

First report of endosymbionts in *Dreissena polymorpha* from Sweden

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Abstract

We report the first results of parasitological study of *Dreissena polymorpha* (zebra mussels) from Sweden. The samples of *Dreissena* were collected from Lake Erken in July 2007. The most common endosymbionts found in the mantle cavity of the mussels were the commensal ciliate *Conchophthirus acuminatus* and free-living nematodes *Chromadorina bioculata* and *Laimydorus* sp. Unidentified chironomid larvae and aquatic mites were also occasionally observed in the mantle cavity. The finding of host-specific ciliate *C. acuminatus* suggests that zebra mussels invaded Lake Erken at the juvenile or adult stage.

Key words: *Chromadorina bioculata*, *Conchophthirus acuminatus*, *Dreissena polymorpha*, *Laimydorus*, Lake Erken, Sweden

The Ponto-Caspian invasive mollusc *Dreissena polymorpha* (Pallas, 1771) is known to serve as the sole or intermediate host for more than 45 taxonomically different endosymbionts, including pathogenic helminth parasites of fish and waterfowl (Molloy et al. 1997; Karatayev et al. 2000; Mastitsky and Gagarin 2004; Mastitsky and Samoilenko 2005). During their own dispersal zebra mussels may play a role as vector for their symbiotic organisms and, in addition, may become hosts to indigenous endosymbionts (Karatayev et al 2000; Mastitsky et al. 2006). As a result, the invasion of zebra mussels into a waterbody may lead to simultaneous co-introduction of several species of their endosymbionts and, at least theoretically, to worsening of the parasitological situation in the invaded waterbody. Information on *D. polymorpha*

endosymbionts, however, is full of gaps, which makes it difficult to assess ecological risks associated with invasions of this mollusc. Several research directions have been proposed to fill these gaps, including extensive studies on geographical distribution and taxonomical diversity of the zebra mussels' parasites and commensals (Mastitsky et al. 2006). Such studies are currently being performed by the International Research Consortium on Molluscan Symbionts (IRCOMS, <http://www.nysm.nysed.gov/ircoms/>), an association of over a dozen scientists from Europe and North America. As a part of the IRCOMS research programme, we conducted the first parasitological study of *D. polymorpha* from Sweden. The mollusc appeared in this country in the 1920s, when introduced into Lake Mälaren. Subsequent spread of the zebra mussels across

Sweden has been very slow and to date they are known to have invaded only three more waterbodies, i.e. Lake Hjälmaren, Lake Erken and Lake Björklinge-Långsjön (Nadaffi 2007).

The mussels for our study were collected from Lake Erken on 1-5 July 2007. This lake is a

mesotrophic, dimictic waterbody located in south-eastern Sweden. Its surface area is 24 km², the mean depth is 9 m, and the maximum depth is 21 m. *Dreissena polymorpha* invaded Lake Erken in 1975, and currently its mean density is about 1700 ind./m² (Nadaffi 2007).

Table 1. Characteristics of the sampling sites

Site #	Coordinates		Substrate	Mean <i>Dreissena</i> shell length, mm (\pm SD)
	Latitude, N	Longitude, E		
1	59°50'12	18°37'44	Stones	17.3 \pm 0.2 ($n = 157$)
2	59°50'21	18°37'43	Stones within a bed of macrophytes	18.1 \pm 0.2 ($n = 199$)
3	59°51'20	18°35'56	Stones overgrown by filamentous algae	16.0 \pm 0.3 ($n = 150$)

Samples of approximately 300 mussels were collected with a scraper (mesh size 1 mm) from a depth of 0.5-0.8 m at three littoral sites of the eastern lake basin (Table 1), stored at room temperature in containers with lake water, and dissected within 48 h. As the level of endosymbionts burden greatly depends on *Dreissena* size (e.g., Burlakova et al. 1998), the size frequency distributions of the mussel's populations at sampling sites were taken into account prior to dissections. Based on the shell length, at least 150 molluscs from each site were sorted into size classes with 5.0 mm intervals. Seven mussels were then randomly taken from the most abundant class; the number of individuals from the other classes was determined according to their proportion in the entire population at a site. In total, 17 mussels were dissected from each sampling site in accordance to the protocol of Karatayev et al. (2000). The intensity of infection with the ciliate *Conchophthirus acuminatus* Claparède et Lachmann, 1858 was estimated using the following rank scale: 'Low' (<100 ciliates/ mussel), 'Moderate' (ca. 100-1000 ciliates/ mussel), 'High' (ca. 1000-3000 ciliates/mussel) and 'Very high' (>3000 ciliate/mussel). Other observed macroendosymbionts were counted totally. The nematodes found were collected and preserved in 70% ETOH for subsequent taxonomical identification. Statistical analysis of obtained data was performed using StatXact-4 software (Cytel Software Corporation 1998). Differences between compared groups were considered statistically significant at $P < 0.05$.

Conchophthirus acuminatus

The commensal ciliate *C. acuminatus* (Figure 1) occurred in the mantle cavity of *D. polymorpha* from all inspected samples. The prevalence of infection was very high and only slightly varied between sites, i.e. from 88.2% (site 2) to 100%

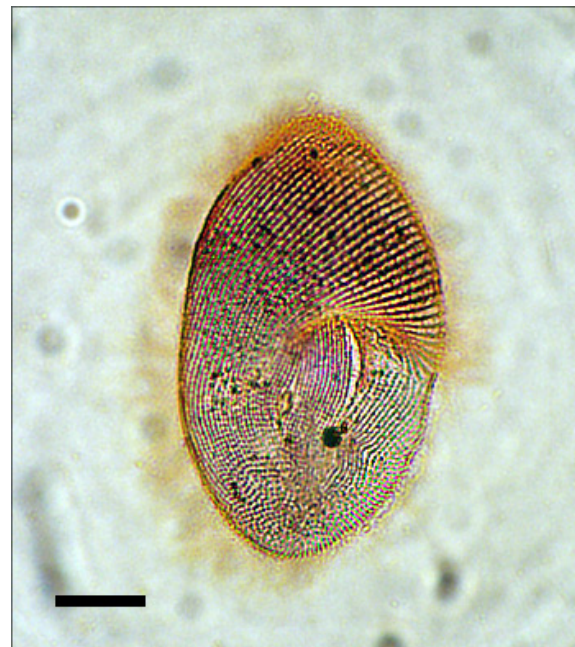


Figure 1. General view of the ciliate *Conchophthirus acuminatus* isolated from *Dreissena polymorpha* inhabiting Lake Erken. Impregnated with silver nitrate. Scale bar is 20 μ m. Photo by S. Mastitsky

(sites 1 and 3) (Monte-Carlo estimate of $P = 0.32$, Fisher-Freeman-Halton exact test). The intensity of *C. acuminatus* infection in most mussels was moderate to high (Table 2) and, in contrast to prevalence, statistically significantly differed between sites (Monte-Carlo estimate of $P = 0.025$, Fisher-Freeman-Halton exact test). The highest intensity was observed at site 1, where about 60% of the mussels harboured approximately 1000 to 3000 ciliates each (Table 2).

Table 2. Intensity of *D. polymorpha* infection with the ciliate *C. acuminatus* (% mussels having a given rank)

Intensity rank	Site # (as in Table 1)		
	1	2	3
Low	0.0	20.0	0.0
Moderate	29.4	46.7	16.7
High	58.8	33.3	55.5
Very high	11.8	0.0	27.8

Table 3. Prevalence and intensity of *D. polymorpha* infection with nematodes

Parameter	Site # (as in Table 1)		
	1	2	3
Prevalence, %	82.4	82.4	16.7
Median intensity, ind./mussel	3.5	3	2

Conchophthirus acuminatus is known to be a highly host-specific commensal ciliate with wide distribution among European populations of *D. polymorpha* (reviewed in Molloy et al. 1997; Karatayev et al. 2007). Denmark is the closest Scandinavian country to Sweden where these ciliates have been recorded (Fenchel 1965). The finding of *C. acuminatus* in Lake Erken supports the pattern of their wide European distribution. At the same time, the ciliate has never been found in North American populations of *D. polymorpha*, which suggests that zebra mussels invaded this continent as larvae – a life stage which is simply too small to be a host of *C. acuminatus* (Karatayev et al. 2000). Our data suggest that Lake Erken was invaded by at least juvenile zebra mussels, some of which were harbouring *C. acuminatus* infection.

Amongst several ecological factors positively affecting the intensity of *C. acuminatus* infection, size of the mussels is one of the most important (Burlakova et al. 1998; reviewed in Karatayev et al., 2007). In Lake Erken, however, we did not observe the positive correlation between *D. polymorpha* shell length and intensity of *C. acuminatus* infection. For example, the largest mussels were collected at site 2 (Table 1) but most of them (about 47%, Table 2) were just moderately infected there (i.e., 100 to 1000 ciliates/mussel). Thus, some other factors, like microhabitat differences in temperature regime, could have a greater impact on the level of *C. acuminatus* infection.

Nematodes

Live nematodes were found in the mantle cavity of *D. polymorpha* from all inspected samples, with up to 24 worms per individual mussel. The prevalence of infection significantly varied between sites (Table 3; Monte-Carlo estimate of $P < 0.001$, Fisher-Freeman-Halton exact test), while the intensity of infection did not (Monte-Carlo estimate of $P = 0.093$, Kruskal-Wallis ANOVA by ranks). The intensity of *D. polymorpha* infection with nematodes in Lake Erken was similar to that observed in a number of other European waterbodies (e.g., Karatayev et al. 2000; Mastitsky and Gagarin 2004). The prevalence of infection at sites 1 and 2, however, was unusually high for the summer period. Congruent high percentage of infected mussels has previously been recorded only for the autumn-winter period in the Drozdy Reservoir, Belarus (Karatayev et al. 2003).

All 65 nematodes collected during dissections appeared to be free-living species commonly found in the periphyton of macrophytes' surfaces. Two of them (3.1%) were *Laimydorus* sp., while the other 63 (96.9%) were *Chromadorina bioculata* (Schultze in Carus, 1857). Similar conspicuous dominance of *C. bioculata* and some other chromadorids (e.g., *C. leuckarti* (de Man, 1876) and *Punctodora ratzeburgensis* (Linstow, 1876)) has previously been observed among free-living nematodes infecting *D. polymorpha* in several Belarusian waterbodies (Karatayev et al. 2003; Mastitsky and Gagarin 2004; S. Mastitsky and V. Gagarin, unpublished data). Thus, our Lake Erken data are further evidence that, in contrast to other nematode species reported from *D. polymorpha*,

C. bioculata and its congeners have a rather high affinity to the mollusc. As suggested by Mastitsky and Gagarin (2004), such affinity can be explained by the oxyphilic nature of chromadorids. Oxygenation of the water caused by filtration activity of zebra mussels may attract these nematodes to *Dreissena* aggregations, and then the worms can inadvertently enter the mantle cavity of a mussel by being sucked in through its inhalant siphon. Alternatively, free-living nematodes may penetrate into the mantle cavity by crawling among byssal threads of the mussels (Molloy et al. 1997).

Other organisms

At sampling site 1, we found an unidentified chironomid larva in the mantle cavity of a mussel 21.5 mm long. Also, a single unidentified aquatic mite was found in the mantle cavity of a mussel 20.8 mm long at site 2. As with nematodes, the penetration of chironomids and mites into the mantle cavity of zebra mussels may occur due to filtration activity of the mollusc (e.g., Mastitsky and Samoilenko 2005).

Concluding remarks

Our data suggest that zebra mussels invaded Lake Erken at the juvenile or adult stage and were co-introduced with at least one more alien species, i.e., their host-specific commensal ciliate *C. acuminatus*. Other organisms found in this study are likely to be indigenous free-living species, which inhabited the lake before the *D. polymorpha* invasion. The zebra mussels in our samples were not infected with trematodes that use waterfowl and fishes as final hosts. This suggests that since its invasion in 1975, *D. polymorpha* has not resulted in the worsening of the parasitological situation in Lake Erken. Further observations, however, could be helpful in supporting this suggestion as our conclusions are based on a single sampling effort implemented within a limited part of the lake.

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