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RESEARCH PAPER OPEN ACCESS

Potamopyrgus antipodarum (Gray, 1843) in Polish waters – its mitochondrial haplotype and role as intermediate host for trematodes

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Abstract – The New Zealand mud snail (*Potamopyrgus antipodarum* (Gray, 1843)) is on the list of one hundred worst invasive species. Researchers point out that genetic variation between populations of *P. antipodarum* manifested in differences in life-history traits. The main objective of our investigation was to gain pioneer knowledge about mitochondrial haplotypes of *P. antipodarum* in Polish waters on the background of these haplotypes recorded in the world and confirmation of the main role of *P. antipodarum* in the life cycle of digenean trematodes. We examined 1000 individuals of *P. antipodarum* from five water bodies in three different parts of Poland for the presence of larval stages of digenean trematodes. For several randomly selected individuals we carried out DNA sequencing of the 16S ribosomal RNA gene as marker of this non-indigenous mollusk. Only one 16S rRNA haplotype of *P. antipodarum* was recorded in Polish waters, defined in this study as haplotype 1 which turned out to be the most widespread in Europe. *Potamopyrgus antipodarum* is a source of trematode metacercariae belonging mainly to the family Echinostomatidae. As a result, we can demonstrate that it plays a role as the second intermediate host of digenean trematodes in European waters.

Keywords: Freshwater snail / New Zealand mud snail / 16S rRNA / metacercariae / biological invasion

Résumé – *Potamopyrgus antipodarum* (Gray, 1843) dans les eaux polonaises – son haplotype mitochondrial et son rôle d'hôte intermédiaire pour les trématodes. L'escargot de vase de Nouvelle-Zélande (*Potamopyrgus antipodarum* (Gray, 1843)) figure sur la liste des cent pires espèces envahissantes. Les chercheurs soulignent que la variation génétique entre les populations de *P. antipodarum* se manifeste par des différences dans les traits du cycle de vie. L'objectif principal de notre étude était d'acquérir des connaissances préliminaires sur les haplotypes mitochondriaux de *P. antipodarum* dans les eaux polonaises sur le fond de ces haplotypes enregistrés dans le monde et de confirmer le rôle principal de *P. antipodarum* dans le cycle de vie des trématodes digéniens. Nous avons examiné 1 000 individus de *P. antipodarum* provenant de cinq plans d'eau dans trois régions différentes de Pologne pour détecter la présence de stades larvaires de trématodes digéniens. Pour plusieurs individus sélectionnés au hasard, nous avons effectué le séquençage de l'ADN du gène ARN ribosomique 16S comme marqueur de ce mollusque non indigène. Un seul haplotype d'ARNr 16S de *P. antipodarum* a été enregistré dans les eaux polonaises, défini dans cette étude comme l'haplotype 1 qui s'est avéré être le plus répandu en Europe. *Potamopyrgus antipodarum* est une source de métacercaires trématodes appartenant principalement à la famille des Echinostomatidae. Nous pouvons ainsi démontrer qu'il joue un rôle en tant que deuxième hôte intermédiaire des trématodes digéniens dans les eaux européennes.

Mots clés : Escargot d'eau douce / escargot de vase de Nouvelle-Zélande / ARNr 16S / métacercaires / invasion biologique

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1 Introduction

The New Zealand mud snail *Potamopyrgus antipodarum* (Gray, 1853) (Gastropoda, Tateidae) has been present in Europe for about 160 yrs (Boycott, 1936), while in Poland, its first occurrence was recorded almost 100 yrs ago (Urbański, 1935). Currently, this is a species with a worldwide distribution (Collado, 2014) that causes numerous ecological and economic problems (Alexandre da Silva *et al*., 2019). *Potamopyrgus antipodarum* has also been presented among the one hundred worst invasive species in Europe (Nentwig *et al*., 2018). The latest models of potential distribution and areas susceptible to the invasion of *P. antipodarum* in South America and worldwide, regardless of positive and negative climate changes, show greater suitability of the environment, also outside the area of its current distribution (Alexandre da Silva *et al*., 2019).

One of the strategies that make the species an effective invader in a new area is parthenogenesis (*i.e.*, asexual reproduction in which development of embryos occur without fertilization) (Jacobsen and Forbes, 1997). In the European populations, there is no evidence of sexual reproduction, although males of *P. antipodarum* have been found (Jacobsen and Forbes, 1997). The populations of parthenogenetic organisms can expand their range more efficiently while adapting to a new environment (Morgan-Richards *et al*., 2010). The parthenogenesis of *P. antipodarum* living outside native boundaries under favourable and stable conditions allows for a fast multiplication of specimens that become quantitative dominants among macroinvertebrates (Alonso and Castro-Díez, 2012). Städler *et al*. (2005) showed a marked divergence between the two European haplotypes of *P. antipodarum*, which means successful colonization by two distinct mitochondrial lineages. However, the samples of Polish origin have not been studied so far.

Ballast water is considered the main cause of the global spread of this invasive species (Alonso and Castro-Díez, 2008). However, the presence of the snail species in Polish water bodies as well as other non-endemic places, may result from other passive modes of its distribution in the spread of the individuals on birds' feathers or in fish intestines (Alonso and Castro-Díez, 2008). The success of *P. antipodarum* in inhabiting non-native areas is associated with a wide tolerance to different physicochemical conditions (*e.g.*, pH, water temperature, dissolved oxygen), as well as the shell morphological adaptations and the lack of native enemies. For example, thanks to the solid operculum and strong shell, snails are able to survive in the digestive system of fish, as a result, these vertebrates are less effective as predators, and on the contrary, they can be the carrier of *P. antipodarum* (Alonso and Castro-Díez, 2012).

Very little has been proven about the use of *P. antipodarum* by parasites in its new areas (Larson and Krist, 2019). The snail is even considered as a potential biological control against some species of parasites such as bird schistosomes (Marszewska *et al*., 2018a). However, it should be taken into account that this snail species, reaching such huge densities (Dorgelo, 1987), may become a convenient host for local parasites. In the work of Kelly *et al*. (2009) a wide range of arguments is presented that nonindigenous animal species may be highly competent hosts for the indigenous ones.

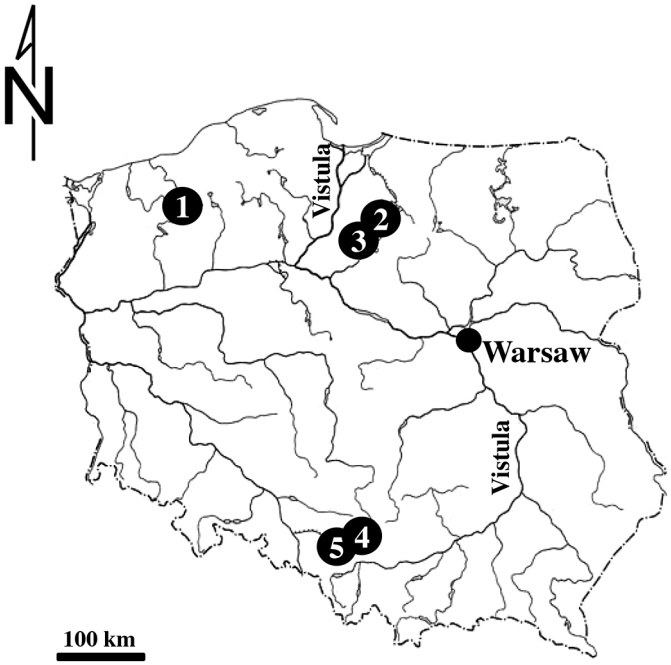

Fig. 1. Study sites in Poland, Central Europe: 1–Lake Czaplino (53°32′59″N, 16°14′59″E), 2–Lake Iławskie (53°35′37″N, 19°36′54″E), 3–Lake Sosno (53°20′15″N, 19°20′55″E), 4–pond in Strzemieszyce Wielkie (district of Dąbrowa Górnicza) (50°18′23″N, 19°19′24″E), 5–a bathing resort in the Valley of Three Ponds area in Katowice (50°14′32″N, 19°02′42″E).

The genetic structure of intermediate hosts of trematodes is of great interest to scientists (Hauswald *et al*., 2011; Tantrawatpan *et al*., 2020). The genetic variation between populations of *P. antipodarum* is reflected *e.g.* in differences in life-history traits (Jacobsen and Forbes, 1997). We examined specimens of this alien species from different localization in Poland to obtain pioneer knowledge about its mitochondrial haplotypes in the area of Central Europe, which we presented on the background of the recorded mitochondrial haplotypes in the world, and we checked the role of *P. antipodarum* as an intermediate host for digenean trematodes.

2 Material and methods

2.1 Research area and sampling

The samples were gathered in September 2018, that is, after months of the strongest release of cercariae from the first intermediate hosts (various species of freshwater snails) (Marszewska *et al*., 2018b; Cichy *et al*., 2019) for which *P. antipodarum* can play the role of the second intermediate host (Żbikowski and Żbikowska, 2009; Cichy *et al*., 2017). The study areas which we used for the research were water bodies from three different parts of Poland: western Poland – Lake Czaplino; central – Lake Iławskie and Lake Sosno; and southern Poland – a pond in Strzemieszyce Wielkie (a district of Dąbrowa Górnicza), as well as a bathing resort in the Valley of Three Ponds area in Katowice (Fig. 1). All of the lakes situated in the north part of Poland are natural water bodies, and the others are anthropogenic reservoirs that seem more

Table 1. The mean values of conductivity and pH from the study sites.

Study sites	Conductivity (μS/cm)	pH	Oxygen (mg l^{-1})	Temperature (°C)
Lake Czaplino	327	7.0	8.3	17
Lake Iławskie	368	8.5	8.6	18
Lake Sosno	369	8.3	8.6	17
Pond in Strzemieszyce Wielkie	810	7.4	7.3	19
Valley of three ponds area in Katowice	740	8.3	7.8	18

attractive to *P. antipodarum* (Johnson *et al*., 2008). Also, the abiotic conditions of the environment are of great importance for the formation of mollusk populations (Larson *et al*., 2020), and therefore the values of physical and chemical parameters of water were measured during the study with the core sampler and a MultiLine P4 (WTW) Universal Pocket Sized Meter (Tab. 1). The sampling of *P. antipodarum* from a sandy bottom (up to a depth of 1.5 m) in each site was carried out using a metal sieve (mesh diameter 3 mm). Then the snails were transported to the laboratory in containers with lake water. The taxonomic affiliation of collected snails as *P. antipodarum* species was verified on the basis of morphological data (Piechocki and Wawrzyniak-Wydrowska, 2016).

2.2 Parasitological examination of snails

In each sample, we randomly isolated 200 individuals with a shell height of 4–6 mm from each research area for an autopsy. The snail shells were removed and the soft parts of the body were carefully examined for the presence of trematodes under a light microscope (Axio Lab.A1), whereas using the Axiocam 105 color camera and ZEN software, photographs and measurements were taken. The identification was made on the basis of the morphological characteristics of live parasite larvae. For this purpose, we used drawings, photos, and descriptions of parasites presented by various authors (Kanev, 1994; Gérard and Le Lannic, 2003; Faltýnková *et al*., 2007; Żbikowski and Żbikowska, 2009; Cichy and Żbikowska, 2016; Cichy *et al*., 2017).

The term "prevalence" is the percentage ratio of the number of infected individuals to the number of all collected *P. antipodarum* in a given water reservoir, calculated according to the formula: $P\ [\%] = n/N \times 100$ (P = prevalence, n = the number of infected individuals in a sample, N = the number of all collected individuals in a sample). The term "average intensity" is the average number of metacercariae recorded per infected individual, calculated according to the formula: $AI = S/n$ (AI = average intensity, S = the sum of all larvae in a sample, n = the number of infected individuals in a sample).

2.3 DNA extraction, amplification and sequencing of *P. antipodarum*

DNA extraction was performed for five individuals of *P. antipodarum* from each research area, with the exception of Lake Sosno, in the case of which, 3 specimens were tested, using the Sherlock AX Kit (A & A Biotechnology, Poland). PCR generated a fragment of the 16S ribosomal RNA gene using the two primers, *S1-Universal* (5′-CGGCCGCC TGTTTATCAAAAACAT-3′) and *S2-Potamo* (5′-GTGGTC GAACAGACCAACCC-3′) (Städler *et al*., 2005). A PCR reaction of each sample was performed in a 20 μl reaction mixture, consisting of 3 μl of template DNA, 0.6 μl of each primer, 2 μl of 10× buffer, 13 μl of ddH_2O, 0.6 μl of 20 mM dNTP (ThermoFisher Scientific, USA) and 0.2 μl of Taq-Polymerase (ThermoFisher Scientific, USA). PCR conditions consisted of 5 min initial denaturation at 92 °C, 30 s denaturation at 92 °C, followed by 60 s annealing at 55 °C, and 90 s elongation at 72 °C for 40 cycles followed by a final elongation step for 5 min at 72 °C. A 3 μl sample of PCR product was run on a 1.5% agarose gel for 30 min at 100 V to check DNA quality. PCR products were cleaned up by using Clean-up Kit (A&A Biotechnology, Poland). A sequencing reaction was performed in 10 μl of the reaction mixture, consisting of 2 μl of PCR product, 0.15 μl of primer, 1 μl of sequencing buffer (Brilliant Dye Terminator Sequencing Kit, Nimagen, The Netherlands), 5.85 μl of ddH_2O and 1 μl of Terminator (Brilliant Dye Terminator Sequencing Kit, Nimagen, The Netherlands). The sequencing programme consisted of four steps: 1 min initial denaturation at 96 °C, followed by 10 s denaturation at 96 °C, 5 sec annealing at 55 °C, and 4 min elongation at 60 °C for 25 cycles. Sequencing products were cleaned up by using ExTerminator (A&A Biotechnology, Poland) and sequenced in one direction. The sequencing reactions were performed in the Genomed company (Warsaw, Poland). Sequences were deposited in GenBank with the following accession numbers: MK578223, MK578224, MK578225, MK578226, MK578227.

2.4 Alignment and statistical analyses

All available mitochondrial 16S ribosomal RNA (rRNA) sequences of *P. antipodarum* were downloaded from GenBank (Tab. 2). These sequences together with 23 newly obtained sequences were aligned by using MAFFT version 7 (Katoh *et al*., 2002; Katoh and Toh, 2008). A 16S dataset (436 bp length) comprises sequences from 16 countries (Tab. 2). To estimate the population genetic parameters (such as the number of haplotypes, polymorphic sites (S), nucleotide diversity (π), haplotype diversity (H)) calculations were performed in DnaSP v.5.10 (Librado and Rozas, 2009). Median Joining haplotype network (436 bp length of alignment) was performed in PopART (Bandelt *et al*., 1999). The haplotype networks were constructed from all haplotype sequences presented in each studied population.

Table 2. Haplotypes of Potamopyrgus antipodarum in certain localities based on 16S ribosomal RNA gene.

Haplotype	Populations	GenBank accession number	References
1	Poland, Pond in Strzemieszyce	MK578223	this study
	Wielkie, Dąbrowa Górnicza	MK578224	this study
	Poland, Dolina Trzech Stawów, Katowice	MK578225	this study
		MK578226	this study
	Poland, Lake Iławskie, Dół	MK578227	this study
	Poland, Lake Sosno, Sosno		
	Poland, Lake Czaplino, Czaplinek		
	Germany	AY314009	Wilke, 2003 (GenBank, unpublished)
	France, River Doubs near Saunieres	JQ346709	Staneviciute *et al*., 2011 (GenBank, unpublished)
	Hungary, Balaton	JQ346708	Staneviciute *et al*., 2011 (GenBank, unpublished)
	Hungary, River Danube near Budapest	JQ346707	Staneviciute *et al*., 2011 (GenBank, unpublished)
	Poland, Lake Wigry	JQ346705	Staneviciute *et al*., 2011 (GenBank, unpublished)
		JQ346704	
	Lithuania, Curonian Lagoon	JQ346703	Staneviciute *et al*., 2011 (GenBank, unpublished)
	Lithuania, Lake Vilkoksnis	JQ346702	Staneviciute *et al*., 2011 (GenBank, unpublished)
	Spain	KU933009	Clusa *et al*., 2016
	UK, London, West India Dock	EU573989	Wilke and Ponder, 2008 (GenBank, unpublished)
	Denmark, Odder Å (Assedrup near Århus)	AY955376	Städler *et al*., 2005
	France, Slack Estuary (near Ambleteuse)		
	Germany, River Rhine (near Wiesbaden)		
	Germany, Görlitz		
	Germany, Lake Mindel (near Lake Constance)		
	Italy, Casarza Ligure (near Genova)		
	Netherlands, Damse Rawart (near Sluis)		
	Switzerland, Lake Zurich (Zurich)		
	UK, London (Bushy Park, SW London)		
	UK, Portmeirion Pond, Wales (near Portmadoc)		
	UK, River Thames (near Sonning)		
	UK, Colinton, Scotland (near Edinburgh)		
	UK, Tiree Island (Inner Hebrides)		
	UK, Loch of Stenness (Orkney)		
2	Estonia, Baltic Sea near Tallinn	JQ346706	Staneviciute *et al*., 2011 (GenBank, unpublished)
	USA, Oregon, Devil's Lake	JN639013	Hoy, 2011 (GenBank, unpublished)
	USA, Great Lakes	KY426909	Klymus *et al*., 2017
	Spain	KU933010	Clusa *et al*., 2016
	Belgium, Willem-Leopold-Polder (Knokke)	AY955393	Städler *et al*., 2005
	Belgium, Nieuve Watergang (Knokke)		
	France, Slack Estuary (near Ambleteuse)		
	UK, Harlech, Wales		
	UK, Loch of Stenness (Orkney)		
3	New Zealand	KU933004	Clusa *et al*., 2016
	New Zealand, Lake Tarawera	AY955391	Städler *et al*., 2005
4	New Zealand	KU933003	Clusa *et al*., 2016
	New Zealand, Lakes: Poerua, Mapourika, Paringa, Wanaka, Alexandrina, Forsyth, Marymere, Clearwater	AY955379	Städler *et al*., 2005

Table 2. (continued).

Haplotype	Populations	GenBank accession number	References
5	New Zealand, Lake Wairarapa	AY955392	Städler *et al.*, 2005
6	New Zealand, Lake Moeraki	AY955390	Städler *et al.*, 2005
7	New Zealand, Lake Paringa	AY955389	Städler *et al.*, 2005
8	New Zealand, Lakes: Paringa, Moeraki, Alexandrina	AY955388	Städler *et al.*, 2005
	New Zealand, Horseshoe Bay	AY634106	Haase, 2005
9	New Zealand, Lake Ianthe	AY955387	Städler *et al.*, 2005
10	New Zealand, Lake Ianthe	AY955386	Städler *et al.*, 2005
11	New Zealand, four small streams at Boat Harbour, Coromandel Peninsula; Lake Mapourika	AY955385	Städler *et al.*, 2005
	New Zealand, Browns	AY634104	Haase, 2005
12	New Zealand, Lake Waikaremoana	AY955384	Städler *et al.*, 2005
13	New Zealand, Lake Moeraki	AY955383	Städler *et al.*, 2005
14	New Zealand, Lake Marymere	AY955382	Städler *et al.*, 2005
15	New Zealand, Lake Alexandrina	AY955381	Städler *et al.*, 2005
16	New Zealand, Lakes: Moeraki, Forsyth	AY955380	Städler *et al.*, 2005
17	New Zealand, Waikato River, Hamilton	AY955378	Städler *et al.*, 2005
18	Tasmania, Tamar River, Launceston	AY955377	Städler *et al.*, 2005
19	New Zealand, Ruakuri Cave	AY634109	Haase, 2005
20	New Zealand	AY634107	Haase, 2005
21	New Zealand, Motu River	AY634080	Haase, 2005
22	New Zealand, Morere Springs	AY634079	Haase, 2005

Haplotypes were shown as black circles, where the size of the circle represents the number of populations where they are present. Haplotypes are identified by numbers with # marks, white circles without a number indicate a hypothetical intermediate haplotype which is necessary to link observed haplotypes. Hatch marks in the network represent single mutations.

3 Results

3.1 Molecular examination

Based on all 16S rRNA sequences of *P. antipodarum* obtained from our samples, we observed that all of them belong to the same haplotype, defined in this study as haplotype 1 (Tab. 2, Fig. 2). Together with all available mitochondrial 16S ribosomal RNA sequences of *P. antipodarum,* we identified 22 distinct haplotypes. Haplotype 1 and 2 were most frequent, present in 11 and 6 countries, respectively. Apart from Poland, haplotype 1 was present in Germany, France, Hungary, Lithuania, Spain, Denmark, Italy, the Netherlands, Switzerland and the United Kingdom (UK), whereas haplotype 2 occurs in Estonia, the USA, Spain, Belgium, France and the UK (Tab. 2). Haplotypes from 3 to 17 and from 19 to 22 were restricted to water bodies of New Zealand, while haplotype 18 occurs in Tasmania (Australia). Population genetic parameters for *P. antipodarum* were S = 22; $\pi = 0.00804$; H = 1.000.

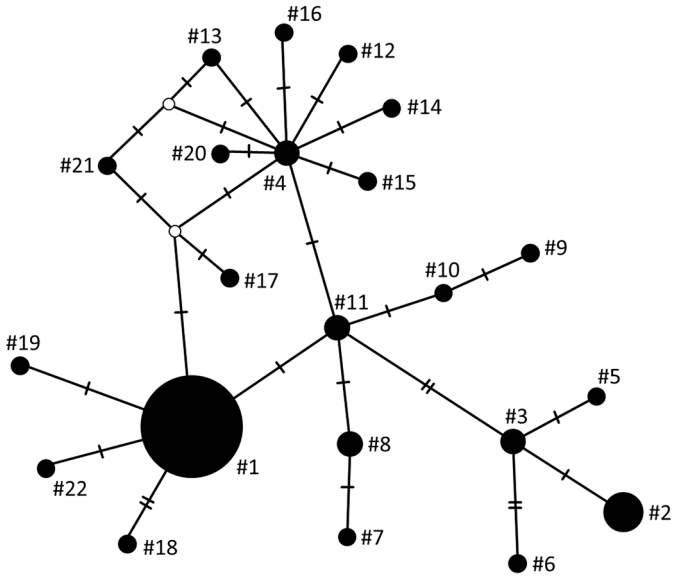

Fig. 2. Haplotype Median Joining network for the 16S ribosomal RNA haplotypes of *Potamopyrgus antipodarum*. Haplotypes are represented by black circles, the size of which is proportional to the number of populations in which a particular haplotype is present. Populations are listed in Table 2. Haplotypes are from #1 to #22; white circles without a number indicate a hypothetical intermediate haplotype linking observed haplotypes. Hatch marks in the network represent single mutations.

Table 3. Infection of metacercariae in *Potamopyrgus antipodarum*.

Study sites	No. of examined snails	No. of infected snails	Metacercariae prevalence (%)	Metacercariae type	Average intensity of metacercariae infection
Lake Czaplino	200	1	0.5	I	1
Lake Iławskie	200	2	1	II	1.5
Lake Sosno	200	2	1	III	2.5
Pond in Strzemieszyce Wielkie	200	12	6	II	1.33
Valley of three ponds area in Katowice	200	0	0	–	–

3.2 Parasitological examination

We found three morphological types of metacercariae (Fig. SM 1, Tab. 3). They differed both in diameter and in the presence/absence of collar spines. The metacercaria of type I (from Lake Czaplino) was characterized by the outer diameter of cyst: 118 × 116 μm. Inside the cyst, we observed two suckers and we could not observe a stylet or collar spines. The metacercariae of type II were found in snails from Lake Iławskie and Pond in Strzemieszyce Wielkie, and they had the outer diameter of cysts: 149 (SE 0.9) × 148 (SE 0.7) μm and 150 (SE 0.5) × 148 (SE 0.5) μm, respectively. The metacercariae of type III recorded in Lake Sosno were much larger than the others (261 (SE 0.6) × 260 (SE 0.7) μm in diameter). All the larvae also had two rows of collar spines, but 4 spines at both edges of the collars were much larger than the other spines. The presence of these larvae was recorded in specimens from four out of the five investigated water bodies (Tab. 3). The prevalence ranged from 0.5% to 6%, and the average intensity from 1 to 2.5 larvae per one host snail (Tab. 3).

4 Discussion

A single parthenogenetic individual of *P. antipodarum* can colonize a new area by itself (Schreiber *et al.*, 1998). These populations consist of "snail clones" because only new introductions and/or mutations can increase the diversity of the gene pool (Weetman *et al.*, 2006). As a result, snail populations living outside their native range are characterized by low genetic diversity (Alonso and Castro-Díez, 2012). All *P. antipodarum* used in our studies represented the same haplotype, despite the fact that they came from five water bodies which are located at a substantial distance from each other. Our data is the first demonstration of the presence of 16S rRNA haplotype in Poland. In the case of the whole Europe, there are several clonal lineages presumably created as a consequence of the long invasion history (Alonso and Castro-Díez, 2012). Our analysis showed that the haplotype found in our research is the most widespread in Europe.

Potamopyrgus antipodarum individuals from our study were infected only with metacercariae. In New Zealand, *P. antipodarum* is widely used by many species of digenean trematodes not only as the second but also as a strongly specific first intermediate host (Hechinger, 2012). For example, Hechinger (2012) listed 20 species of digenean trematodes for which *P. antipodarum* plays the role of an intermediate host in its native area, with the prevalence up to 7%. By contrast, outside the native range, pre-patent or patent infection (infections with sporocysts, rediae or immature cercariae) is extremely rare (Evans *et al.*, 1981; Gérard and Le Lannic, 2003; Żbikowski and Żbikowska, 2009). Recorded cases of such digenean infections found in *P. antipodarum* are the result of an introduction from their native areas (Gérard *et al.*, 2017). We recorded a low prevalence of snails infected with metacercariae, similar to our previous study presented by Cichy *et al.* (2017). Because too few morphological features have been observed to identify the species of metacercariae found in Lake Czaplino, we were unable to determine whether it posed a medical or veterinary threat. Other metacercariae detected in *P. antipodarum* from our study were identified to belong to the family Echinostomatidae (Kanev, 1994; Faltýnková *et al.*, 2007; Cichy and Żbikowska, 2016). Given the characteristics of metacercariae, we suspect that these larvae represent the genus Echinoparyphium and/or Echinostoma (Kanev, 1994; Faltýnková *et al.*, 2007; Cichy and Żbikowska, 2016). There are several species of Echinostoma (*e.g. E. revolutum*, *E. paraulum*) and Echinoparyphium (*e.g. E. recurvatum*, *E. mordwilkoi*) in Europe, which at the stage of metacercaria cannot be distinguished without molecular methods (Georgieva *et al.*, 2014). Members of these genera are quite common in the environment and use common snail species as first or second intermediate hosts (Cichy and Żbikowska, 2016). Echinostomes are widespread flukes causing intestinal diseases and they are mainly important in wildlife diversity (Saijuntha *et al.*, 2010).

In conclusion, our research indicates the presence of only one 16S rRNA haplotype of *P. antipodarum* in Poland. *Potamopyrgus antipodarum* outside its natural occurrence is used as the second intermediate host of digenean trematodes; however, the detection of the association between the mtDNA haplotype of *P. antipodarum* and metacercarial infection should be investigated in detail in the future.

Supplementary Material

Fig. SM 1. Metacercariae recorded in Potamopyrgusantipodarum from - A) Lake Czaplino, B) Lake Sosno, C) Pond in StrzemieszyceWielkie, Poland.

The Supplementary Material is available at https://www.kmae-journal.org/10.1051/kmae/2020040/olm.

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Author contribution statement

Anna Stanicka and Elżbieta Żbikowska designed the study. Anna Stanicka, Kamila Stefania Zając, Dorota Lachowska-Cierlik, Anna Cichy and Janusz Żbikowski executed the study. Anna Stanicka and Kamila Stefania Zając analyzed and interpreted the data and wrote the manuscript. Elżbieta Żbikowska provided substantive contributions and critical review.

Conflicts of interest

The authors declare no conflict of interest.

References

Alexandre da Silva MV, Nunes Souza JV, Souza JRB, Vieira LM. 2019. Modelling species distributions to predict areas at risk of invasion by the exotic aquatic New Zealand mud snail *Potamopyrgus antipodarum* (Gray 1843). *Freshw Biol* 64: 1504–1518.

Alonso A, Castro-Díez P. 2008. What explains the invading success of the aquatic mud snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca)? *Hydrobiologia* 614: 107–116.

Alonso Á, Castro-Díez P. 2012. The exotic aquatic mud snail *Potamopyrgus antipodarum* (Hydrobiidae, Mollusca): state of the art of a worldwide invasion. *Aquat Sci* 74: 375–383.

Bandelt HJ, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol* 16: 37–48.

Boycott AE. 1936. The habitats of fresh-water Mollusca in Britain. *J Anim Ecol* 5: 116–186.

Cichy A, Żbikowska E. 2016. Atlas of Digenea developmental stages: The morphological characteristics and spread within the populations of freshwater snails from the Brodnickie Lakeland, Poland, NCU Press, Torun.

Cichy A, Marszewska A, Parzonko J, Żbikowski J, Żbikowski E. 2017. Infection of *Potamopyrgus antipodarum* (Gray, 1843) (Gastropoda: Tateidae) by trematodes in Poland, including the first record of aspidogastrid acquisition. *J Invertebr Pathol* 150: 32–34.

Cichy A, Marszewska A, Ciapka P, Żbikowska E. 2019. Different thermal conditions of lakes affect host–parasite systems: a case study of *Viviparus contectus* (Millet, 1813) and digenean trematodes. *Freshw Biol* 42: 417–425.

Clusa L, Ardura A, Gower F, Miralles L, Tsartsianidou V, Zaiko A, Garcia-Vazquez E. 2016. An easy phylogenetically informative method to trace the globally invasive *Potamopyrgus* mud snail from river's eDNA. *PLoS One* 11: e0162899.

Collado GA. 2014. Out of New Zealand: molecular identification of the highly invasive freshwater mollusk *Potamopyrgus antipodarum* (Gray, 1843) in South America. *Zool Stud* 53: 1821.

Dorgelo J. 1987. Density fluctuations in populations (1982–1986) and biological observations of *Potamopyrgus jenkinsi* in two trophically differing lakes. *Hydrobiol Bull* 21: 95–110.

Evans NA, Whitfield PJ, Dobson AP. 1981. Parasite utilization of a host community: the distribution and occurrence of metacercarial cysts of *Echinoparyphium recurvatum* (Digenea: Echinostomatidae) in seven species of mollusc at Harting Pond, Sussex. *Parasitology* 83: 1–12.

Faltýnková A, Nasincová V, Kablásková L. 2007. Larval trematodes (Digenea) of the great pond snail, *Lymnaea stagnalis* (L.), (Gastropoda, Pulmonata) in Central Europe: a survey of species and key to their identification. *Parasite* 14: 39–51.

Georgieva S, Faltýnková A, Brown R, Blasco-Costa I, Soldánová M, Sitko J, Scholz T, Kostadinova A. 2014. *Echinostoma 'revolutum'* (Digenea: Echinostomatidae) species complex revisited: species delimitation based on novel molecular and morphological data gathered in Europe. *Parasit Vectors* 7: 520.

Gérard C, Le Lannic J. 2003. Establishment of a new host–parasite association between the introduced invasive species *Potamopyrgus antipodarum* (Smith) (Gastropoda) and *Sanguinicola* sp. Plehn (Trematoda) in Europe. *J Zoology* 261: 213–216.

Gérard C, Miura O, Lorda J, Cribb TH, Nolan MJ, Hechinger RF. 2017. A native-range source for a persistent trematode parasite of the exotic New Zealand mud snail (*Potamopyrgus antipodarum*) in France. *Hydrobiologia* 785: 115–126.

Haase M. 2005. Rapid and convergent evolution of parental care in hydrobiid gastropods from New Zealand. *J Evol Biol* 18: 1076–1086.

Hechinger RF. 2012. Faunal survey and identification key for the trematodes (Platyhelminthes: Digenea) infecting *Potamopyrgus antipodarum* (Gastropoda: Hydrobiidae) as first intermediate host. *Zootaxa* 3418: 1–27.

Hauswald AK, Remais JV, Xiao N, Davis GM, Lu D, Bale MJ, Wilke T. 2011. Stirred, not shaken: genetic structure of the intermediate snail host *Oncomelania hupensis robertsoni in* an historically endemic schistosomiasis area. *Parasit Vectors* 4: 206.

Jacobsen R, Forbes VE. 1997. Clonal variation in life-history traits and feeding rates in the gastropod, *Potamopyrgus antipodarum*: performance across a salinity gradient. *Funct Ecol* 11: 260–267.

Johnson PTJ, Olden JD, Vander Zanden MJ. 2008. Dam invaders: impoundments facilitate biological invasions into freshwaters. *Front Ecol Environ* 8: 357–363.

Kanev I. 1994. Life-cycle, delimitation and redescription of *Echinostoma revolutum* (Froelich, 1802) (Trematoda: Echinostomatidae). *Syst Parasitol* 125–144.

Katoh K, Toh H. 2008. Recent developments in the MAFFT multiple sequence alignment program. *Brief Bioinform* 9: 286–298.

Katoh K, Misawa K, Kuma K, Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Res* 30: 3059–3066.

Kelly DW, Paterson RA, Townsend CR, Poulin R, Tompkins DM. 2009. Parasite spillback: a neglected concept in invasion ecology? *Ecology* 90: 2047–2056.

Klymus KE, Marshall NT, Stepien CA. 2017. Environmental DNA (eDNA) metabarcoding assays to detect invasive invertebrate species in the Great Lakes. *PLOS One* 12: e0177643.

Larson MD, Krist AC. 2019. Trematode prevalence and an invasive freshwater snail: fewer infections and parasites likely contribute to the success of an invasive snail. *Biol Invasions* 48: 275.

Larson MD, Dewey JC, Krist AC. 2020. Invasive *Potamopyrgus antipodarum* (New Zealand mud snails) and native snails differ in sensitivity to specific electrical conductivity and cations. *Aquat Ecol* 54: 103–117.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451–1452.

Marszewska A, Cichy A, Bulantová J, Horák P, Żbikowska E. 2018a. Potamopyrgus antipodarum as a potential defender against swimmer's itch in European recreational water bodies-experimental study. *PeerJ* 6: e5045.

Marszewska A, Strzała T, Cichy A, Dąbrowska GB, Żbikowska E. 2018b. Agents of swimmer's itch-dangerous minority in the Digenea invasion of Lymnaeidae in water bodies and the first report of *Trichobilharzia regenti* in Poland. *Parasitol Res* 117: 3695–3704.

Morgan-Richards M, Trewick SA, Stringer IAN. 2010. Geographic parthenogenesis and the common tea-tree stick insect of New Zealand. *Mol Ecol* 19, 1227–1238.

Nentwig W, Bacher S, Kumschick S, Pyšek P, Vilà M. 2018. More than "100 worst" alien species in Europe. *Biol Invasions* 20: 1611–1621.

Piechocki A, Wawrzyniak-Wydrowska B. 2016. Guide to freshwater and marine Mollusca of Poland. Poznan: Bogucki, 280 p.

Saijuntha W, Sithithaworn P, Andrews RH. 2010. Genetic differentiation of *Echinostoma revolutum* and *Hypodereaum conoideum* from domestic ducks in Thailand by multilocus enzyme electrophoresis. *J Helminthol* 84: 143–148.

Schreiber ESG, Glaister A, Quinn GP, Lake PS. 1998. Life history and population dynamics of the exotic snail *Potamopyrgus antipodarum* (Prosobranchia: Hydrobiidae) in Lake Purrumbete, Victoria, Australia. *Mar Freshwater Res* 49: 73.

Städler T, Frye M, Neiman M, Lively CM. 2005. Mitochondrial haplotypes and the New Zealand origin of clonal European *Potamopyrgus*, an invasive aquatic snail. *Mol Ecol* 14: 2465–2473.

Tantrawatpan C, Saijuntha W, Bunchom N, Suksavate W, Pilap W, Walalite T, Agatsuma T, Tawong W, Sithithaworn P, Andrews RH, Petney TN. 2020. Genetic structure and geographical variation of *Bithynia siamensis goniomphalos* sensu lato (Gastropoda: Bithyniidae), the snail intermediate host of *Opisthorchis viverrini* sensu lato (Digenea: Opisthorchiidae) in the Lower Mekong Basin revealed by mitochondrial DNA sequences. *Int J Parasitol* 50: 55–62.

Urbański J. 1935. Dwa ciekawe gatunki ślimaków w Wielkopolsce. *Wydawnictwa Okręgowej Komisji Ochrony Przyrody w Poznaniu* 5: 108–114.

Weetman D, Hauser L, Carvalho GR. 2006. Heterogeneous evolution of microsatellites revealed by reconstruction of recent mutation history in an invasive apomictic snail, *Potamopyrgus antipodarum*. *Genetica* 127: 285–293.

Żbikowski J, Żbikowska E. 2009. Invaders of an invader-trematodes in *Potamopyrgus antipodarum* in Poland. *J Invertebr Pathol* 101: 67–70.

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