

**University of South Bohemia in České Budějovice,  
Faculty of Science, Department of Parasitology**

**TREMATODE DIVERSITY IN FRESHWATER PULMONATE SNAILS  
FROM THE ST LAWRENCE WETLANDS, CANADA**

**Master thesis**

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#### **ANNOTATION**

This study applies a molecular approach addressing the identification of trematode larval stages (sporocysts, rediae and cercariae) parasitising four freshwater pulmonate snails (*Stagnicola elodes*, *Radix auricularia*, *Planorbella trivolvis* and *Physella gyrina*) collected in the wetlands of St Lawrence River in Canada. A total of 20 genetically distinct species or species level lineages were identified *via* phylogenetic analyses based on, depending on the taxonomic group, mitochondrial (*cox1* and *nad1*) and nuclear (ITS-5.8S-ITS2 and 28S rDNA) sequences integrated with data from the recently generated sequence libraries for the European and some North American trematodes. Phylogenetic analyses and distance-based approaches allowed molecular identification of six described/named and 14 putative new species. These included species of six digenean families (Clinostomidae, Derogenidae, Echinostomatidae, Plagiorchiidae, Schistosomatidae and Strigeidae).

#### **DECLARATION**

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České Budějovice, 19 April 2017

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## 1. INTRODUCTION

Digenean trematodes are a large widespread and entirely parasitic group within the phylum Platyhelminthes (Bray, 2008). The subclass comprises of *c.*2,500 nominal genera including *c.*18,000 nominal species (Cribb et al., 2001; Bray, 2008; Kostadinova & Pérez-del-Olmo, 2014), obligatory parasitic in invertebrate intermediate hosts, usually molluscs and found as adults in all vertebrate classes (Bush et al., 2001). Digenean trematodes are characterised with complex life-cycles involving different host species (Bush et al., 2001; Poulin & Cribb, 2002; Galaktionov & Dobrovolskij, 2003).

The typical three-host digenean life-cycle involves two free-living, but short-lived stages, the miracidium (hatched from the eggs released by the adult worms) and cercaria (emerging from the first intermediate host) and four parasitic stages, the sporocyst and redia (developmental stages in the first intermediate host), metacercaria (developmental stage in the second intermediate host) and adult (parasitic in the definitive host). In the first intermediate host the miracidium develops into a mother sporocyst, which subsequently produces the next larval stages through asexual reproduction, daughter sporocysts or rediae in which cercariae are developed. Cercariae emerge from the snails as a consequence of a stimuli-dependent process (photoperiod) and this emergence is usually synchronised with the chronobiological behaviour of the next host in the parasite's life-cycle (Combes et al., 1994). The second intermediate host, either invertebrate or a vertebrate, is usually actively penetrated by the cercariae and the development into a metacercarial stage takes place. The final transmission to the definitive host where the worms mature and sexually reproduce is usually *via* predation of the second intermediate host by the definitive host. However, there are many examples where the typical three-host life-cycle has been truncated into two or a single host species, or alternatively, has been evolved into a four-host life-cycle incorporating an additional paratenic host, where a mesocercarial stage has been evolved (Galaktionov & Dobrovolskij, 2003).

Deeply involved for millions of years in the trematode life-cycles (Cribb et al., 2003), snails act as first intermediate hosts for the majority of the known species. No other parasites are so inextricably linked to a single group of hosts as the digenean trematodes to their snail hosts (Esch et al., 2001). As obligate hosts, snails are considered keystone species for the digenean trematodes; this is further emphasised by their strict host specificity. Most of the digenean species have been reported from a single snail species (Esch et al., 2001); however, a single snail species can be a host of a wide range of digenean trematodes. Thus the distribution of the trematodes is shaped by the distribution of the compatible snail

intermediate host. However, the distribution of the definitive host governs the larval trematode infections in snails (Hechinger & Lafferty, 2005). Digenean trematode diversity appears to have been addressed predominantly in studies on the definitive or the second intermediate host (typically fishes, in relation to diseases caused by the metacercariae). Studies on the larval life-cycle stages and life-cycles are relatively few, more extensively developed in the middle of the 20th Century (see Faltýnková et al., 2016 for a detailed review).

Linking life-cycle stages, and especially those in the snail and vertebrate definitive hosts is especially difficult and represents a significant obstacle to the identification of larval trematodes and their role in the freshwater ecosystems. Trematode taxonomy is based on the morphology of the adult stage and linking the larval and adult stages can be achieved in experimental completion of the life-cycle. This laborious and difficult to conduct approach is being rapidly replaced by a modern approach made possible by the decreasing cost of DNA sequencing (Blasco-Costa & Poulin, 2017). Matching sequences from different life-cycle stages has become the most common approach in trematode life-cycle studies (see Blasco-Costa et al., 2016 for a review and recent studies of large freshwater trematode groups in Europe by Georgieva et al., 2013a, b; 2014; Pérez-del-Olmo et al., 2014; Zikmundová et al., 2014; Soldánová et al., 2017).

Trematodes in the freshwater environment have been studied for nearly three centuries since Swammerdam's (1737) first record of cercariae from a dissected snail. Most extensive studies on the different life-cycle stages in the first snail intermediate hosts have been carried out in Europe (see Faltýnková et al., 2016 for an extensive review). The first contributions to the knowledge of the trematode larval fauna in North America date from the early 1800s with the record of *Cercaria hyalocauda* Haldeman, 1840 from *Physa heterostropha* (Say) by Haldeman (1840), followed by the work of Leidy (1877) who described *Cercaria ascoidea* Leidy, 1877 from two host species, *Planorbis parvus* Say and *Lymnaea elodes* Say, and *Cercaria lucania* Leidy, 1877 from *P. parvus* Say in 1877 (see Leidy, 1877). These were followed by significant contributions by Cort, Faust, O'Roke and Kull in the beginning of the 20th Century in their pioneer studies on the larval trematode morphology and life-cycle descriptions including more than 100 cercariae and several trematode life-cycles in the USA. Cort carried out studies on the North American larval trematode fauna in parallel with the extensive studies in Europe at that time. However, most of the records of larval trematode infections in snails are from limited geographical areas exclusively in the USA.

New insights into the genetic trematode diversity on a global scale have been achieved by the application of molecular approaches. Studies including molecular data on larval trematode stages from North America were initiated by Sorensen et al. (1998) who provided the first sequences for larval *Echinostoma* spp. in two naturally infected snail species, *Stagnicola elodes* and *Planorbella trivolvis* (Say), from Indiana, USA. Thereafter, data on specific trematode groups have been accumulated (see Supplementary Table 1 for details). Important contributions to the molecular taxonomy and systematics of the avian schistosomes including sequencing of isolates of cercariae and adults and thus linking life-cycle stages, were made by Brant and colleagues (see Brant & Locker, 2009; Brant et al., 2011, 2013). These authors provided *cox1*, ITS, 28S rDNA and 18S rDNA sequence data for the larval stages of 12 North American bird schistosomes. These include three species, *Anserobilharzia brantae* Brant, Jouet, Ferte & Locker, 2013, *Dendritobilharzia pulverulenta* (Braun, 1901) and *Dendritobilharzia* sp., recoded from the small planorbid *Gyraulus parvus* (Say); three species occurring in physid snail hosts, *Gigantobilharzia huronensis* Najim, 1950 from *Physella gyrina* (Say), *Trichobilharzia physellae* (Talbot, 1936) from *Ph. gyrina* and *Physella parkeri* (Currier), and *Trichobilharzia querquedulae* (McLeod, 1937) from *P. gyrina*; four additional species recorded in lymnaeid snail hosts, *Trichobilharzia stagnicolae* (Talbot, 1936) from *Stagnicola emarginata* (Say), *S. elodes* (Say) and *Stagnicola* sp., *Trichobilharzia szidati* Neuhaus, 1952 from *L. stagnalis* (L.) and *S. elrodi* (Baker & Hendereson), plus two otherwise unidentified but genetically distinct species, *Trichobilharzia* sp. D and *Trichobilharzia* sp. E from *Stagnicola* sp. (Brant & Locker (2009) (see Supplementary Table 1).

Another important contribution is that of Detwiler et al. (2010, 2012) who carried out extensive surveys of the echinostome trematodes in two intermediate snail hosts, *S. elodes* and *P. trivolvis*, predominantly in localities in Indiana and Minnesota (USA). Totals of 37 (*cox1*) and 52 (*nad1*) sequences were generated from cercarial isolates of North American species of three genera, *Echinostoma*, *Echinoparyphium* and *Hypoderaeum*, by Detwiler et al. (2010) (see Supplementary Table 1). These authors also carried out experimental infections to aid the identification and test the specificity of the echinostomes to their definitive hosts (birds *versus* mammals) and also sequenced adult worms from one echinostome definitive host, i.e. naturally infected muskrats *Ondatra zibethicus*, from Indiana, USA. Detwiler et al. (2010, 2012) characterised molecularly 11 genetically distinct species/species level lineages: (i) *Echinostoma revolutum* (*sensu lato*); *Echinostoma robustum/friedi* Lineages A and C; *Echinostoma trivolvis* Lineages B and C; *Echinoparyphium* sp. Lineage 2; and *Hypoderaeum* sp. Lineage 1 from *S. elodes*; (ii) *E.*



*revolutum* (s.l.); *E. trivolvis* Lineage A; and *Echinoparyphium* sp. Lineage 3 from *P. trivolvis*; (iii) *E. robustum/friedi* Lineage D ex *Biomphalaria glabrata* (isolate from Brazil); (iv) and *Echinostoma revolutum* (s.l.), *E. trivolvis* Lineage A, *E. trivolvis* Lineage B, *E. trivolvis* Lineage C and *Echinoparyphium* sp. Lineage 1 from *Ondatra zibethicus* (see Supplementary Table 1).

The most recent study on larval trematode diversity in North American snails was carried out by Gordy et al. (2016) who examined large samples (a total of 8,910 snails) of five snail host species, *P. trivolvis*, *Planorbula armigera* (Say), *Ph. gyrina*, *S. elodes* and *Lymnaea stagnalis*, in Alberta, western Canada. These authors sampled in six Albertan lake ecosystems bi-weekly over the course of two years (2013–2014) and reported 39 trematode species. Gordy et al. (2016) sequenced representative samples which were predominantly identified by BLAST searches of the GenBank database (as the most similar GenBank entry). However, similarities ranged from 74.7 to 100% and complete match was detected for just six species (*Diplostomum baeri*, *Diplostomum* spp. 3 and 4, *Neopetasiger islandicus*, *Petasiger* sp. 4 and *Trichobilharzia stagnicola*). Gordy et al. (2016) indicated that the lack of genetic data for many trematode families hampers the identification to the species level.

To summarise, although a significant progress has been made towards studying the taxonomic and genetic diversity of trematodes in the freshwater ecosystems of North America, there is still an urgent need of additional studies to expand the genetic datasets, link parasite life-cycle stages and assess species/lineages distinctness, preferably in phylogenetic analyses. This was the main aim of the present study based on an existing collection of larval trematodes from four species of snail in the wetlands of St Lawrence River near Montreal. Previous studies on larval trematodes in fishes in the fluvial section of St Lawrence River near Montreal using only sequence data, revealed an immense diversity of species of the order Diplostomida Olson, Cribb, Tkach, Bray & Littlewood, 2003 (47 species/species level lineages) (Moszczyńska et al., 2009; Locke et al., 2010a, b). However, there is a single trematode species, *Diplostomum* sp. 1 with sequence data available (KR271236) from larval stages in a snail host of the St Lawrence River (Locke et al., 2015).

## 2. AIM AND OBJECTIVES

The study aimed to assess the larval trematode diversity in samples of four freshwater pulmonate snails, *Stagnicola elodes* (Say), *Radix auricularia* (L.), *Planorbella trivolvis* (Say) and *Physella gyrina* (Say), collected in St Lawrence Wetlands near Montreal, Canada.

### OBJECTIVES

- (i) To sort and classify the cercarial isolates to the level of family/genus based on the morphology of the fixed material and the photomicrographs of live material where available.
- (ii) To generate partial sequences of the mitochondrial cytochrome *c* oxidase subunit 1 (*cox1*) and nicotinamide adenine dinucleotide dehydrogenase subunit 1 (*nad1*) genes and of the nuclear 28S rRNA gene (domains D1-D3) and the entire ribosomal internal transcribed spacer region ITS1-5.8S-ITS2 for selected isolates depending on the preliminary identification to the level of family/genus.
- (iii) To conduct phylogenetic analyses aiming molecular identification of the newly sequenced representative isolates.

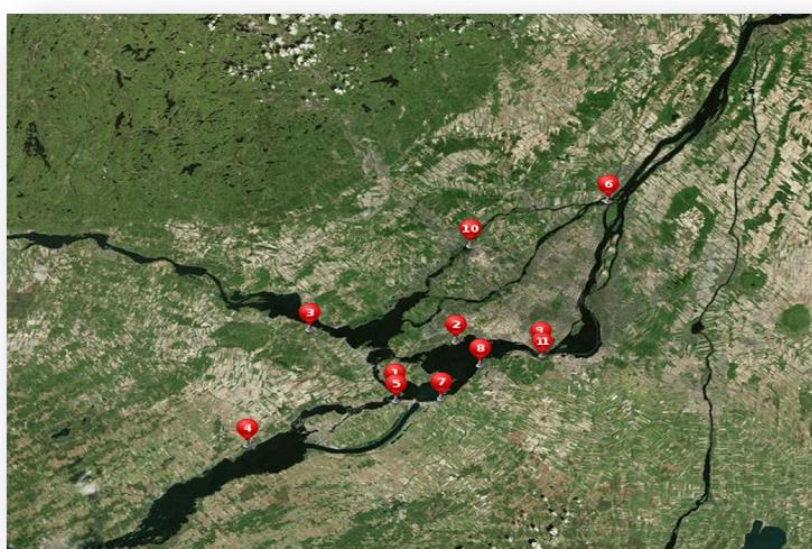
### 3. MATERIALS AND METHODS

#### 3.1. SAMPLE LOCALITIES AND COLLECTION OF TREMATODE SAMPLES

Sampling was carried out in August 2013 by Drs Simona Georgieva and Miroslava Soldánová, in the Wetlands of St Lawrence River, near Montreal, Canada. A total of 1,100 snails belonging to four species, i.e. *Stagnicola elodes* (Say) ( $n = 611$ ), *Radix auricularia* (L.) ( $n = 235$ ), *Planorbella trivolvis* (Say) ( $n = 211$ ) and *Physella gyrina* (Say) ( $n = 43$ ) were collected at 11 sampling sites (see Table 1 for localities and coordinates and a map in Fig. 1).

**Table 1** Coordinates of the sampling localities in St Lawrence Wetlands, Canada

Locality number	Locality name/code	Coordinates	
1	Pointe-des-Cascades	45°20'6.01"N	73°57'20.28"W
2	Beaconsfield	45°25'52.51"N	73°49'6.67"W
3	Pine Lake	45°27'10.6"N	74°08'52.5"W
4	Site L1	45°13'37.84"N	74°17'25.06"W
5	Site L4	45°18'54.98"N	73°57'12.23"W
6	Site L8	45°42'9.52"N	73°28'37.47"W
7	Site L9	45°19'9.70"N	73°51'8.53"W
8	Site L10	45°23'4.01"N	73°45'50.34"W
9	Site L11	45°24'14.12"N	73°37'30.78"W
10	Site L12	45°37'3.20"N	73°47'8.30"W
11	Site L13	45°24'12.95"N	73°37'27.60"W



**Fig. 1** Map showing sampling localities in the St Lawrence Wetlands, near Montreal, Canada

In the laboratory, snails were identified according to Burch (1982, 1989) and other sources, measured, placed in individual containers and subjected to light stimulation for cercarial emergence. All snails which did not emit cercariae for several days were dissected and examined to check for the presence of prepatent trematode infections. Cercariae and sporocysts/rediae were studied as live and fixed material. Preliminary identification was attempted using relevant primary sources (Cort, 1914; Miller, 1935, 1936; Fried et al., 1998; Bolek et al., 2010). Live cercariae were stained with Neutral Red and Nile Blue sulphate. For further morphological examination, representative photomicrographs of live sporocysts/rediae and cercariae were taken with a digital camera on a Leica microscope. Upon preliminary identification, subsamples of cercariae, sporocysts and/or rediae were fixed in both, molecular-grade ethanol for DNA isolation and in 4% cold formaldehyde solution for subsequent morphological examinations. Formalin-fixed cercariae were stained with iron-acetocarmine, dehydrated through alcohol series (70–100%), cleared in dimethyl phthalate and examined as permanent mounts in Canada balsam. Light photomicrograph series of the fixed isolates were taken for a representative subset of the samples with a digital camera of an Olympus BX51 microscope.

### **3.2. GENERATION OF MOLECULAR DATA**

Total genomic DNA was isolated from a pool of 20–100 ethanol-fixed cercariae or individual rediae/sporocysts using Chelex® extraction. Specimens were placed in 200 µl of a 5% suspension of deionised water and Chelex® containing 0.1 mg/ml proteinase K and incubated at 56 °C overnight; this was followed by boiling at 90 °C for 8 min and centrifugation at 16,000 *g* for 10 min. Four different fragments were amplified depending on the parasite family-level group in order to achieve species identification. These included partial fragments of two mitochondrial genes, i.e. the nicotinamide adenine dinucleotide dehydrogenase subunit 1 (*nad1*) and cytochrome *c* oxidase subunit 1 (*cox1*); the nuclear 28S rRNA gene (domains D1-D3) and the entire ribosomal internal transcribed spacer region ITS1-5.8S-ITS2. Polymerase chain reaction (PCR) amplifications were performed in 25 µl reactions using illustra puReTaq Ready-To-Go PCR beads (GE Healthcare, UK) containing c.2.5 units of puReTaq DNA polymerase, 10 mM Tris-HCL (pH 9.0), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 200 mM of each dNTP and stabilisers including BSA, 10 mM of each primer, and about 50 ng of template DNA. Alternatively, PCR amplifications were carried out in a total volume of 20 µl containing 10 µl 2× MyFi™ Mix (Bioline, USA), 10 pmol of each primer

and ~50 ng of genomic DNA. Primers combinations and annealing temperatures thermocycling conditions are detailed in Table 2 and PCR reaction profiles used for individual gene fragment amplifications using different primer combinations are shown in Fig. 2.

PCR amplicons were visualised by agarose gel electrophoresis (1% agarose gels stained with GelRed) and purified with QIAquick PCR purification kit (Qiagen Ltd, UK) following the manufacturer's instructions. DNA quantification (ng/μl) was carried out with NanoDrop 1000 Spectrophotometer using the programme ND1000. PCR amplicons were sequenced directly from both strands using the PCR primers with ABI BigDye chemistry (ABI Perkin-Elmer), alcohol-precipitated, and run on an ABI Prism 3130xl or 3730xl automated sequencers. Contiguous sequences were assembled, edited and aligned in MEGA v. 7 (Kumar et al., 2016). Sequence identity of the newly generated sequences was verified by the Basic Local Alignment Tool (BLAST) ([https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE\\_TYPE=BlastSearch&LINK\\_LOC=blasthome](https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome)).

### 3.3. PHYLOGENETIC ANALYSES

Phylogenetic analyses were performed using separate alignments according to the trematode group and gene/region fragment amplified. Alignments were built with MUSCLE (Edgar, 2004a, b) implemented in MEGA v. 7 under default parameter values. The alignments for protein-coding genes included no insertions or deletions and were aligned with reference to the amino acid translation, using the echinoderm and flatworm mitochondrial code (translation table 9; <https://www.ncbi.nlm.nih.gov/Taxonomy/Utils/wprintgc.cgi#SG9>).

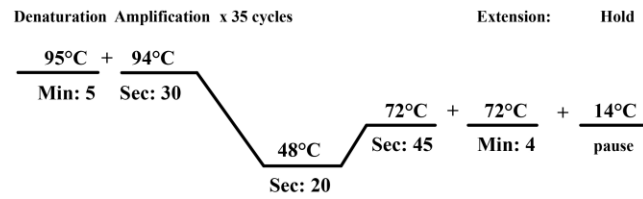
Five alignments were analysed. The alignments included members of three families, i.e. the Echinostomatidae Looss, 1899, the Strigeidae Railliet, 1919, and the Derogenidae Nicoll, 1910, and members of the genus *Plagiorchis* Lühe, 1899. Up to three representative published sequences per species/lineage with the longest possible length were selected to build the alignments of the mitochondrial datasets. Outgroup selection was based on previous studies on the above mentioned groups.

Alignment 1 (480 nt positions; 92 sequences) comprised 27 newly generated *nad1* sequences and 64 sequences retrieved from GenBank for species of the family Echinostomatidae. This alignment represented the data currently available for 37 species: 10

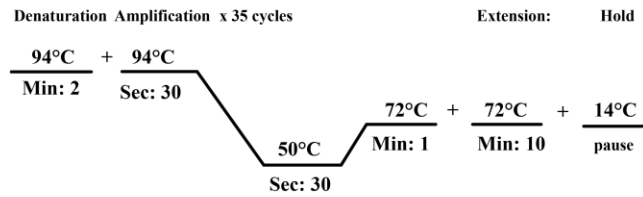
**Table 2** Primers used for amplification (PCR) and/or sequencing (Seq)

Target gene or region/Primer	Sequence (5'-3')	Direction	Application	Annealing temperature	Family	Source
<b><i>cox1</i></b>						
JB3	TTTTTTGGGCATCCTGAGGTTTAT	Forward	PCR+Seq	55 °C	Plagiorchiidae	Bowles et al. (1995)
JB4.5	TAAAGAAAGAACATAATGAAAATG	Reverse	PCR+Seq		Plagiorchiidae	Bowles et al. (1995)
DiploCO1 (F)	CGTTTRAATTATACGGATCC	Forward	PCR+Seq	50 °C	Strigeidae	Moszczyńska et al. (2009)
DiploCO1 (R)	AGCATAGTAATMGCAGCAGC	Reverse	PCR+Seq		Strigeidae	Moszczyńska et al. (2009)
<b>28S</b>						
LSU5'	TAGGTCGACCCGCTGAAYTTAAGCA	Forward	PCR+Seq	55 °C	Derogenidae, Echinostomatidae, Schistosomatidae	Littlewood et al. (2000)
ZX-1	ACCCGCTGAATTTAAGCATAT	Forward	PCR+Seq			Bray et al. (2009)
1500R	GCTATCCTGAGGGAAACTTCG	Reverse	PCR+Seq			Tkach et al. (2003)
<b><i>nad1</i></b>						
NDJ11	AGATTCGTAAGGGGCCTAATA	Forward	PCR+Seq	48 °C	Echinostomatidae	Kostadinova et al. (2003)
NDJ2a	CTTCAGCTTCAGCATAAT	Reverse	PCR+Seq		Echinostomatidae	Kostadinova et al. (2003)
<b>ITS1-5.8S-ITS2</b>						
its5Trem	GGAAGTAAAAGTCGTAACAAGG	Forward	PCR+Seq	50 °C	Clinostomidae, Schistosomatidae	Dvořák et al. (2002)
its4Trem	TCCTCCGCTTATTGATATGC	Reverse	PCR+Seq			Dvořák et al. (2002)

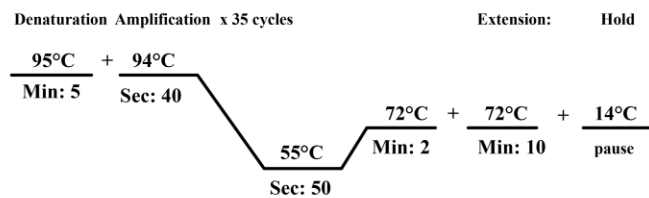
*nad1* (NDJ11 and NDJ2a)



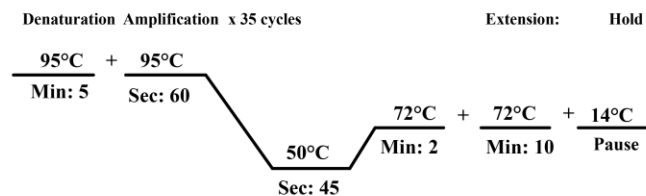
*cox1* (Plat-diploCOX1F and Plat-diploCOX1R)



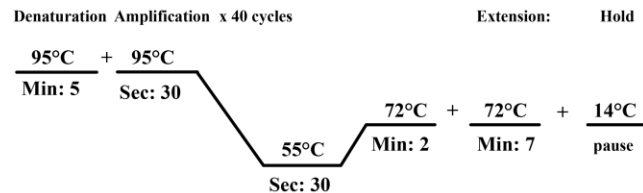
*cox1* (JB3 and JB4.5)



ITS1-5.8S-ITS2 rRNA gene cluster (its5Term and its4Term)



28S rRNA gene (variable domains D1-D3; primers LSU5'/ZX-1 and 1500R)



**Fig. 2** PCR thermocycle profiles and primer combinations used for amplification of the four genetic markers

from North America, 14 from Europe, 5 from Australia and New Zealand, 4 from South America, 2 from Africa and 2 from Asia.

Alignment 2 (1,261 nt positions; 54 sequences) comprised 11 newly generated 28S DNA sequences representing a subset of the *nad1*-derived clades of the Echinostomatidae and included data for 42 species: 13 from North America, 23 Europe, 3 New Zealand, 2 from Asia and a single species from Africa.

Alignment 3 (407 nt positions; 58 sequences) represented a *cox1* dataset for the family Strigeidae, including 3 newly generated sequences and 54 sequences for 29 species or species level lineages retrieved from GenBank. Of these, sequences for 24 species/species level lineages originate from North America, 3 from Europe and 2 from New Zealand.

Alignment 4 (423 nt positions; 31 sequences) comprised 2 newly generated *cox1* sequences for *Plagiorchis* spp. and encompassed the available data for 13 species/species level lineages. Of these, sequences for 12 species originate from Europe and one sequence for a single species originates from Asia.

Alignment 5 (1,317 nt positions; 6 sequences) comprised a small dataset of 28S rDNA for the hemiuroidean family Derogenidae and included 2 newly generated and 3 published sequences for species from North America, Europe and Australia.

Species boundaries were assessed using Bayesian inference (BI) analysis. Prior to analysis, the appropriate models of base substitution were estimated with jModelTest 2.1.1 (Guindon & Gascuel, 2003, Darriba et al., 2012) under Akaike Information Criterion with correction for small sample sizes (AICc) (Sugiura, 1978). BI analyses were run on MrBayes v. 3.2.2 (Ronquist et al., 2012) as online execution on the Cipres Science Gateway v. 3.1 ([http://www.phylo.org/sub\\_sections/portal/](http://www.phylo.org/sub_sections/portal/)) (Miller et al., 2010), using MrBayes (3.2.6) on XSEDE. Log likelihoods were estimated over 10,000,000 generations using MCMC searches on two simultaneous runs of four chains, sampling every 1,000 generations. The 'burn-in' was set for the first 25% of the sampled trees. Consensus topology and nodal support estimated as posterior probability values (Huelsenbeck et al., 2001) were calculated from the remaining trees. FigTree v.1.4.2 (Rambaut, 2014) was used for tree visualisation (see Table 3 for more details on the specific data analyses).



**Table 3** Details for the alignments used in the phylogenetic analyses

<b>Trematode group</b>	<b>Gene</b>	<b>Alignment</b>	<b>No. of newly generated sequences</b>	<b>No. of sequences retrieved from GenBank<sup>a</sup></b>	<b>No. of species<sup>a, b</sup></b>	<b>Outgroup</b>	<b>Alignment length (nt)</b>	<b>Model<sup>c</sup></b>
Family Echinostomatidae	<i>nad1</i>	1	27	64	34	<i>Fascioloides magna</i>	480	GTR+I+ $\Gamma$
Family Echinostomatidae	28S rRNA	2	11	42	42	<i>Notocotylus attenuatus</i>	1,261	GTR+I+ $\Gamma$
Family Strigeidae	<i>cox1</i>	3	3	54	22	<i>Tylodelphys clavata</i>	407	HKY+I+ $\Gamma$
Genus <i>Plagiorchis</i> (Plagiorchiidae)	<i>cox1</i>	4	2	28	13	<i>Choledocystus hepaticus</i>	423	GTR+I+ $\Gamma$
Family Derogenidae	28S rRNA	5	2	3	3	<i>Otodistomum cestoides</i>	1,317	GTR+ $\Gamma$

<sup>a</sup> Ingroup; <sup>b</sup> Sequences retrieved from GenBank; <sup>c</sup> GTR+I+ $\Gamma$ , general time reversible model including estimates of invariant sites and gamma distributed among-site variation; GTR+ $\Gamma$ , general time reversible model and gamma distributed among-site variation; HKY+I+ $\Gamma$ , Hasegawa-Kishino-Yano model including estimates of invariant sites and gamma distributed among-site rate variation

[„následující pasáž o rozsahu 13–53 (41) stran je obsažena pouze v archivovaném originále diplomové práce uloženém na Přírodovědecké Fakultě Jihočeské Univerzity”]

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## **8. SUPPLEMENT**

**Supplementary Table 1** Summary of the molecular evidence available for larval digenean trematodes from North America at the onset and during the course of the present study

Species	Host	Locality	No. of sequences				Source
			<i>cox1</i>	<i>nad1</i>	ITS	28S	
<b>Azygiidae Lühe, 1909</b>							
<i>Proterometra epholkos</i> Wombe, Oréelis-Ribeiro & Bullard, 2015	<i>Pleurocera cf. modesta</i> (Lea)	USA: Terrapin Creek (South Fork), Cleburne County, Alabama			1		Womble et al. (2015)
<b>Family Diplostomidae Poirier, 1886</b>							
<i>Diplostomum baeri</i> Dubois, 1937	<i>Stagnicola elodes</i> (Say)	Canada: Isle Lake	1				Gordy et al. (2016)
<i>Diplostomum indistinctum</i> (Guberlet, 1923)	<i>Stagnicola elodes</i>	Canada: Gull Lake	1				Gordy et al. (2016)
<i>Diplostomum huronense</i> (La Rue, 1927)	<i>Stagnicola elodes</i>	Canada: Gull Lake	1				Gordy et al. (2016)
<i>Diplostomum</i> sp. 1	Lymnaeidae	Canada: Montreal	1				Locke et al. (2015)
	<i>Stagnicola elodes</i>	Canada: Wabamun Lake	1				Gordy et al. (2016)
<i>Diplostomum</i> sp. 2	<i>Stagnicola elodes</i>	Canada: Alberta	1				Gordy et al. (2016)
<i>Diplostomum</i> sp. 3	<i>Lymnaea stagnalis</i> (L.)	Canada: Wabamun Lake	1				Gordy et al. (2016)
<i>Diplostomum</i> sp. 4	<i>Stagnicola elodes</i>	Canada: Alberta	1				Gordy et al. (2016)
<i>Diplostomum</i> sp. 8	<i>Stagnicola elodes</i>	Canada: Isle Lake	1				Gordy et al. (2016)
<i>Ornithodiplostomum</i> sp. 2	<i>Physella gyrina</i> (Say)	Canada: Wabamun Lake	1				Gordy et al. (2016)
<i>Ornithodiplostomum</i> sp. 4	<i>Physella gyrina</i>	Canada: Wabamun Lake	1				Gordy et al. (2016)
<i>Ornithodiplostomum</i> sp. 8	<i>Physella gyrina</i>	Canada: Pigeon Lake	1				Gordy et al. (2016)
<i>Tylodelphys scheuringi</i> (Hughes, 1929)	<i>Planorbella trivolvis</i> (Say)	Canada: Wabamun Lake	1				Gordy et al. (2016)
<b>Family Echinostomatidae Looss, 1899</b>							
<i>Drepanocephalus auritus</i> Kudlai, Kostadinova, Pulis & Tkach, 2015	<i>Physella gyrina</i>	Canada: Buffalo Lake; The Narrows (site 11)	1				Gordy et al. (2016)
<i>Echinoparyphium rubrum</i> (Cort, 1914)	<i>Planorbella trivolvis</i>	USA				1	Tkach et al. (2012)
<i>Echinoparyphium</i> sp.	<i>Stagnicola elodes</i>	Canada: Gull Lake	1				Gordy et al. (2016)
<i>Echinoparyphium</i> sp. Lineage 2	<i>Stagnicola elodes</i>	USA: Indiana	5	13			Detwiler et al. (2010)
<i>Echinoparyphium</i> sp. Lineage 2c	<i>Stagnicola elodes</i>	USA: Indiana	2	2	2		Detwiler et al. (2010)
<i>Echinoparyphium</i> sp. Lineage 3d	<i>Planorbella trivolvis</i>	USA: Indiana	1	2	1		Detwiler et al. (2010)
<i>Echinostoma caproni</i> Richard, 1964	<i>Stagnicola elodes</i>	Canada: Buffalo, Pelican Point (site 9)	1				Gordy et al. (2016)
<i>Echinostoma revolutum</i> (Frölich, 1802) ( <i>sensu lato</i> )	<i>Stagnicola elodes</i>	USA: Indiana	25	34	3	1	Soerensen et al. (1998); Detwiler et al. (2010)
	<i>Planorbella trivolvis</i>	USA: Indiana		1			Detwiler et al. (2010)

Supplementary Table 1 Continued

Species	Host	Locality	No. of sequences				Source
			<i>cox1</i>	<i>nad1</i>	ITS	28S	
<i>Echinostoma robustum/friedi</i> Lineage A	<i>Stagnicola elodes</i>	USA: Minnesota	2	1	1		Detwiler et al. (2010)
<i>Echinostoma robustum/friedi</i> Lineage C	<i>Stagnicola elodes</i>	USA: Minnesota		1			Detwiler et al. (2010)
<i>Echinostoma</i> sp.	<i>Stagnicola elodes</i>	Canada: Alberta	1				Gordy et al. (2016)
	<i>Physella gyrina</i>	Canada: Alberta	1				Gordy et al. (2016)
<i>Echinostoma trivolvis</i> (Cort, 1914)	<i>Planorbella trivolvis</i>	USA: Indiana			2		Soerensen et al. (1998)
<i>Echinostoma trivolvis</i> Lineage A	<i>Planorbella trivolvis</i>	USA: Indiana	2	2	2		Detwiler et al. (2010)
<i>Echinostoma trivolvis</i> Lineage B	<i>Stagnicola elodes</i>	USA: Indiana		1	1	1	Detwiler et al. (2010)
<i>Echinostoma trivolvis</i> Lineage C	<i>Stagnicola elodes</i>	USA: Indiana			1		Detwiler et al. (2010)
<i>Hypoderaeum</i> sp.	<i>Stagnicola elodes</i>	Canada: Alberta	1				Gordy et al. (2016)
<i>Hypoderaeum</i> sp. Lineage 1	<i>Stagnicola elodes</i>	USA: Indiana	7	12	1		Detwiler et al. (2010)
<i>Neopetasisiger islandicus</i> (Kostadinova & Skírnisson, 2007)	<i>Planorbula armigera</i> (Say)	Canada: Wabamun Lake		1			Gordy et al. (2016)
<i>Neopetasisiger</i> sp.	<i>Planorbella trivolvis</i>	Canada: Alberta				1	Gordy et al. (2016)
<i>Neopetasisiger</i> sp. 4	<i>Planorbella trivolvis</i>	Canada: Alberta		1			Gordy et al. (2016)
<i>Ribeiroia ondatrae</i> (Price, 1931)	<i>Planorbella trivolvis</i>	USA: Warren County, New Jersey; Shady Lakes, Albuquerque, NM			2		Keeler et al. (2012); Wilson et al. (2005)
<b>Family Gorgoderidae Looss, 1899</b>							
<i>Gorgoderina</i> sp.	<i>Stagnicola elodes</i>	Canada: Alberta	1				Gordy et al. (2016)
<b>Family Notocotylidae Lühe, 1909</b>							
<i>Notocotylus</i> sp.	<i>Stagnicola elodes</i>	Canada: Alberta	1				Gordy et al. (2016)
<b>Family Philophtalmidae Looss, 1899</b>							
<i>Pseudopsilostoma varium</i> (Linton, 1928)	<i>Planorbella trivolvis</i>	Canada: Alberta	1				Gordy et al. (2016)
<b>Family Plagiorchiidae Lühe, 1909</b>							
<i>Plagiorchis</i> sp.	<i>Stagnicola elodes</i>	Canada: Alberta	1				Gordy et al. (2016)
<b>Family Schistosomatidae Stiles &amp; Hassal, 1898</b>							
<i>Anserobilharzia brantae</i> Brant, Jouet, Ferte & Locker, 2013	<i>Gyraulus parvus</i> (Say)	Canada: Alberta; USA	2		6	3	Brant et al. (2013)

**Supplementary Table 1 Continued**

Species	Host	Locality	No. of sequences				Source
			<i>cox1</i>	<i>nad1</i>	ITS	28S	
<i>Dendritobilharzia pulverulenta</i> (Braun, 1901)	<i>Gyraulus parvus</i>	USA			1	1	Brant et al. (2011); Jothikumar et al. (2015)
<i>Dendritobilharzia</i> sp.	<i>Gyraulus parvus</i>	USA	1				Brant et al. (2013)
<i>Gigantobilharzia huronensis</i> Najim, 1950	<i>Physella gyrina</i>	USA			1		Brant et al. (2011)
Schistosomatidae gen. sp.	<i>Stagnicola elodes</i>	Canada: Alberta	1				Gordy et al. (2016)
<i>Schistosomatium douthitti</i> (Cort, 1914)	<i>Stagnicola elodes</i>	Canada: Gull Lake	1				Gordy et al. (2016)
<i>Trichobilharzia physellae</i> (Talbot, 1936)	<i>Physella gyrina</i>	USA: New Mexico, Canada: Lac la Nonne	3		3		Brant & Loker (2009); Gordy et al. (2016)
	<i>Physella parkeri</i> (Currier)	USA: Michigan	1			1	Brant & Locker (2009)
<i>Trichobilharzia querquedulae</i> (McLeod, 1937)	<i>Physella gyrina</i>	USA			1		Brant et al. (2011)
<i>Trichobilharzia stagnicolae</i> (Talbot, 1936)	<i>Stagnicola</i> sp.	USA: Montana, New Mexico	2		2	1	Brant & Locker (2009)
	<i>Stagnicola emarginata</i> (Say)	USA: Michigan, Minnesota	3		3	1	Brant & Locker (2009)
	<i>Stagnicola elodes</i>	Canada: Isle Lake	1				Gordy et al. (2016)
<i>Trichobilharzia szidati</i> Neuhaus, 1952	<i>Lymnaea stagnalis</i>	USA: Michigan; Canada: Gull; Buffalo; The Narrows (site 11)	2		1	1	Brant & Locker (2009); Gordy et al. (2016)
	<i>Stagnicola elrodi</i> (Baker and Henderson)	USA: Montana	1		1		Brant & Locker (2009)
<i>Trichobilharzia</i> sp. D	<i>Stagnicola</i> sp.	Canada	1		1	1	Brant & Locker (2009)
<i>Trichobilharzia</i> sp. E	<i>Stagnicola</i> sp.	Canada	2		1	1	Brant & Locker (2009)
<b>Family Strigeidae Railliet, 1919</b>							
<i>Apatemon</i> sp. 1	<i>Stagnicola elodes</i>	Canada: Isle Lake	1				Gordy et al. (2016)
<i>Apharyngostrigea pipientis</i> (Faust, 1918)	<i>Planorbella trivolvis</i>	Canada: Alberta	1				Gordy et al. (2016)
<i>Australapatemon burti</i> (Miller, 1923)	<i>Stagnicola elodes</i>	Canada: Alberta	1				Gordy et al. (2016)

**Supplementary Table 1 Continued**

Species	Host	Locality	No. of sequences				Source
			<i>cox1</i>	<i>nad1</i>	ITS	28S	
<i>Bolbophorus</i> sp.	<i>Planorbella trivolvis</i>	Canada: Buffalo Lake; The Narrows (site 11)	1				Gordy et al. (2016)
<i>Corylurus gallinulae</i> Lutz, 1928	<i>Stagnicola elodes</i>	Canada: Alberta	1				Gordy et al. (2016)
<i>Icthyocotylurus</i> sp. 3	<i>Stagnicola elodes</i>	Canada: Alberta	1				Gordy et al. (2016)
Strigeidae gen. sp. 9	<i>Stagnicola elodes</i>	Canada: Isle Lake	1				Gordy et al. (2016)
<b>Family Telorchiidae Looss, 1899</b>							
<i>Telorchis</i> sp.	<i>Stagnicola elodes</i>	Canada: Buffalo Lake; Pelican Point (site 9)	1				Gordy et al. (2016)



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