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Invaders of an invader – Trematodes in *Potamopyrgus antipodarum* in Poland

Janusz Żbikowski [a], Elżbieta Żbikowska [b,*]

[a] Department of Hydrobiology, Institute of Ecology and Environmental Protection, Nicolaus Copernicus University Toruń, Poland
[b] Department of Invertebrate Zoology, Institute of General and Molecular Biology, Nicolaus Copernicus University Toruń, Poland

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ABSTRACT

The subject of the following study was the natural and experimental invasion of trematode larvae in *Potamopyrgus antipodarum* from Bory Tucholskie National Park (Poland). Only one out of the 14,908 dissected specimens had oval sporocysts and mature cercariae of fish fluke, which belongs to the Sanguinicolidae family. It is the first recorded case in the European population of *P. antipodarum* living in inland water. The experimental study showed the possibility of native metacercariae (*Echinostoma revolutum, Echinoparyphium aconiatum* and *Hypoderaeum conoideum*) settlement in those immigrant snail species; however, exposure to parasites resulted in an increase in snail mortality. The three out of six used cercariae species were able to transform into metacercariae in *P. antipodarum* as in the second intermediate host, but the exposure to parasitic larvae of four of the used species resulted in an increase in snails' mortality. It may suggest that not only metacercariae settlement but also the attack of cercariae (*Rubenstrema opisthovitellinum* at a temperature of 22 °C) affected the low survival of experimental snails in comparison to control animals. The subject of discussion presented in this paper is also the hypothesis on probable effect of the interaction between *P. antipodarum* and native snail species (as a source of invasive larvae of parasites) living in the same habitat.

1. Introduction

Potamopyrgus antipodarum (Gray, 1843) is nowadays one of the common prosobranch snail species in Europe (Kerney, 1999; Morley, 2008). This mollusc came from New Zealand over 100 years ago (Kerney, 1999). The introduction, which has been considered, occurred via shipping from New Zealand ports. Snails were probably taken into containers with fresh or ballast water, and were liberated in European harbours during washing or filling of tanks (Ponder, 1988; Morley, 2008). In Poland, snails of this species were first noted by Urbański (1938) in Trląg Lake (northern Poland) and have spread in different types of water bodies: lakes, ponds, streams, dams, etc. Their abundance in the shore-zone can be very high, and their density can reach 30,000/m^2 (Jeleń Lake, northern Poland) (Piechocki and Kaleta, 2001). The expansion of *P. antipodarum* into new lands results from its high tolerance to water salinity, capability of parthenogenetic reproduction and small size (Ponder, 1988; Piechocki and Kaleta, 2001; Gerard and Dussart, 2003; Vinson, 2004; Kerans et al., 2005). A very interesting phenomenon is that in New Zealand the natural invasion of trematodes into *P. antipodarum* is very common. Fourteen trematodes are known to use this snail species as an intermediate host, and the prevalence of invasion in some snail populations exceeds 80% (Winterbourn, 1973). In Europe there is only one finding of natural trematode invasion of this snail species used as the first intermediate host (Gerard and Le Lannic, 2003). Authors suggest that the parasite which was found in the *P. antipodarum* community from four localities in the Mont Saint Michel Bay belongs to *Sanguinicola* sp. They considered that this species was probably a native and used the immigrant snail host to create a new snail–trematode association. Morley (2008) suggests an alternative interpretation for the occurrence of this parasite in European *P. antipodarum* populations. The author postulates that this cercaria belonging to the Sanguinicolidae family may be of the eel blood fluke *Paracardicoloides yamagutii*, a common parasite in New Zealand. Both interpretations are valid and, the answer to this problem needs the continuation of studies in different parts of Europe. On the other hand, there are more reports in Europe concerning *P. antipodarum* as a second intermediate host of trematodes (Evans et al., 1981; Morley, 2008), but still no detailed information on the prevalence and intensity of invasion in snail populations (Morley, 2008).

Considering the necessity of studies in different European countries on *P. antipodarum*–trematodes interaction, the main aim of our research was to check: (1) the prevalence of natural flukes invasion in several populations of this snail species and (2) the possibility of experimental invasion of *P. antipodarum* by cercariae emerged from common snail species (*Lymnaea stagnalis* and *Planorbarius corneus*) in Poland.

* Corresponding author.
E-mail addresses: jzbikow@biol.uni.torun.pl (J. Żbikowski), ezbikow@biol.uni.torun.pl (E. Żbikowska).

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2. Materials and methods

2.1. Field studies

The snails were collected from 15 locations in water bodies of Bory Tucholskie National Park in northern Poland (53°60′N, 17°50′E). There were localities in the shore-zone of eight inland (freshwater) lakes and seven localizations in a small stream Struga Siedmiu Jezior connecting those lakes. The samples were gathered with a metal sieve (1 mm) monthly from May to September 2006–2007. All 10 samples were taken from shallow parts of water bodies. In the lakes there was a depth of 1 m, and in the stream 0.1 m. The water temperature during the snails' sampling depended on the season and ranged from +16 °C in May and September to +22 °C in July and August. The samples were transported in boxes to the laboratory, and the snails were systematically identified under a stereoscopic microscope and measured with an electronic caliper (0.1 mm). All sampled individuals were checked as potential trematode hosts. At the beginning, for the non-invasive method of parasite detection every tenth snail was placed in small dishes filled with 1 ml of tap water and exposed to white light for 1 h. After that, all the individuals which did not release cercariae were subsequently dissected. The presence of embryos in snails was also checked. The prevalence of parasites was calculated as the percentage of infected snails in the group of collected animals.

2.2. Experimental invasion

Two groups of *P. antipodarum* individuals (240 individuals each) from Bory Tucholskie were sampled additionally in September 2007, and were exposed to cercariae invasion: small individuals of 2.75 ± 0.3 mm, and large individuals of 5.0 ± 0.2 mm shell height. Cercariae of six parasite species were used: *Echinostoma revolutum* (Frolich, 1802), *Echinoparyphium aconiatum* Dietz, 1909, *Hypoderaeum conoideum* (Bloch, 1782), *Paryphostomum radiatum* (Dujardin, 1845) (freshly emerged from naturally infected *L. stagnalis* (Linnaeus, 1758) specimens) and *Rubenstrema opisthovitellinum* Soltys, 1954, *Tylodelphys excavata* (Rudolphi, 1803) (from *P. corneus* (Linnaeus, 1758)). The parasites under study are very common trematode species in host snail populations in Poland, and all of them have a second intermediate host in their life cycle (Nasincova, 1992; Żbikowska, 2007). The invasion experiment was conducted in two thermal variants: +22 °C and +4 °C. The first one, +22 °C was the highest measured temperature during the field study, and +4 °C was used to underline the cold effect on cercariae invasion into the second intermediate host and on the host survival.

Twenty snails of each experimental group were placed in 10 ml beakers filled with tap water, and then 500 freshly emerged cercariae of the same trematode species were added. The individuals placed in dishes without parasitic larvae served as a control group. After 24 h of exposition the snails were removed, rinsed and placed in conditioned tap water for further observation. The snails under study were kept without food, and every 24 h the water in the beakers was changed, and all dead individuals were dissected. The presence of metacercariae in snail bodies was checked. The observation was conducted until the death of the last snail.

In statistical analysis data concerning only 436 out of 480 experimental snails were used: all control individuals and those exposed to *P. radiatum, T. excavata* or *R. opisthovitellinum*, and only hosts of *E. aconiatum, E. revolutum* or *H. conoideum* metacercariae. Average snail longevity for each group was calculated. Initial observations showed no differences between small and large snails (pair-wise *t*-tests, $p > 0.05$) so these two groups were connected to enhance the statistical power of the ANOVA test. In ANOVA analysis two factors were taken into account: parasitic invasion and temperature.

3. Results

3.1. Field studies

During studies in 2006 – 2336 and in 2007 – 12,572 *P. antipodarum* individuals were collected. The most of them (71%) had 3.3–5.2 mm of shell height. All the snails were dissected. None individual was infected by metacercariae, and only one specimen, which was found in August 2006 in Charzykowskie Lake (prevalence 3.3%), had sporocysts and active swimming cercariae. The infected snail had 5.0 mm of shell height. In its dissected body there were no host embryos, but there were seven regular oval sporocysts and dozens of cercariae. On the morphological description of cercaria we classified this larva as the parasite belonging to the Sanguinicolidae family. The furco-lophocercous cercaria was non-ocellate, apharyngate, sucker-less and had a large fold on the dorsal part of the body and two fin-folds on furca (Fig. 1). A biometrical description of sporocyst and cercaria is presented in Table 1.

3.2. Metacercariae settlement

Three out of six trematode species used in laboratory experiments were able to penetrate the snail body, and to create metacercariae: *E. aconiatum, H. conoideum* and *E. revolutum*. The average number of metacercariae found in snails experimentally exposed to cercariae is presented in Table 2.

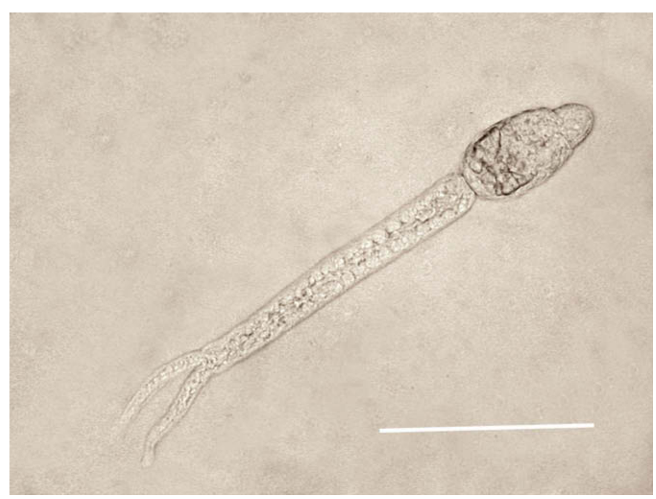

Fig. 1. Light microscopy photograph of cercaria found in *Potamopyrgus antipodarum* from Charzykowskie Lake. Scale bar = 100 μm.

Table 1
Measurements of living larval stages of parasite found in dissected *Potamopyrgus antipodarum* individual from Charzykowskie Lake.

	Mean size (μm)	SD	Number of larvae measured
Cercaria			
Body length	74	2.5	30
Tail stem length	136	5.7	30
Tail furca length	93	3.1	30
Sporocyst			
Longer diameter	288	8.9	7
Shorter diameter	141	4.6	7

SD – standard deviation.