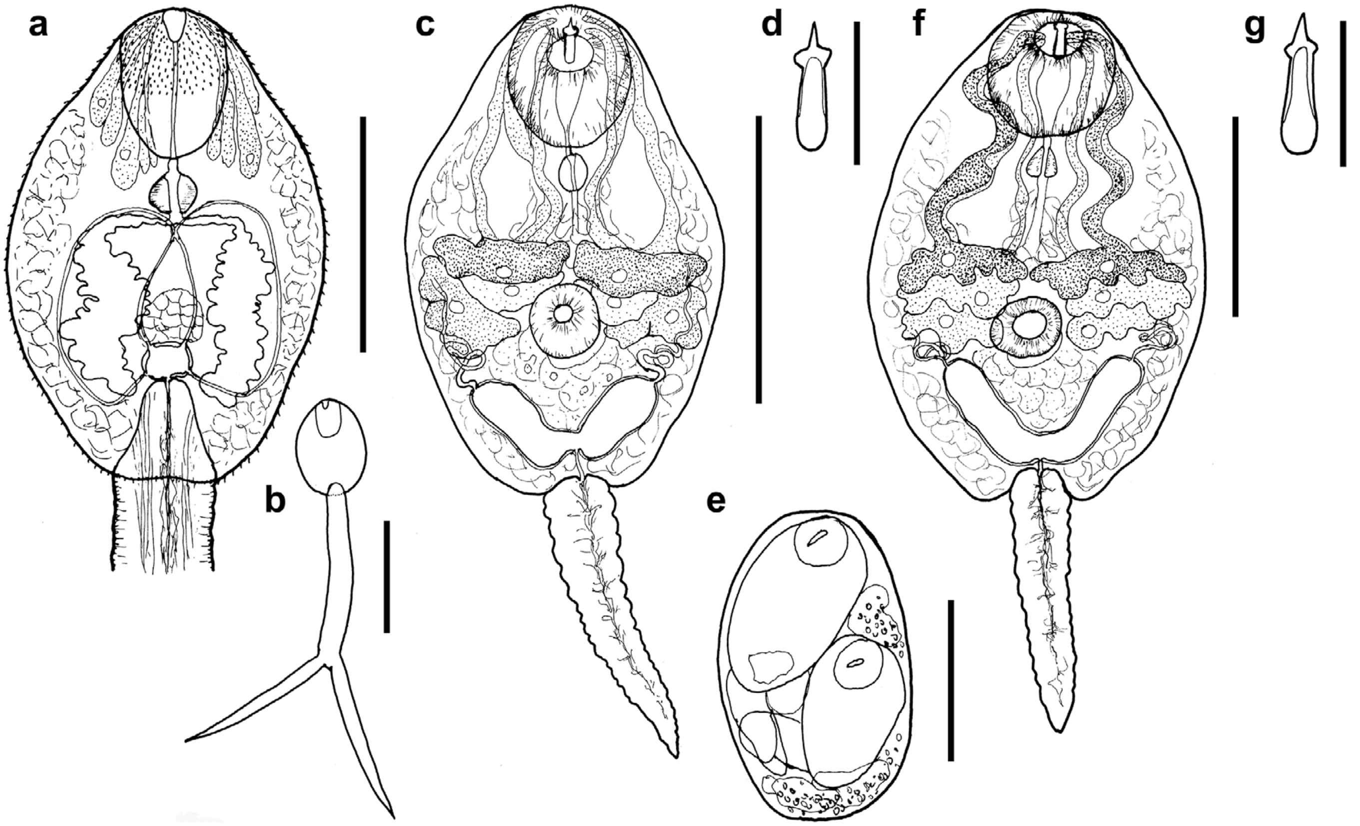

Figure 7. Morphology of trematodes ex *Bithynia tentaculata.* (A, B) *Cyathocotyle prussica* (A, ventral view of body; B, total view); (C–E) *Lecithodendrium linstowi* (C, total view; D, stylet; E, sporocyst); (F, G) *Lecithodendrium* sp. (F, total view; G, stylet). Scale bars: A, C, E, 100 μm; B, 200 μm; D, G, 20 μm; F, 50 μm.

system with main collecting vessels united in a central anastomosis, a forked tail, and the absence of a ventral sucker (Yamaguti 1975). Schwelm *et al.* (2020) summarised that nine species of cyathocotylids have been reported from *B. tentaculata* in Europe (*C. bithyniae* Sudarikov, 1974, *C. bushiensis* (Khan, 1962), *C. prussica*, *Holostephanus cobitidis* Opravilová, 1968, *H. curonensis* (Szidat, 1933), *H. dubinini* Vojtek & Vojtková, 1968, *H. luehei* Szidat, 1936, *H. volgensis* (Sudarikov, 1962), *Prohemistomum vivax* (Sonsino, 1892)) and added two more species, *Cyathocotyle* sp. 1 and *Cyathotocyle* sp. 2, of which we consider *Cyathocotyle* sp. 1 conspecific with our material (see above). The morphology of our cercariae agrees well with that of *Cyathocotyle* sp. 1 of Schwelm *et al.* (2020) in possessing ten rows of postoral spines (vs. 10–12), a short but distinct oesophagus, furcae without fin-folds, and conspicuous caeca with quite wide lumen and undulating edges. Also, the material overlaps in dimensions, although our cercariae exhibit higher minima and maxima for body, tail stem, furca, and anterior organ size. The life cycle of *C. prussica* was described by Mathias (1935) (originally as *C. gravieri* Mathias, 1935). The dimensions of our cercariae agree well with those provided by Mathias (1935). Kanev (1984) completed the life cycle of *C. prussica* experimentally, based on material of cercariae from naturally infected *B. tentaculata* in Bulgaria. The dimensions of our material overlap with those of Kanev (1984), although the body of our cercariae is larger (162–218 vs. 136–169). Our material of *C. prussica* differs from *C. bithyniae* of Niewiadomska (1980) in the absence of fin-folds on furca (vs. small triangular fin-folds at the tips of furca), in a larger body (162–218 vs. 161–187) and longer tail stem (299–325 vs. 192–204) and furca (202–261 vs. 119–153). From *C. bushiensis* of Khan (1962) our cercariae differ in the number and arrangement of penetration gland cells (most probably four, reaching up to pharynx or slightly beyond vs. numerous, in three groups reaching to caeca, 'between the intestinal caeca'), in a larger body (162–218 vs. 103–133), in a longer and narrower tail stem (299–325 × 29–39 vs. 266–286 × 40–56), i.e. the tail-stem of *C. bushiensis* appears as more robust and wide in relation to body. In Europe, *C. prussica* was much more commonly recorded as adults from waterfowl or as metacercariae in fishes or frogs (Razmashkin 1975; Sitko *et al.* 2006; Sulgostowska 2007; Rząd *et al.* 2013; Kvach *et al.* 2016). The first DNA sequences were obtained from metacercariae ex *Gasterosteus aculeatus* in Germany by Locke *et al.* (2018); however, no sequences of adults exist.

Lecithodendriidae Lühe, 1901

Lecithodendrium linstowi Dollfus, 1931

First intermediate host: Bithynia tentaculata (L.) (Gastropoda, Littorinimorpha).

Site of infection: hepatopancreas.

Locality: Lough Corrib, Ireland.

Prevalence: 1.6% (2 of 125 snails).

Representative DNA sequences: PP849700, PP849701 (28S), PP849715, PP849716 (ITS2), PP844658, PP844659 (*cox*1).

Voucher material: cercariae (isogenophores) in ethanol in two vials (Coll. No. NMINH: 2024.5.2., NMINH: 2024.5.3., coll. 31.vii. 2019).

Cercaria (Figures 7C–E)

[Description and measurements based on four live specimens.] Body oval, wider in anterior half, maximum width at its mid-level; 140–163 × 66–114 (151 × 93). Tegumental spines fine, dense, backwards directed, on whole body. Oral sucker well-developed, muscular, rounded, with sub-terminal mouth opening, 30–48 × 24–42 (42 × 36). Stylet dorsal to mouth opening, robust, sharply pointed, with well-developed anterior lateral thickening of similar width as base, base well sclerotised, 17–19 × 4–5 (18 × 5). Virgula absent. Ventral sucker well-developed, rounded, just postequatorial, 18–23 × 15–30 (21 × 23). Ventral sucker smaller than oral sucker, VSW/OSW 1:0.53–0.77 (1:0.61). Prepharynx very short, shorter than pharynx. Pharynx rounded to oval, feebly muscular, 8–17 × 7–14 (12 × 11). Oesophagus short, narrow. Intestinal bifurcation and caeca not observed. Cystogenous gland cells throughout body, numerous, delicate, transparent. Penetration gland cells large, of three pairs, with large nuclei, first pair anterior to ventral sucker, with coarse granular content; second pair anterolateral to ventral sucker, with almost transparent content; third pair lateral to ventral sucker, with fine granular content; ducts narrow, passing laterally and medially in body, opening anterolaterally to stylet. Genital anlagen just posterior to ventral sucker. Excretory vesicle V-shaped, thin-walled, arms extending up to posterior level of ventral sucker, 37 long. Ascending excretory vessels forming a glomerulus on both sides posterior to penetration gland cells. Flame cell formula not observed. Tail simple, contractile, with slightly undulating margins, tapered distally, 70–108 × 16–25 (85 × 20). Tail shorter than body, TL/BL ratio 1:0.48–0.76 (0.57).

Measurements of cercariae fixed in ethanol (based on 18 specimens; not all specimens contributed a data point to all metrical variables): Body 93–135 × 48–89 (111 × 66). Oral sucker 21–35 × 20–39 (28 × 31). Ventral sucker 15–20 × 17–24 (28 × 31). VSW/OSW 1:0.51–0.77 (1:0.87). Stylet 11–17 × 3–4 (15 × 4). Pharynx 8 × 7–8. Tail 71–120 × 13–18 (96 × 15). TL/BL ratio 1:0.73–114 (0.87).

Sporocyst (Figure 7E)

[Based on four live specimens.] Body small, oval, 162–192 × 103–122 (179 × 110); containing 1–2 cercariae of advanced development.

Remarks

The morphology of the present cercariae agrees well with that of the genus *Lecithodendrium* Looss, 1896 in the presence of a stylet, a V-shaped excretory vesicle and penetration gland cells anterolateral to ventral sucker (Kudlai *et al.* 2015). The virgula, which was considered the most typical character for the Lecithodendriidae and other families considered as the 'virgulate group', is absent; this was discussed recently by Kudlai *et al.* (2015) and Enabulele *et al.* (2018), who hinted that the virgula cannot be considered a synapomorphic feature any longer. Cercariae of *L. linstowi* were recorded by Enabulele *et al.* (2018) from the United Kingdom and by Schwelm *et al.* (2020) from Germany who both provided a morphological characterisation linked to DNA sequences. While Schwelm *et al.* (2020) recorded *B. tentaculata* as first intermediate host, Enabulele *et al.* (2018) reported *Radix balthica*, which is peculiar. The present cercariae correspond well in morphology and in dimensions with the description of Schwelm *et al.* (2020), exhibiting lower minima for body size and a higher maximum for tail length. However, we cannot reliably compare our material with that of Enabulele *et al.* (2018), as the description is quite general, and the figures do not reveal any details useful for identification. Moreover, the cercariae of Enabulele *et al.* (2018) are all conspicuously smaller than our material and that of Schwelm *et al.* (2020), raising doubts on fixation methods. Adults of *Lecithodendrium linstowi* are among the most common parasites of a wide spectrum of bats in Eurasia (Esteban *et al.* 2001; Lord *et al.* 2012; Horvat *et al.* 2017). In Europe, they are widely distributed, and Kudlai *et al.* (2015) pointed out that they found identical *L. linstowi* from different bat species in the United Kingdom and in Ukraine. Aquatic insect larvae are used as second intermediate hosts, which can ensure wide dispersion when they metamorphose to imagoes (Kudlai *et al.* 2015; Enabulele *et al.* 2018).

***Lecithodendrium* sp.**

First intermediate host: Bithynia tentaculata (L.) (Gastropoda, Littorinimorpha).

Site of infection: hepatopancreas.

Locality: Lough Corrib, Ireland.

Prevalence: 0.8% (1 of 125 snails).

Representative DNA sequences: PP849702 (28S), PP849717 (ITS2), and PP844660 (*cox*1).

Voucher material: cercariae (isogenophores) in ethanol in one vial (Coll. No. NMINH: 2024.5.4., coll. 31.vii. 2019).

Cercaria (Figures 7F, G)

[Description and measurements based on two live specimens.] Body elongate-oval, with maximum width at its mid-level, 126–134 × 57–106 (130 × 75). Tegumental spines minute, dense, on whole body. Oral sucker well-developed, muscular, rounded to oval, with sub-terminal mouth opening, 36–39 × 31. Stylet dorsal to mouth opening, robust, relatively large, sharply pointed, with well-developed anterior lateral thickening, base well sclerotised, 18–19 × 5. Ventral sucker well-developed, muscular, rounded to transversely oval, postequatorial, 18–25 × 20–22 (22 × 21). Ventral sucker smaller than oral sucker, VSW/OSW 1:0.65. Prepharynx short. Pharynx small, feebly muscular, 7 × 6. Oesophagus, intestinal bifurcation, and caeca not observed. Cystogenous gland cells numerous, delicate, transparent. Penetration gland cells of three pairs, quite large, first pair anterior to ventral sucker, with darkish, coarse granular content; second and third pair smaller, anterolateral to ventral sucker, with fine granular content; ducts passing laterally and medially in body, opening anterolaterally to stylet. Genital anlagen just posterior to ventral sucker. Excretory vesicle V-shaped, thin-walled, arms extending up to one third of body length, 33–37 long. Ascending excretory vessels forming a glomerulus laterally, posterior to penetration gland cells. Flame cell formula not observed. Tail simple, contractile, with undulating margins, shorter than body, tapered distally, 86–141 × 16–18 (105 × 17).

Measurements of cercariae fixed in ethanol (based on 22 specimens; not all specimens contributed a data point to all metrical variables): Body 101–137 × 45–96 (124 × 65). Oral sucker 21–36 × 23–39 (29 × 30). Ventral sucker 15–21 × 16–23 (18 × 20). VSW/OSW 1:0.41–0.91 (1:0.64). Stylet 12–17 × 3–4 (16 × 4). Pharynx 8–9 × 7–8 (8 × 7). Tail 60–138 × 12–18 (93 × 15). TL/BL ratio 1:0.49–1.15 (0.74).

Remarks

The present cercariae agree well in their morphology with that of the genus *Lecithodendrium* in characters as stated above (Kudlai *et al.*

2015). The present cercariae of *Lecithodendrium* sp. are very similar in morphology and dimensions to *L. linstowi*, but they differ in the shape of the stylet (the lateral thickening is less prominent in *Lecithodendrium* sp.; see Figures 7D and 7G) and in the content of penetration gland cells (second and third pair with the same fine granular content vs. second pair with finer, more transparent content than third pair). The present cercariae are identical with those ex *B. tentaculata* from Lithuania of Kudlai *et al.* (2015) (see molecular part), which were originally described as *Cercaria helvetica* XII by Dubois (1928) from Switzerland and later reported from the Curonian Bay in Lithuania by Bykovskaya-Pavlovskaya & Kulakova (1971). Another recent record is from *B. tentaculata* in Denmark, where *Lecithodendrium* sp. was reported as the most common by Duan *et al.* (2021), who however, erroneously identified them as *L. linstowi*. It seems that this species is widely distributed and common in Europe as *L. linstowi*, but because they are very closely related, careful identification via an integrative approach is necessary.

Opecoelidae Ozaki, 1925

Sphaerostoma bramae (Müller, 1776) Lühe, 1909

First intermediate host: Bithynia tentaculata (L.) (Gastropoda, Littorinimorpha).

Site of infection: hepatopancreas.

Locality: Lough Corrib, Ireland.

Prevalence: 1.6% (2 of 125 snails).

Representative DNA sequences: PP849703, PP849704 (28S) and PP849718, PP849719 (ITS2).

Voucher material: cercariae (isogenophores) in ethanol in two vials (Coll. No. NMINH: 2024.5.5., NMINH: 2024.5.6., coll. 31.vii. 2019).

Cercaria (Figures 8A, B)

[Description and measurements based on two live specimens.] Cotylomicrocercous cercaria. Body elongate-oval, maximum width at anterior level of ventral sucker; 295–331 × 126–155 (313 × 141). Tegument thick, devoid of spines; numerous sensory papillae on body surface and on both suckers. Oral sucker well-developed, muscular, sub-spherical, with sub-terminal mouth opening, with 3–5 rows of minute spines, 61–62 × 55–61 (61 × 58). Stylet dorsal to mouth opening, minute, pointed, with slight anterior lateral thickening, base well sclerotised, 10–11 × 5. Ventral sucker large, well-developed, muscular, sub-spherical, just postequatorial, with c. three rows of minute spines, 67–72 × 73–75 (70 × 74). Ventral sucker larger than oral sucker, VSW/OSW 1:1.23–1.32 (1.27). Prepharynx long, narrow, curved. Pharynx well-developed, sub-spherical, muscular, 22 × 23. Oesophagus long, narrow, curved. Intestinal bifurcation anterior to ventral sucker. Caeca narrow, obscured by gland cells, extent not observed. Cystogenous gland cells small, numerous, delicate, transparent. Penetration gland cells small, of five pairs, with fine granular content, anterior to ventral sucker; ducts narrow, passing laterally in two strands each, opening anterolaterally to stylet. Genital anlagen of two, compact, transparent masses of cells, first anterodorsal to ventral sucker, second posterodorsal to ventral sucker. Excretory vesicle large, thick-walled, strawberry-shaped, filling most space of hindbody, 81–88 × 86–90 (84 × 88). Flame cell formula not observed. Cotylocercous tail simple, knob-like, with posterior concavity, 27–39 × 40–46 (33 × 43). Tail much shorter than body, TL/BL ratio 1:0.08–0.13 (0.11).

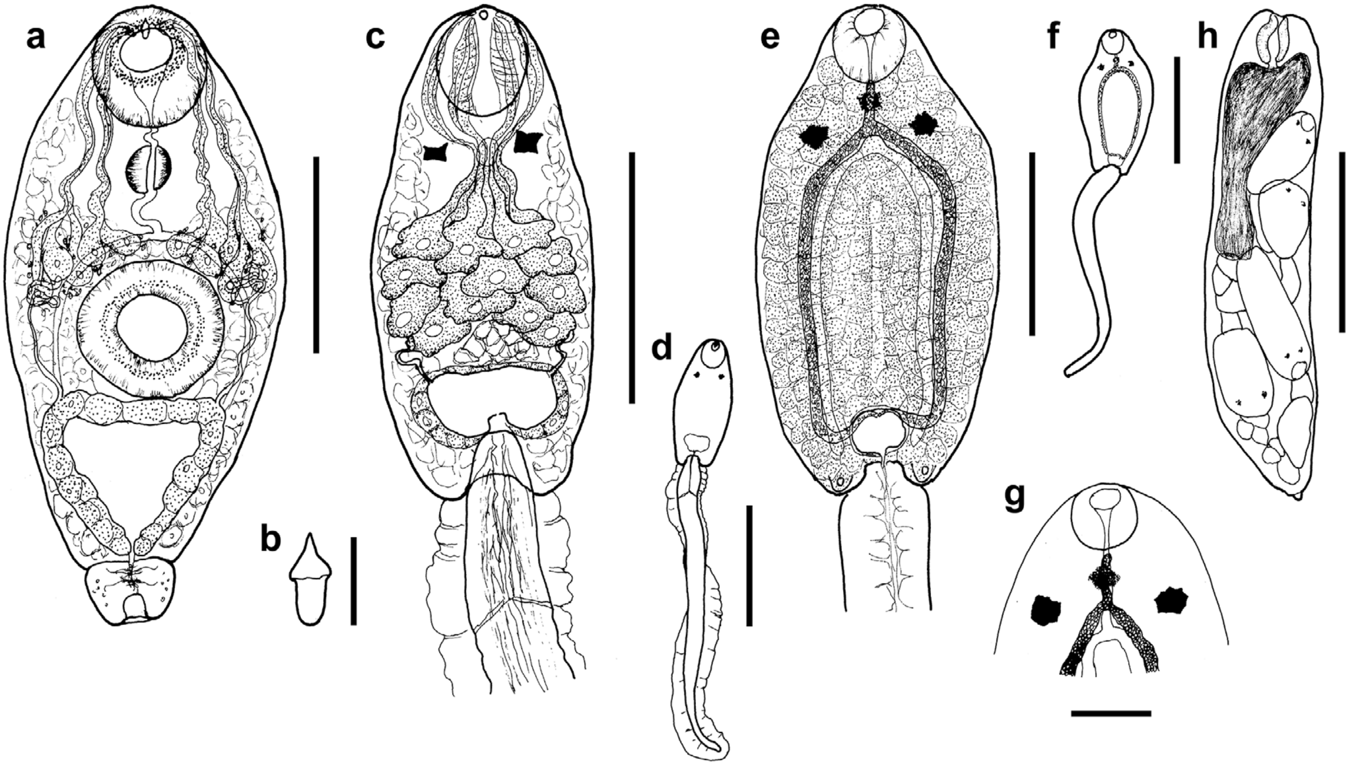

Figure 8. Morphology of trematodes ex *Bithynia tentaculata.* (A, B) *Sphaerostoma bramae* (A, total view; B, stylet); (C, D) *Metorchis xanthosomus* (C, ventral view of body; D, total view); (E–H) *Notocotylus* sp. (E, ventral view of body; F, total view; G, detail of eyespots and Yenchingensis-type of excretory system; H, redia). Scale bars: A, C, E, 100 μm; D, F, 200 μm; G, 50 μm; B, 10 μm; H, 400 μm.

Measurements of cercariae fixed in ethanol (based on 12 specimens; not all specimens contributed a data point to all metrical variables): Body 221–319 × 82–115 (274 × 99). Oral sucker 49–60 × 46–59 (55 × 50). Ventral sucker 54–77 × 55–71 (63 × 63). VSW/OSW 1:1.20–1.40 (1:1.27). Stylet 10–13 × 3–4 (11 × 3). Prepharynx 18–24 (20) long. Pharynx 19–25 × 16–28 (23 × 22). Excretory vesicle 64–96 × 55–75 (78 × 65). Tail 26–45 × 29–37 (35 × 34). TL/BL ratio 1:0.11–0.15 (0.13).

Remarks

The present cercariae correspond well in morphology to that of *Sphaerostoma* Rudolphi, 1809 in the presence of a short, cotylocercous tail, a minute stylet, well-developed, muscular suckers and pharynx, and the ventral sucker being larger than the oral sucker (Pike 1967; Bykhovskaya-Pavlovskaya & Kulakova 1971). In morphology and dimensions, our cercariae correspond to those of *Sphaerostoma* sp. of Schwelm *et al.* (2020), apart from the number of penetration gland cells, of which we observed five pairs (vs. four pairs). This difference could be due to the fact that the penetration gland cells are difficult to observe as they are partly obscured by cystogenous gland cells. Most of the other authors providing a description of *S. bramae* (Wesenberg-Lund 1934; Wikgren 1956; Chernogorenko-Bidulina & Bliznyuk 1960; Zdun 1961; Pike 1967; Bykhovskaya-Pavlovskaya & Kulakova 1971) reported four pairs, except for Našincová (1992), who observed five pairs in her record of *Sphaerostoma* sp. from *B. tentaculata* in the Czech Republic. Our material of cercariae overlaps in dimensions with those of the cercariae provided by the above authors (partly reported as *Cercaria micrura* Filippi, 1857); however, some authors (Wesenberg-Lund 1934; Wikgren 1956; Pike 1967) give much higher maxima of body size (up to 450 μm). Also, a differing number of tegumental spines on suckers has been reported, i.e. three rows of spines on both suckers by Bykhovskaya-Pavlovskaya & Kulakova (1971) and two and three rows, respectively by Pike (1967), while we observed 3–5 rows on the oral sucker and three rows of spines on the ventral sucker. Koval (1971) and Našincová (1992) doubted that the above-mentioned records of cercariae of *S. bramae* would all belong to one species only. Indeed, the wide size range and the differences in morphology mentioned could indicate that more species are involved. *Sphaerostoma bramae* is a common fish parasite in Europe, and its cercariae were frequently reported ex *B. tentaculata* in Denmark, Finland, Great Britain, Lithuania, Netherlands, Russia, and Ukraine (Wesenberg-Lund 1934; Wikgren 1956; Chernogorenko-Bidulina 1958; Ginetsinskaya 1959; Zdun 1961; Pike 1967; Bykhovskaya-Pavlovskaya & Kulakova 1971; Keulen 1981; Petkevičiūtė et al. 1995; Schwelm *et al.* 2020). Moreover, Chernogorenko-Bidulina (1958) and Zdun (1961) reported apart from *B. tentaculata* also *Lithoglyphus naticoides* (Pfeiffer) and *Theodoxus fluviatilis* (L.) as first intermediate hosts. In Europe, there were four other species of *Sphaerostoma* recorded, *S. globiporum* (Rudolphi, 1802), *S. minus* Szidat, 1944, *S. romanicum* Dinulescu, 1942, and *S. salmonis* Slusarski, 1958, but their life cycles are thus far unknown. From Ireland, adults of *S. bramae* were reported by Vickers (1951) and Kane (1966) from bream, rudd, and pike; in the United Kingdom, *S. bramae* was reported from fishes in several places (Nicoll 1924; Kennedy 1974), indicating that it is a common species in Western Europe.

Opisthorchiidae Looss, 1899
***Metorchis xanthosomus* (Creplin, 1846) Braun, 1902**

First intermediate host: Bithynia tentaculata (L.) (Gastropoda, Littorinimorpha).

Site of infection: hepatopancreas.

Locality: Lough Corrib, Ireland.

Prevalence: 2.4% (3 of 125 snails).

Representative DNA sequences: PP849705, PP849706 (28S) and PP840845, PP840846 (*cox*1).

Voucher material: cercariae (isogenophores) in ethanol in two vials (Coll. No. NMINH: 2024.5.7., NMINH: 2024.5.8., coll. 31.vii. 2019).

Cercaria (Figures 8C, D)

[Description and measurements are based on seven live specimens. Not all specimens contributed a data point to all metrical variables.] Pleurolophocercaria. Body elongate-oval, with maximum width in mid-body, with brownish pigment located in body posterior to oral sucker; 150–264 × 61–102 (203 × 85). Tegument thick. Tegumental spines minute, arranged in rows, extending from oral sucker level up to eyespots laterally. Eyespots conspicuous, with black pigment, crescent to square-shaped, in first half of forebody, 6–9 × 8–13 (8 × 10). Oral sucker sub-spherical, muscular, 38–56 × 35–46 (43 × 39), with sub-terminal mouth opening and protrusible anterodorsal tip. Anterior part of protrusible tip armed with spines larger than tegumental spines, arranged in three rows with seven, six, and four spines. Ventral sucker rudimentary, postequatorial. Prepharynx very short, 5 long. Pharynx small, feebly muscular, posterior to oral sucker, 10–12 × 11–13 (11 × 12). Oesophagus, intestinal bifurcation, and caeca not observed, obscured by ducts of penetration gland cells. Cystogenous gland cells large, with coarse granular content, posterior to eyespots, extending to posterior extremity of body. Penetration gland cells large, of six pairs, with coarse granular content, in median part of body; ducts wide, median, dividing posterior to oral sucker into two strands on each side, opening anterolaterally to tip of oral sucker. Genital anlagen just anterior to excretory vesicle, of transparent, compact cells. Excretory vesicle large, rounded, thick-walled, 38 × 34. Flame cell formula not observed. Caudal excretory pore forked, opening in proximal fifth of tail. Tail, long, simple, contractile, with tapered tip, 382–455 × 23–24 (415 × 24). Tail longer than body, TL/BL ratio 1:1.57–2.55 (2.12). Tail with fin-folds; in distal half of tail, conspicuous dorso-ventral fin-fold, 22–33 (27) wide, surrounding tip of tail; proximal half of tail with a dorso-ventral dilatation of tegument (fin-fold-like), 12–23 (16) wide.

Measurements of cercariae fixed in ethanol (based on 18 specimens; not all specimens contributed a data point to all metrical variables): Body 161–203 × 50–71 (182 × 70). Oral sucker 28–43 × 27–37 (37 × 30). Eyespot 6–11 × 7–13 (8 × 9). Pharynx 8–10 × 7–10 (9 × 9). Excretory vesicle 33 × 36. Tail 314–387 × 21–36 (341 × 25). TL/BL ratio 1:1.55–2.24 (1.87). Width of dorso-ventral fin-fold of tail 6–8 (7). Width of dorso-ventral dilatation of tegument in proximal part of tail 11–18 (14).

Remarks

The morphology of the present cercariae corresponds well in characters with the genus *Metorchis* Looss, 1899, i.e. in the pleurolophocercaria-type with eyespots, a rudimentary ventral sucker and long tail with fin-folds (Heinemann 1937). However, because of close similarity to cercariae of *Opisthorchis*, a confirmation with molecular genetic methods is of vital importance.

In Europe, four species of *Metorchis* have been reported from *B. tentaculata*: *Metorchis bilis* (Braun, 1790), *M. intermedius* Heinemann, 1937, *M. xanthosomus*, and *Metorchis* sp. (summarised by Schwelm *et al.* 2020; see also Zdun 1961; Bykhovskaya-Pavlovskaya & Kulakova 1971; Cichy *et al.* 2011). As adults, there have been several known species of *Metorchis*, most of which are now considered synonyms either of *M. bilis* (Braun, 1790), (*M. albidus* (Braun, 1893), and *M. crassiusculus* (Rudolphi, 1809) or of *M. xanthosomus* (*M. intermedius*) (for details see Sitko *et al.* 2016), indicating the complex taxonomy within this genus. *Metorchis intermedius* was originally described by Heinemann (1937) from *B. tentaculata* in the Curonian Lagoon, and adults were obtained experimentally from ducks (*Anas boschas*). Heinemann (1937) considered the characters of his species close to *M. xanthosomus* (obtained previously by Szidat from the same place but not published) but described the species as new because of differences in body spination in adults. We are of the opinion that the worms of Heinemann (1937) are identical to those of *M. xanthosomus* as defined by Sitko *et al.* (2016) (extent of reproductive organs relative to body length). Our material of cercariae corresponds well in morphology and dimensions to those provided by Heinemann (1937), only we observed a differing number of penetration gland cells (six pairs vs. seven pairs). The present cercariae are similar to those of Opisthorchiidae gen. sp. of Schwelm *et al.* (2020): the dimensions of body overlap, the tail of our cercariae is slightly shorter (314–387 vs. 437–494), and the number of penetration gland cells corresponds (six pairs).

Species of *Metorchis* are parasites of birds and mammals, including man, and can cause serious chronic diseases (Sitko *et al.* 2016). *Metorchis bilis* is considered much more common in Europe than *M. xanthosomus*; their cercariae are morphologically very close, and they both use *B. tentaculata* as the first intermediate host (Mordvinov *et al.* 2012; Sitko *et al.* 2016). The two species differ in the definitive host spectrum: *M. xanthosomus* uses ducks and coots as definitive hosts, whereas *M. bilis* uses both mammals and birds (birds of prey and cormorants). Not only cercariae, but adults are also difficult to identify by morphology; therefore, the identity of opisthorchiid trematodes should always be verified by molecular genetic methods.

Psilostomidae Looss, 1900

***Sphaeridiotrema* sp.**

First intermediate host: Bithynia tentaculata (L.) (Gastropoda, Littorinimorpha).

Site of infection: mantle cavity.

Locality: Lough Corrib, Ireland.

Prevalence: 3.2% (4 of 125 snails).

Representative DNA sequences: PP849707, PP849708, PP849709 (28S) and PP856383, PP856384, PP856385 (*nad*1).

Voucher material: metacercariae (isogenophores) in ethanol in one vial (Coll. No. NMINH: 2024.5.9., coll. 31.vii. 2019).

Metacercaria

[Description and measurements based on two live specimens.] Metacercaria rounded, 130–142 × 122–143 (136 × 136). Cyst wall relatively thick, 7–8 (8), difficult to excyst. Oral sucker 50 × 51; ventral sucker 39 × 55; pharynx 31 × 21. Excretory vesicle inconspicuous, with dilated main collecting channels ascending to oral sucker. Conspicuous excretory granules c. 15–21 in number in main collecting channels, 9–17 × 7–10 (11 × 9).

Remarks

The present metacercariae were found encysted in the tissue of snails. They were identified based on results of phylogenetic analyses. When comparing their morphology with that of the members of the genus *Sphaeridiotrema* Odhner, 1913, they agree in the shape of the cyst, thickness of the wall, and the presence of conspicuous excretory granules in large collecting excretory channels (Yamaguti 1975; Kalinina *et al.* 2022); however, these characters are too general for any exact identification. Recently, Schwelm *et al.* (2020) recorded cercariae of *Sphaeridiotrema* sp. in Germany and summarised the occurrence of species of the genus *Sphaeridiotrema* in *B. tentaculata* in Europe: *S. globulus* being recorded in Bulgaria, Finland, Russia, and United Kingdom, and *Sphaeridiotrema* sp. of Tkach *et al.* (2016) from Lithuania (for detailed references see Schwelm *et al.* 2020). Based on molecular genetic results, our material is close to *S. aziaticum* Kalinina, Tatonova & Besprozvannykh, 2022 described from the Russian Far East, which is peculiar. To clarify this case, more sequences of members of the Psilostomidae from different geographical locations are necessary. Species of the genus *Sphaeridiotrema* are pathogenic parasites of waterfowl (anatids, charadriids). They are widespread in Europe, Asia, and North America (Kostadinova 2005; Bergame *et al.* 2011). As many of the bird hosts are migratory, they could ensure a potentially wide dispersion of the trematodes.

Lissorchiidae Magath, 1917

***Asymphylodora progenetica* Serkova & Bykhowskiy, 1940**

First and second intermediate host: Bithynia tentaculata (L.) (Gastropoda, Littorinimorpha).

Site of infection: hepatopancreas (cercariaeum), mantle cavity (metacercaria).

Locality: Lough Corrib, Ireland.

Prevalence: 1.6% (2 of 125 snails).

Representative DNA sequences: PP849710, PP849711 (28S), PP849720, PP849721 (ITS2), and PP840847, PP840848 (*cox*1).

Cercariaeum and metacercaria

No sufficiently representative morphological data linked to DNA sequences could be obtained for description of the tailless cercariaeum. Therefore, identification is based solely on molecular genetic data. For metacercariae, no DNA sequences could be obtained; neither was the material sufficient for morphology.

Remarks

In the present study, the cercarieum found in *B. tentaculata* was identical to those found in the same snail species in Germany and Lithuania by Schwelm *et al.* (2020) and Petkevičiūtė et al. (2020). Previously, *A. progenetica* has been recorded in the Curonian Lagoon and in the region of St. Petersburg, Russia (Niewiadomska 2003). Its life cycle, completed by Serkova & Bykhovsky (1940), is characterised by the presence of a progenetic metacercaria bearing eggs – we did observe such a specimen in the present study, but we failed to obtain DNA sequences. Recently, Petkevičiūtė et al. (2022) clarified the assignment of *A. progenetica* to the genus *Asymphylodora.* As before, it has been recognised as *Parasymphylodora* Szidat, 1943 by some authors (see Niewiadomska 2003; and for details, Petkevičiūtė et al. 2022). Thus far, no species of *Asymphylodora* have been reported from

Ireland, but there are records from fishes in the United Kingdom of *A. kubanica* Issaitschikov, 1923 and *A. tincae* (Modeer, 1790).

***Parasymphylodora parasquamosa* Kulakova, 1972**

Synonym: *Asymphylodora parasquamosa* (Kulakova, 1972)

First intermediate host: Bithynia tentaculata (L.) (Gastropoda, Littorinimorpha).

Site of infection: mantle cavity.

Locality: Lough Corrib, Ireland.

Prevalence: 0.8% (1 of 125 snails).

Representative DNA sequences: PP849712 (28S), PP849722 (ITS2), and PP840849 (*cox*1).

Metacercaria

No sufficiently representative morphological data could be obtained for description of the metacercaria, and identification is based solely on molecular genetic data.

Remarks

The present metacercariae found in *B. tentaculata* are identical to those recorded in Lithuania by Petkevičiūtė et al. (2022). By molecular genetic analysis, Petkevičiūtė et al. (2022) clarified that the present species belongs to the genus *Parasymphylodora* Szidat, 1943 and confirmed that *B. tentaculata* is used as the first and second intermediate host. *Parasymphylodora parasquamosa* has so far been reported to occur in Central and Eastern Europe (Niewiadomska 2003); thus, our present record extends its occurrence to Western Europe.

Notocotylidae Lühe, 1909

***Notocotylus* sp.**

First intermediate host: Bithynia tentaculata (L.) (Gastropoda, Littorinimorpha).

Site of infection: hepatopancreas.

Locality: Lough Corrib, Ireland.

Prevalence: 0.8% (1 of 125 snails).

Representative DNA sequences: PP849713 (28S).

Voucher material: cercariae (isogenophores) in ethanol in two vials (Coll. No. NMINH: 2024.5.10., coll. 31.vii. 2019).

Cercaria (Figures 8E–H)

[Description and measurements based on three live specimens.] Cercaria monostome, triocellate. Body elongate-oval, with maximum width at its mid-level, 210–259 × 115–145 (234 × 133). Body with dark brownish pigment; at its posterior extremity, two protrusible dorsal adhesive pockets located postero-laterally. Tegument devoid of spines. Eyespots three, with black pigment, two lateral ones posterior to oral sucker, median eyespot with less dense pigment just posterior to oral sucker (Figure 8G), 12–22 × 13–16 (16 × 14). Oral sucker muscular, rounded to oval, 35–46 × 31–37 (38 × 33). Pharynx and ventral sucker absent. Oesophagus short. Intestinal bifurcation just posterior to lateral eyespots. Caeca reaching to excretory vesicle. Cystogenous gland cells with dark content, densely filling whole body. Anlagen of reproductive organs in median line of body as a chain of transparent cells. Excretory vesicle thin-walled, rounded. Main collecting excretory channels filled with numerous, small, refractive excretory granules. Main collecting excretory channels ascending to anterior part of body, fusing at level of lateral eyespots, and forming a finger-like diverticulum reaching to the front of the median eyespot, just posterior to oral sucker (Figure 8G, Yenchingensis morphotype). Flame cells obscured by cystogenous gland cells; flame cell formula not elucidated. Tail long, simple, transparent, highly contractile, with tapered tip, 316–422 × 36–49 (373 × 43). Tail longer than body, TL/BL ratio 1:1.37–2.01 (1.67).

Measurements of cercariae fixed in ethanol (based on 14 specimens; not all specimens contributed a data point to all metrical variables): Body 186–263 × 82–143 (214 × 120). Oral sucker 32–41 × 33–43 (35 × 37). Eyespot 12–18 × 14–23 (14 × 18). Excretory vesicle 27–32 × 23–26 (29 × 29). Tail 202–363 × 36–49 (304 × 43). TL/BL ratio 1:0.91–1.95 (1.47).

Redia (Figure 8H)

Body elongate, with brownish pigment, without locomotory appendages; anterior extremity blunt, posterior extremity with a pointed tip. Pharynx muscular, relatively small. Sac-like intestine with brownish content, reaching up to mid-level of body. Containing cercariae of different stages of development.

Measurements of rediae fixed in ethanol (based on seven specimens): Body 568–779 × 173–202 (649 × 185). Pharynx 79–101 × 68–88 (89 × 75).

Remarks

The features of the present cercariae, which are the lack of ventral sucker and pharynx, the presence of three conspicuous eyespots, excretory system united behind oral sucker, dark pigment in whole body and prominent cystogenous gland cells, correspond well with the morphology of cercariae of the family Notocotylidae (Filimonova 1985; Gonchar *et al.* 2019). The present cercariae belong to the Yenchingensis morphotype (excretory system with a diverticulum in anterior part of fusion vs. the Monostomi and Imbricata morphotypes, see Gonchar *et al.* 2019). The Yenchingensis morphotype was observed in marine notocotylids using hydrobiid snails, but also in freshwater notocotylids using bithyniid snails as intermediate hosts (Gonchar *et al.* 2019). The taxonomy of the Notocotylidae is complex and controversial (Gonchar *et al.* 2019). Notocotylid cercariae are particularly difficult to distinguish morphologically, and in reality, they can only be reliably identified to family level, as their morphology is strikingly uniform, and observation is impeded by the dark pigment and cystogenous gland cells (Gonchar *et al.* 2019). From *B. tentaculata* in Europe, there have been several species of notocotylid cercariae reported: *Catatropis verrucosa* (Frölich, 1789), *N. imbricatus* (Looss, 1893), *N. parviovatus* Yamaguti, 1934, *N. ponticus* Tschiaberashwili, 1964, *N. triserialis* Diesing, 1839 (Filimonova 1985; Kanev *et al.* 1994; Morley *et al.* 2004), and two unidentified species with DNA sequences provided by Schwelm *et al.* (2020) from Germany. The above list of species reflects the complex systematics of this genus, as *N. triserialis* is now considered a synonym of *N. attenuatus*, whereas *N. attenuatus* is known to utilise only lymnaeid snails as intermediate hosts (Filimonova 1985; Gonchar *et al.* 2019). Species of the genus *Notocotylus* Diesing, 1839 use a wide range of snails as intermediate hosts and birds and mammals as definitive hosts; they are distributed worldwide and can be pathogenic to their bird hosts (Barton & Blair 2005; Gonchar *et al.* 2019). In Galway Bay, Ireland, adults of *C. verrucosa* and *Notocotylus* sp. from charadriid birds were recorded by Cabot (1969).

Discussion

A diverse range of larval trematode parasites was observed in the faucet snail, *Bithynia tentaculata*, in two lakes in Ireland. A total of nine trematode species of seven families were discovered as cercariae (seven species), metacercariae (three species), or both (one species). While this list represents a small part of the high species richness of trematodes thus far known from *B. tentaculata*, it is an important result for an initial study on this snail species in Ireland. All but one (*S. bramae*) of the trematode parasites identified are new species records for Ireland. Notably, our study identifies the most western distribution for the trematode species within Europe. The species list from Ireland almost fully overlaps with that found in Germany and Lithuania by Schwelm *et al.* (2020), who discovered 20 species belonging to 10 families in their spatially and temporally intensive study concentrated on *B. tentaculata*.

The species found are known parasites of fish (*A. progenetica*, *P. parasquamosa*, and *S. bramae*), birds (*C. prussica*, *M. xanthosomus*, *Notocotylus* sp., and *Sphaeridiotrema* sp.), and insectivorous mammals/bats (*L. linstowi* and *Lecithodendrium* sp.) (Yamaguti 1971; Niewiadomska 2003; Kudlai *et al.* 2015). A common feature of the present species is that the adult trematodes are generalists in definitive host use. For example, *L. linstowi* was recorded in various bat species (Esteban *et al.* 2001), *C. prussica* is a common parasite of waterfowl (Sitko *et al.* 2006), and *S. bramae* was reported from a wide variety of fish species (Kennedy 1974; Niewiadomska 2003). Another common feature of the present species is that they are widespread in Europe, with many of them (*L. linstowi*, *A. progenetica*, *P. parasquamosa*, and *S. bramae*) recorded in northern (Finland), eastern (Lithuania, Russia, Ukraine), and western Europe (United Kingdom), complemented now by the present results from the western edge of Europe. Trematodes using birds as definitive hosts can be well disseminated along bird migration routes. As Ireland is situated on the East Atlantic flyway, it is a location used for nesting, wintering, or staging of many migratory birds (Cramp & Simmons 1980). In previous studies on larval trematodes from Irish freshwater snails, an overlap in species of bird trematodes with samples from Iceland and continental Europe was observed (Kudlai *et al.* 2021; Pantoja *et al.* 2021; Faltýnková et al. 2022). Trematode parasites using less mobile small insectivorous mammals as definitive hosts might rely on life cycle stages other than adults for effective dispersion. The first intermediate host, *B. tentaculata* itself, has a high dispersion potential and can be introduced to new areas by human activity (e.g. via cargo ships), as well as by migratory birds (Weeks *et al.* 2017; Van Leeuwen et al. 2012). The second intermediate hosts harbouring metacercariae can contribute to dispersion too. For example, *L. linstowi* uses aquatic insect larvae as an intermediate host, which can ensure transmission either in water or in the terrestrial environment after host metamorphosis, thus expanding the range of potential definitive hosts (Kudlai *et al.* 2015).

The range of *B. tentaculata* expands from Europe to the West Siberian Plain in Asia (Welter-Schultes 2012), and from there it has been reported as an important intermediate host of trematode parasites in the local ecosystems, including *M. xanthosomus* and other cercariae of the Opisthorchiidae and Notocotylidae (Serbina 2014, 2016, 2022). From the Russian southern Far East, *Sphaeridiotrema aziaticum* from the snail *Boreoelona ussuriensis* (Büttner & Ehrmann) has been reported by Kalinina *et al.* (2022), which seems closely related to our material of *Sphaeridiotrema* sp. and that from Germany and Lithuania (Tkach *et al.* 2016; Schwelm *et al.* 2020). However, to clarify this, still more integrative taxonomic studies using mitochondrial markers are needed from both Europe and Asia.

Many of the trematode species found by us and by Schwelm *et al.* (2020) belong to groups with unclarified or controversial taxonomy. A typical example is the species of the genus *Notocotylus*, where cercariae and adults have few distinguishing features and there is conflicting evidence on species identity and life cycle stages (Gonchar *et al.* 2019). Also, for *S. bramae*, the suspicion exists that it might be a complex of multiple species (Koval 1971; Našincová 1992). Alas, the life cycle stages of the other species are still unknown; therefore, no comparative morphological data are available. Also, there is still a lack of robust phylogenetic information for most of the groups, and molecular genetic data are limited. For instance, for the Cyathocotylidae, Achatz *et al.* (2019) was the first to examine the phylogeny of the family based on molecular genetic data; Sitko *et al.* (2016) revised the species of the genus *Metorchis* but based predominantly on data from Europe; and for the Notocotylidae, a complex molecular phylogenetic study is still pending. To clarify the status and species diversity of trematode genera, a more intensive sampling campaign of hotspot regions is necessary. We expect that a more species-rich trematode parasite fauna could be found in Ireland during a longer and more geographically spread investigation, as is likely also the case for *B. tentaculata* in other parts of Europe.

Bithynia tentaculata hosts an array of trematode parasites that can be harmful to definitive host animals. At least four trematode species, including *Cyathocotyle bushiensis* Khan, 1962 and *Sphaeridiotrema globulus*, were introduced to North America together with *B. tentaculata* and are causing severe mortality in American migratory birds (Sauer *et al.* 2007; Bergame *et al.* 2011; Roy *et al.* 2016; Weeks *et al.* 2017). Similarly, members of the Notocotylidae at high intensities can be pathogenic to birds including domestic waterfowl (Filimonova 1985; Gonchar *et al.* 2019). Zoonotic trematodes that can affect humans are also hosted by *B. tentaculata*, e.g. opisthorchiids (*Metorchis*, and *Opisthorchis*) (Serbina 2022; Katokhin & Serbina 2023). In Russia, up to West Siberia, opisthorchiasis is common and is particularly linked to the presence of *B. tentaculata* and related species (Katokhin & Serbina 2023). While it is difficult to distinguish species of *Opisthorchis* from those of *Metorchis*, it is of particular importance to carefully identify them for correct assessment of infection foci and for epidemiological measures (Sitko *et al.* 2016).

Our findings support the statement by Schwelm *et al.* (2020) that *B. tentaculata* hosts a unique diversity of trematode species that do not overlap with those of the better-known trematode fauna of pulmonate snails (Heterobranchia). There is some overlap at family level (e.g. Notocotylidae); however, several of the trematode families (Cyathocotylidae, Echinochasmidae, Prosthogonimidae, Psilostomidae, and Opisthorchiidae) are known to use almost exclusively non-pulmonate snails of the subclass Caenogastropoda as intermediate hosts (Niewiadomska 2002, Scholz 2008, Tkach *et al.* 2016). Further investigations are required to examine the evolutionary and ecological factors responsible for these divergent trematode faunas.

We can affirm that *B. tentaculata* is a particularly suitable intermediate host for trematode parasites using a wide range of definitive hosts. Thus, this snail species plays an important role in ecosystem functioning as a central hub in a range of trematode life cycles. These trematode parasites are likely to be useful ecological indicators, as demonstrated for trematode parasites in previous studies (e.g. Byers *et al.* 2011; Shea *et al.* 2012; Taglioretti *et al.* 2018). There is an ongoing need for precise identification of trematode species via integrative taxonomic approaches to ensure that wider ecological contexts can be understood and biodiversity and disease threats can be accordingly evaluated. In the face of changing environments and increasing environmental pressures, the faucet snail, *B. tentaculata*, and its trematode fauna offer a useful study system due to the versatility and resistance of the snail species as well as its strong potential for colonising new areas. Future studies that could benefit from using this system include those investigating trends in biodiversity, ongoing ecological change, and changes in disease risk for wildlife.

Data availability statement. Data are available from the authors upon reasonable request; sequences are made available via GenBank.

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Ethical standard. Not applicable.

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