

AN ATTEMPT TO DETERMINE THE INTERMEDIATE HOST
FOR *POMPHORHYNCHUS LAEVIS* (ACANTHOCEPHALA)
IN THE BALTIC SEA

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Background. A number of fish species in the Baltic Sea are known as definitive hosts for *Pomphorhynchus laevis* but it is unclear which of the *Gammarus* species is the intermediate host of this parasite. The aim of the present paper was to identify this host in brackish waters of the Baltic Sea.

Material and methods. A total of 531 scuds (*G. salinus*, *G. zaddachi*, and *G. duebeni*) were collected from the Gulf of Gdańsk and the Pomeranian Bay, to determine the infection rate of those amphipods in the natural environment. Under experimental condition the scuds were exposed to infection with *P. laevis* in two different arrangements. In treatment one, the amphipods were kept, from May to July, in the same tank with infected flounder. In treatment two, 197 scuds were exposed to eggs of *P. laevis*, taken from dissected female acanthocephalans.

Results. Scuds sampled from two areas of the Baltic Sea were not infected with *P. laevis*. Out of three *Gammarus* species cohabiting with infected flounder only *G. zaddachi* became infected. None of the scuds exposed directly to the eggs of the parasites became intermediate host of the acanthocephalan studied.

Conclusion. *Gammarus zaddachi* is probably an intermediate host for *Pomphorhynchus laevis* in the Baltic Sea.

Key words: fish, flounder, *Platichthys flesus*, parasite, *Pomphorhynchus laevis*, Acanthocephala, intermediate host, *Gammarus zaddachi*, Amphipoda, Baltic Sea

INTRODUCTION

A number of marine and freshwater fishes have been reported as definitive hosts of acanthocephalan *Pomphorhynchus laevis* (Zoega in Müller, 1776). The occurrence of this parasite in the waters of the Polish Exclusive Economical Zone of the Baltic Sea has been associated mainly with the flounder, *Platichthys flesus* (Linnaeus, 1758)

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(cf. Mulicki 1947, Sulgostowska et al. 1987, Sulgostowska and Styczyńska-Jurewicz 1996, Ziółkowska et al. 2000, Chibani et al. 2001).

In flounder, this parasite attaches in the terminal portion of the intestine, perforating its wall, with the proboscis. As a consequence of the perforation, the proboscis protrudes into the peritoneal cavity of the fish, while the rest of the body remains in the lumen of the intestine. Under conditions of very intensive infections, the worms may completely obstruct the patency of the intestine. In rare instances, single *P. laevis* can be found on the surface of the liver.

In freshwater bodies of the Great Britain the intermediate host of this acanthocephalan was identified as *Gammarus pulex* (cf. Hine and Kennedy 1974) and *G. duebeni* (cf. Kennedy et al. 1978). In France the freshwater intermediate hosts were *G. pulex* and *G. roeseli* (cf. Bauer et al. 2000), whereas in Italy—*Echinogammarus stammeri* (cf. Dezfuli et al. 2000). According to Jażdżewski (1975) *G. pulex* has been considered a collective species.

MATERIAL AND METHODS

The present paper was divided into three parts.

Part one: Natural infection of *Gammarus* spp. with larvae of *P. laevis* in the southern Baltic Sea.

The scuds were sampled from a site in the Gulf of Gdańsk, within the period of September 2001–August 2002 with the aid of a dredge towed by a fishing cutter. A total of 531 scuds were collected and they were later kept in aerated tanks, filled with sea water, taken from the sampling site. The amphipods were fed every 4 days with a feed for ornamental fishes. A total of 346 scuds were randomly selected for two infection experiments involving eggs of *P. laevis*. The remaining 185 *Gammarus* specimens were necropsied to determine their infection level with larvae of the acanthocephalan studied, which would reflect the infection level of scuds in the natural environment.

In August 2002, a total of 36 scuds were collected in the same way as before, from the Pomeranian Bay (fisheries sub-divisions J-4 and J-5). They were necropsied, following their identification up to species level.

Part two: infection of scuds with the larvae of *P. laevis* under laboratory conditions

Experiment one

Forty live flounder were collected in March 2002 from the fisheries base at Unieście. The fish were caught in fisheries sub-divisions J-4 and J-5. Ten randomly selected flounder were examined to prove their 100% infection with *P. laevis*. The remaining fish were transported in aerated tanks to a laboratory in Gdańsk. A total of nine fish arrived safely in good condition and they were placed in a 400-l, aerated tank with a constant water temperature of 10°C. Throughout the entire time-period of this experiment the fish were fed live shrimp, every 5 days, which also coincided with

the water change in the tank. In May 2002, a total of 149 specimens of *Gammarus* spp. caught in the Gulf of Gdańsk were placed in the tank with the flounder. The scuds were protected by a cubic steel-wire cage (15 × 15 × 15 cm). The cage was covered by a plastic net. The stretched mesh size was 1 mm. Such arrangement enabled undisturbed flow of water and acanthocephalan eggs and it also protected the scuds against flounder predation. This culture was continued until July 2002. Past this date, the flounder were necropsied to determine the level of their infection. The fish were measured and aged, based on their otoliths. After their identification up to species level, the scuds were dissected in search for *P. laevis* larvae.

Experiment two

A total of 218 mature females of *P. laevis* were recovered from 50 flounder caught in fisheries sub-divisions J-4 and J-5 in June 2002. The parasites were placed in a physiological solution in a petri dish, ruptured, and their eggs, containing acanthor were released. The dish was subsequently transferred to a brackish-water tank containing 197 scuds. The scuds were necropsied after 30 days, following their specific identification.

Part three: determining the specific composition of the flounder food. Thirty-two flounder were collected from the fisheries sub-divisions J-4 and J-5 and necropsied to determine their food in the natural environment.

RESULTS

Part one.

Among the 50 scuds collected from the Gulf of Gdańsk in the autumn-winter season and 135 in the summer, no one was infected with the larvae of *P. laevis*. In this number were 41 *G. salinus*, 52 *G. zaddachi*, and 92 *G. duebeni*. A similar result was obtained after examination of *G. salinus* from the fisheries sub-divisions J-4 and J-5.

Part two

Experiment one

After two-month-long cohabitation of scuds (*G. salinus*, *G. zaddachi*, and *G. duebeni*) and flounder harbouring the adults of *P. laevis*, only one species of the amphipods (*G. zaddachi*) became infected with the acanthocephalan (Table 1). The infected scuds hosted from 1 to 5 encysted larvae of *P. laevis* in their hemocoel. The infection experiment yielded a total of 60 specimens of *P. laevis*, representing various phases of development. The length of the smallest cyst was 488 µm, while its width was 126 µm. The dimensions of the larva inside the cyst were 177 and 66 µm, respectively (Fig. 1). Larger cysts contained larger larvae and the increment in size was associated with progressive complication of their structure. A cyst, which was 771 µm long and 246 µm wide, contained a larva measuring 721 µm in length and 136 µm in width. Observations of its structure revealed the presence of partly developed proboscis and primordial testes—the anterior- and posterior one (Fig. 2).

Table 1Results of infecting *Gammarus* spp. with *Pomphorhynchus laevis*

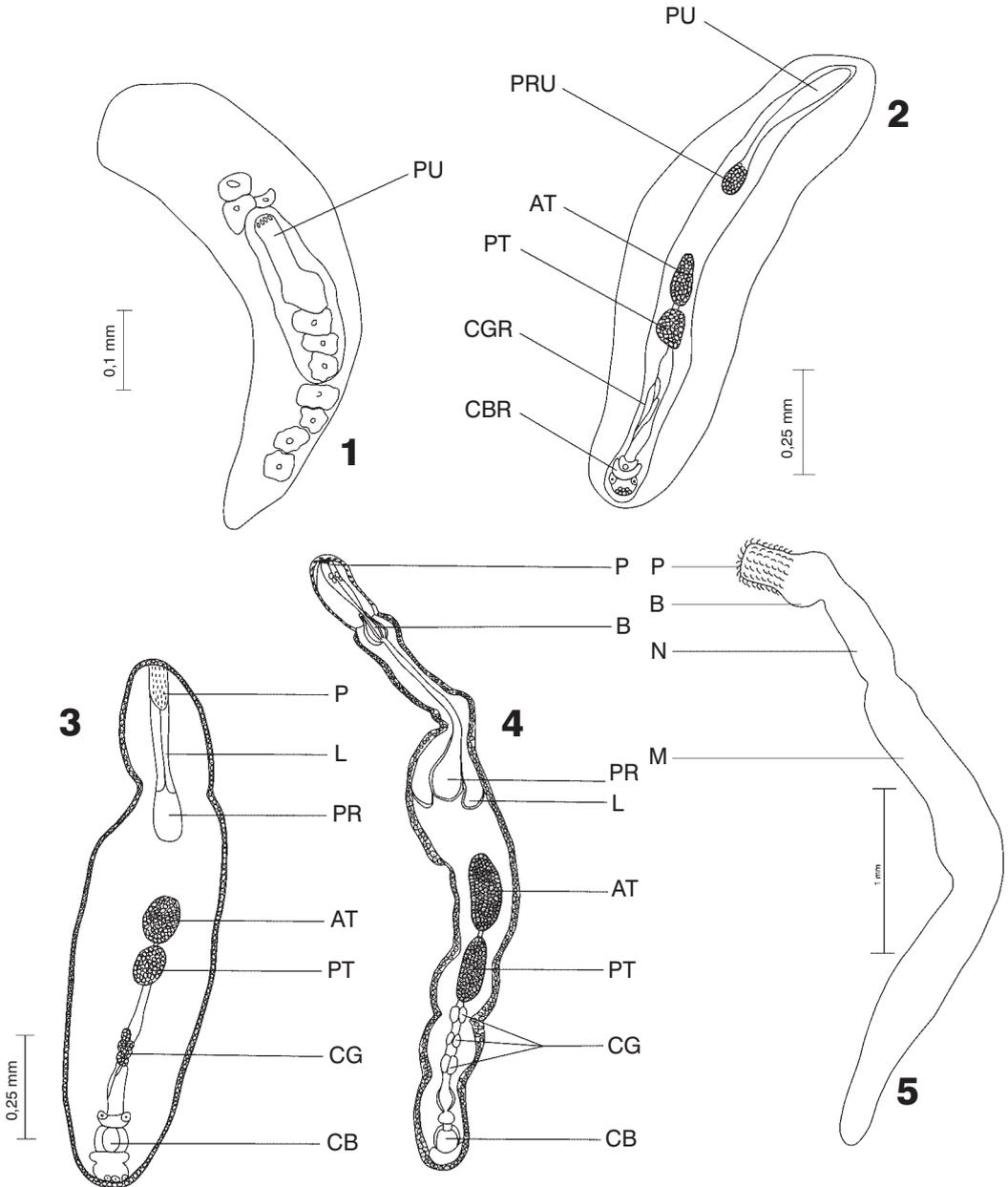
	<i>G. salinus</i>	<i>G. duebeni</i>	<i>G. zaddachi</i>
Number of examined specimens of <i>Gammarus</i> spp.	47	70	32
Number of infected specimens	0	0	32
Mean intensity of infection	—	—	1.9

The subsequent phases of development are marked by a gradual filling of the space inside the cyst by the body of *P. laevis* larva (Fig. 3). The length of such larvae ranged from 1085 to 1348 μm . At this stage the body of the larva was distinctly divided into the anterior part (presoma), consisting of the proboscis and the neck and the posterior one (metasoma) containing primordia of the reproductive system. The length of presoma was 278 μm , while its width reached 139 μm .

Further complication of the external morphology of the larva consisted in appearance of the characteristic widening of the neck forming a spherical bladder called bulbus. At the same time the proboscis structure became also more complex, showing the presence of hooks. The reproductive system developed further and the larva was much larger than those described above. Depending on the specimen it was 2061–3619 μm long, with the proboscis attaining 254–291 μm . The bulbus diameter was about 120 μm (Fig. 4).

The length of larva, the most advanced in its development, was 4015 μm . Its proboscis was 259 μm long and 182 μm wide. The diameter of the bulbus was 240 μm . The neck was 605 μm long and 240 μm wide and the metasoma was 360 μm wide. The proboscis was armed with 18 longitudinal rows of hooks, 12 hooks in each row (Fig. 5).

By the end of this experiment, all flounder were necropsied and all of them harboured adult forms of *P. laevis*. A total of 178 *P. laevis* were found (71 males and 107 females). The mean infection intensity of this parasite was 19.8. The maximum intensity reached 32. In addition to the acanthocephalans, the fish also hosted the following two nematode species in their intestines: *Cucullanus heterochrous* and *Hysterothylacium aduncum*. The livers contained third stage larvae of *H. aduncum*. Four fish showed symptoms of lymphocystis. The fish were 3- to 5-year-old and their length ranged from 22 to 34 cm.



Figs. 1–5. Larval stages of *Pomphorhynchus laevis*. Fig. 1. Early acanthella. Fig. 2. Early acanthella (male). Fig. 3. Acanthella (male). Fig. 4. Young cystacanth (male). Fig. 5. Cystacanth. AT, anterior testis; B, bulbus; CB, copulatory bursa; CBR, copulatory bursa rudiment; CG, cement gland(s); CGR, cement gland rudiment; L, lemniscus; M, metasoma; N, neck; P, proboscis; PR, proboscis receptacle; PRU, proboscis receptacle rudiment; PT, posterior testis; PU, proboscis rudiment

Experiment two

After 30 days of the experiment, no larvae of *P. laevis* were found inside the scuds exposed. All 197 scuds were identified as *G. duebeni*.

Part three

The diet of flounder caught in the fisheries sub-divisions J-4 and J-5 consisted mainly of blue mussel, *Mytilus edulis*, which was in most cases accompanied by *Gammarus salinus* and *Gammarus* sp. Less frequently found were *Macoma balthica*, *Mya arenaria*, *Crangon crangon*, and in one case—*Cardium glaucum*. Each of the flounder studied hosted specimens of *P. laevis*. The mean infection intensity was 31.4. The maximum value of the infection intensity was 101. Other endoparasites found were: acanthocephalan *Echinorhynchus gadi* and nematodes *Cucullanellus minutus*, *C. heterochrous*, and *H. aduncum*. The length of the flounder ranged from 20 to 27 cm and they were 3 to 5 years old.

DISCUSSION

In the course of the present study, no scuds infected with larvae of *P. laevis* were found in the natural environment. Under experimental conditions we were able to infect only one species—*G. zaddachi*.

Among the amphipods reported to be intermediate hosts of *P. laevis* in freshwater bodies were: *G. pulex* (cf. Hine and Kennedy 1974, Kennedy 1984, Kennedy 1996, Siddal and Sures 1998, Cézilly et al. 2000), *G. pulex* and *G. roeseli* (cf. Bauer et al. 2000), *G. pulex* and *G. duebeni* (cf. Kennedy et al. 1978), and *Echinogammarus stammeri* (cf. Maynard et al. 1998, Dezfuli et al. 2000). On the other hand, Kennedy (1996) was not able to find *P. laevis* larvae in freshwater *G. zaddachi*. This problem can be solved only after detailed studies on the biology of the intermediate- and final hosts and the parasite itself, because the successful transmission and development of *P. laevis* depends on their reciprocal relations.

A number of authors who studied this acanthocephalan paid attention to differences between *Pomphorhynchus* specimens acquired from marine environment with those from freshwater. Lundström (1942) and Kennedy (1984) believed that saltwater- and freshwater forms are different, though difficult to differentiate. Engelbrecht (1957) identified the specimens of *Pomphorhynchus* recovered by him from the Baltic Sea as *P. laevis* forma *tereticollis*, whereas Gibson (1972) reported acanthocephalans from the North Sea simply as *Pomphorhynchus* sp. If we assume that the two forms of this acanthocephalan represent separate sub-populations it is possible that two different environments affect the process of intermediate hosts selection by *P. laevis*. Another piece of evidence to support the above hypothesis is the fact that presently described attempts of infecting *G. duebeni* in brackish-water environment failed, while this amphipod, collected from a river, harboured larvae of *P. laevis* (cf. Kennedy et al. 1978).

It cannot be concluded, based on the above-mentioned data, that *P. laevis* is a non-specific parasite capable of infecting different species of the genus *Gammarus*

(cf. Bauer et al. 2000). On the contrary, it is likely that different populations of *P. laevis* select a defined scud species, which guarantees them a successful completion of their life cycle. *P. laevis* is able to affect the behaviour of its intermediate hosts, which is a proof of its narrow specificity. The invertebrates hosting larvae of this acanthocephalan demonstrated an increased activity, positive reaction to light (swimming towards the light source), and drifting. Non-infected scuds exhibited the opposite reactions (Maynard et al. 1998, Cézilly et al. 2000, Dezfuli et al. 2000). Such behaviour of those invertebrates certainly increases the chances of transmission of *P. laevis*. It is easier for a fish to notice (and catch) a scud, intensively moving in the water column, rather than that penetrating the bottom. The behavioural changes of the infected amphipods can explain the high prevalence of *P. laevis* in flounder, despite the failure in finding the infected scuds in the bottom samples. It is presently suggested that the infection rate of *Gammarus* spp. with *P. laevis* in the Baltic Sea is low. Sulgostowska and Vojtková (1992) in their parasitological survey of 863 specimens of *Gammarus* spp. from the Gulf of Gdańsk and the Bay of Puck, were not able to find even a single scud infected with *P. laevis*. A similar result obtained Voigt (1991) who studied parasite fauna of scuds from the eastern Baltic Sea.

The chances of infection of flounder with this parasite can also be affected by migrations of this fish. In late autumn they migrate from the shore towards the closest depth, where they spawn in spring and after that they came back to the coastal feeding grounds (Cięglewicz 1947). Their diet changes, on their route, which is associated with the distribution of invertebrates on the sea floor. According to Mulicki (1947) the food composition of flounder depends on the living area. During their regular migrations in the sea they may encounter a population of *G. zaddachi*, which is infected with larvae of *P. laevis*. They may also infect the scuds with eggs of *P. laevis*, which established themselves in the flounder intestines in the previous year.

CONCLUSION

The results of the present study suggest that *Gammarus zaddachi* is probably an intermediate host for *Pomphorhynchus laevis* in the Baltic Sea.

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