Some new data on the intermediate and paratenic hosts of the nematode *Anguillicola crassus* Kuwahara, Niimi et Itagaki, 1974 (Dracunculoidea), a swimbladder parasite of eels

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Abstract. Three species of planktonic crustaceans, *Cyclops strenus* and *Macrocyclops albidas* (Copepoda) and *Notodromas monacha* (Ostracoda), were experimentally infected with the eggs and second-stage larvae of the swimbladder nematode *Anguillicola crassus* originating from eels from Neusiedler Lake in Austria. At 20-22°C, third-stage larvae of the parasite developed in all these invertebrate hosts within 16-20 days p.i. Ostracods harbouring the nematode third-stage larvae (33 days p.i.) were fed to small eels (*Anguilla anguilla*), while infected copepods (20 days p.i.) to seven other fish species. By these experiments, the larvae from ostracods proved to be infective for the definitive host and the ostracod was thus confirmed as a true intermediate host of *Anguillicola crassus*. *Notodromas monacha* represents a new experimental intermediate host of *A. crassus* and the second known invertebrate other than a copepod in which the larval development of this nematode up to the infective stage takes place. Five species of fish, *Cyprinidae* *Tinca tinca*, *Alburnus alburnus*, *Gobio gobio* and *Alburnoides bipunctatus* (the latter representing a new host record), and guppy, *Poecilia reticulata*, were found to serve as experimental paratenic hosts for *A. crassus*, in which the live nematode infective larvae were recorded 49 days p.i.

At present the nematode genus *Anguillicola* Yamaguti, 1935 comprises five species of pathogenic swimbladder parasites of eels, originally distributed in East Asia, Africa, Australia and New Zealand (*Moravec* and *Taraschewski* 1988). No species of this genus occurred in Europe until two species, *Anguillicola crassus* Kuwahara, Niimi et Itagaki, 1974 from East Asia and *A. novaezelandiae* Moravec et Taraschewski, 1988 from New Zealand were introduced there some ten years ago. While the distribution of *A. novaezelandiae* in Europe remains restricted to one lake (L. Bracciano) in Italy, *A. crassus* has quickly spread among eel populations in many European and North African countries (see *Moravec* 1992).


MATERIALS AND METHODS

Gravid females of *Anguillicola crassus* with eggs containing motile second-stage larvae were recovered from the swimbladder of eels, *Anguilla anguilla* (L.), caught in Neusiedler Lake in Austria in September 1992. The live nematodes removed from the host's body as well as eel swimbladders containing large numbers of *Anguillicola* eggs were placed in plastic vials containing physiological saline. Then the vials cooled with ice were transported to the laboratory of the Institute of Parasitology, ASCR, in České Budějovice (Czech Republic), where the experiments were made. After transferring the nematode females to petri dishes filled with water, they readily laid their larvated eggs. Feeding experiments were made in petri dishes (diameter 16-19 cm) filled with water, each containing a few hundreds of copepods or ostracods to which always about one thousand of parasite's larvated eggs and free second-stage larvae were added. The copepod *Cyclops strenus* Fischer and the ostracod *Notodromas monacha* Müller originating from a pond and a periodical pool, respectively, near České Budějovice, South Bohemia, and *Macrocyclops albidas* (Jurine) from a laboratory culture, were used as experimental intermediate hosts. These were kept at the laboratory temperatures of 20-22°C and were examined for the presence of *Anguillicola* larvae on every second day. The remaining crustaceans were used in subsequent experiments.

Feeding experiments with fishes were carried out in small aquaria in laboratory where fishes were fed spontaneously either the infected copepods (20 days p.i.) or ostracods (33 days p.i.)
(exposure of a few tens of crustaceans per fish). Afterwards, the fishes were fed with tubificids and commercial pellet food. The following fishes were used: 2 eels, Anguilla anguilla (L.), (body length 9-13 cm) originating from Italy and kept at low temperature (10°C) in the laboratory in České Budějovice for one year; 5 carps, Cyprinus carpio L., (7-8 cm), 5 tenches, Tinca tinca (L.), (7-8 cm) and 5 barbels, Barbus barbus (L.), (6-7 cm), all from fish nursery; 4 bleak, Alburnus alburnus (L.), (5-8 cm), 2 spirlins, Alburnoides bipunctatus (Bloch), (4 cm) and 1 gudgeon, Gobio gobio (L.), (5 cm) from the Rokytná River, South Moravia, where Anguillicola does not occur; and 5 guppies, Poecilia reticulata (Peters), (4-5 cm) from a laboratory culture. The nematode larvae were fixed in hot 4% formaldehyde.

RESULTS

Experimental infections of intermediate hosts

Both copepod species employed in experiments, Cyclops strenus and Macrocylops albidos, as well as the ostracod Notodromas monacha were successfully infected with the Anguillicola crassus eggs containing motile second-stage larvae and free second-stage larvae. There was observed a high degree of mortality of intermediate host crustaceans, particularly of copepods, during the first two days after infection. The rate of the development of nematode larvae was similar in all three intermediate host species and similar to that observed by Moravec et al. (1993) in conspecific larvae in copepods. After two weeks, approximately 60% of surviving copepods of both species and about 40% of ostracods were infected with the usual intensity 1-3 Anguillicola larvae per crustacean. While all larvae recorded 14 days p.i. were still inside the loosened cuticle from the second moult, the third-stage larvae already liberated from this cuticular sheath were first observed on day 16 p.i. By day 20 p.i., all third-stage larvae were fully developed and liberated from the old cuticle and, at that time, those from copepods were used for feeding experiments with fishes. The morphology and measurements of infective third-stage larvae from both copepods and ostracods were the same as observed by Moravec et al. (1993). The larvae in ostracods were followed up to 33rd day p.i., but no morphological or biometrical differences compared to those from copepods were found in them (for measurements see Table 1). At that time, only about 20% of remaining ostracods harboured the nematode larvae (intensity 1-2 larvae) in their body cavity (in dorsal part of body) where these were, similar to those in copepods, freely coiled, without any encapsulation; these ostracods were used for feeding experiments with small eels.

Experimental infection of the definitive host

In order to prove that the Anguillicola crassus third-stage larvae from ostracods are actually infective for the definitive host, it was necessary to make a feeding experiment with eels. However, due to a limited number of infected ostracods available (33 days p.i.), these could be fed to only two small eels. The first experimental eel was examined 4 days p.i. and 2 free A. crassus third-stage larvae were found in its abdominal cavity; the larvae were both morphologically and biometrically identical with those from ostracods. The second eel died on 33rd day p.i., but no Anguillicola specimens were found in it.

Experimental infections of paratenic hosts

A total of twenty nine fishes belonging to seven species of the families Cyprinidae and Poeciliidae were tested as potential paratenic hosts of Anguillicola crassus. A survey of these feeding experiments is given in Table 2.

Of the fish species used in our experiments, only carp (C. carpio) and barbel (B. barbus) were found unaffected; all other species proved to be suitable paratenic hosts for this nematode parasite. The experiments showed that after the ingestion of infected intermediate host by fish, the third-stage larvae of A. crassus penetrate into the body cavity of the fish host where they can survive unencapsulated for a period of at least two months; the larvae were frequently found near the host’s swimbladder. The larvae localized on the surface of the intestine were usually

Table 1. Measurements of a few Anguillicola crassus third-stage larvae from the ostracod intermediate host (33 days p. i.) and from experimental fishes (49 days p. i.) (in mm).

<table>
<thead>
<tr>
<th>Host</th>
<th>Notodromas monacha</th>
<th>Tinca tinca</th>
<th>Alburnus alburnus</th>
<th>Gobio gobio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body length</td>
<td>1.020</td>
<td>1.006</td>
<td>0.979-1.088</td>
<td>0.993</td>
</tr>
<tr>
<td>Body width</td>
<td>0.036</td>
<td>0.041</td>
<td>0.039-0.041</td>
<td>0.041</td>
</tr>
<tr>
<td>Length of oesophagus</td>
<td>0.300</td>
<td>0.286</td>
<td>0.264-0.288</td>
<td>0.273</td>
</tr>
<tr>
<td>Maximum width of oesophagus</td>
<td>0.024</td>
<td>0.021</td>
<td>0.024</td>
<td>0.024</td>
</tr>
<tr>
<td>Distance of nerve ring</td>
<td>0.087</td>
<td>0.129</td>
<td>0.117-0.129</td>
<td>0.117</td>
</tr>
<tr>
<td>Distance of excretory pore</td>
<td>0.132</td>
<td>0.171</td>
<td>0.165-0.168</td>
<td>0.165</td>
</tr>
<tr>
<td>Cephalic sclerotized apparatus</td>
<td>- length</td>
<td>0.009</td>
<td>0.009</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>- width</td>
<td>0.015</td>
<td>0.015</td>
<td>0.015</td>
</tr>
<tr>
<td>Genital primordium from posterior extremity</td>
<td>0.366</td>
<td>0.384</td>
<td>0.362-0.371</td>
<td>0.345</td>
</tr>
<tr>
<td>Length of tail</td>
<td>0.087</td>
<td>0.093</td>
<td>0.096-0.105</td>
<td>0.096</td>
</tr>
</tbody>
</table>

66
found encapsulated (Fig. 1), covered by a thin connective layer apparently produced by host’s tissues; the encapsulated larvae were often found dead (Fig. 2), sometimes with already decomposing body. No larvae were found in the lumen or walls of the host’s swimbladder.

The morphology and measurements of all A. crassus third-stage larvae recorded from fish paratenic hosts were almost identical with those from intermediate hosts (Table 1). It indicates that no development or growth occur in these larvae in the paratenic hosts tested.

### DISCUSSION


<table>
<thead>
<tr>
<th>Fish host</th>
<th>No. of fish in experiment</th>
<th>No. of fish infected</th>
<th>Intensity</th>
<th>Localization</th>
<th>Examination of fish (days p. i.)</th>
<th>Intermediate host used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anguilla anguilla</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>body cavity</td>
<td>4</td>
<td>Notodromas monacha</td>
</tr>
<tr>
<td>Cyprinus carpio</td>
<td>5</td>
<td>0</td>
<td>-</td>
<td></td>
<td>49</td>
<td>Cyclops strenuus</td>
</tr>
<tr>
<td>Tinca tinca</td>
<td>5</td>
<td>2</td>
<td>2-3</td>
<td>2 free larvae in body cavity, and 3 dead larvae on gut surface</td>
<td>49</td>
<td>Cyclops strenuus</td>
</tr>
<tr>
<td>Barbus barbus</td>
<td>5</td>
<td>0</td>
<td>-</td>
<td></td>
<td>49</td>
<td>Cyclops strenuus</td>
</tr>
<tr>
<td>Alburnus alburnus</td>
<td>4</td>
<td>1</td>
<td>6</td>
<td>3 free larvae in body cavity, and 3 dead larvae on gut surface</td>
<td>49</td>
<td>Macrocyclops albidus</td>
</tr>
<tr>
<td>Alburnoides bipunctatus</td>
<td>2</td>
<td>1</td>
<td>7</td>
<td>5 free larvae on surface of swimbladder and in body cavity, and 2 larvae encapsulated on gut surface</td>
<td>49</td>
<td>Macrocyclops albidus</td>
</tr>
<tr>
<td>Gobio gobio</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>2 free larvae in body cavity, and 2 larvae encapsulated on gut surface</td>
<td>49</td>
<td>Macrocyclops albidus</td>
</tr>
<tr>
<td>Poeclilla reticulata</td>
<td>5</td>
<td>2</td>
<td>1-4</td>
<td>3 free larvae in body cavity, and 2 larvae encapsulated on gut surface</td>
<td>19; 49</td>
<td>Macrocyclops albidus</td>
</tr>
</tbody>
</table>
Figs. 1, 2. Third-stage larvae of *Anguillicola crassus* on the intestinal surface of experimentally infected fish paratenic hosts (fresh mounts). **Fig. 1.** Encapsulated larva on intestinal surface of sprat, *Alburnoides bipunctatus*, on day 49 p.i. (x 200). **Fig. 2.** Dead larva on intestinal surface of tench, *Tinca tinca*, on day 49 p.i. (x 250).

(Copepoda, Calanoidea) could migrate to the haemocoele but were not found there 24 hours post infection. *Kennedy* and *Fitch* (1990) report that they successfully infected juvenile *Gammarus pulex* (Amphipoda) and even a single specimen of the brackish-water *Eurytemora affinis* (Calanoidea) with *A. crassus* larvae. But it was not confirmed that the parasite larvae could develop to the third stage in these hosts and that the larvae were then capable of infecting eels.

Experimental results presented in this paper show clearly that *Notodiromas monacha* is another ostracod species which can be easily infected with *A. crassus* and that the parasite larvae develop in it up to the third stage, becoming thus infective for the definitive host (eel). Consequently, *N. monacha* can be considered a true intermediate host of *A. crassus*. Although *Pettet et al.* (1990) observed the third-stage larvae of *A. crassus* in *Cypria ophthalmitica* as early as on day 6 p.i. at 18-29°C, we found the fully developed, already unsheathed third-stage larvae in *N. monacha*, as well as in copepods, kept at 20-22°C, only 16 days p.i. The results obtained by French authors (Pettet et al. 1990, Bonneau et al. 1991) as well as those presented in this paper suggest that, in addition to copepods, the ostracods may also play an important role as the intermediate hosts of *Anguillicola crassus*, being the source of infection for both the definitive host (eels) and paratenic hosts (prey fish).

The life-history of *Anguillicola crassus* includes an obligate crustacean intermediate host, but small fish may act as paratenic hosts, in which the infective third-stage larvae remain alive and keep their capability to infect the definitive host. Such paratenic hosts of *A. crassus* were found both in experimental conditions (Lebistes reticulatus (= Poecilia reticulata), Cyprinus carpio, Leuciscus idus - see Pettet et al. 1989; De Charleroy et al. 1990; Thomas and Ollevier 1992) and in the natural environment in Belgium, Netherlands, France and Sweden (Osmerus eperlanus, Cyprinus carpio, Phoxinus phoxinus, Gobio gobio, Leuciscus cephalus, L. leuciscus, L. idus, Scardinius erythrophthalmus, Rutilus rutilus, Chondrostoma nasus, Alburnus alburnus, Tinca tinca, Ictalurus nebulosus, Perca fluviatilis, Stizostedion lucioperca, Gymnocephalus cernuus, Lepomis gibbosus, Oreochromis niloticus, Gasterosteus aculeatus, Pungitius pungitius - see Belpaire et al. 1989; Haenen and van Banning 1990, 1991; De Charleroy et al. 1990; Blanc et al. 1992; Höglund and Thomas 1992; Thomas and Ollevier 1992).

In addition to the above fish species, *Alburnoides bipunctatus* also can serve as a paratenic host for *A. crassus*, as revealed by our experiments; the other three experimentally infected species, *Alburnus alburnus, Gobio gobio* and *Tinca tinca*, have already been recorded as the natural intermediate hosts of *A. crassus* in Belgium by Thomas and Ollevier (1992). The hitherto
records of *A. crassus* larvae from 23 species of fishes serving as paratenic hosts and belonging to 9 families (Osmeridae, Cyprinidae, Ictaluridae, Pocellidae, Percidae, Cichlidae, Centrarchidae, Gasterosteidae and Gobiidae) suggest that there is only a low degree of host specificity in *A. crassus* at the level of paratenic hosts. This presumption is supported by recent information of Dr. K. Molnár (pers. comm.) that almost all small-sized fishes of different species occurring in Lake Balaton in Hungary harbour *A. crassus* infective larvae.

In our experiments, the third-stage larvae of *A. crassus* mostly remained in the body cavity of fish paratenic hosts, not penetrating into their swimbladder, where they remained free (unencapsulated) and viable for at least two months. Some individuals could also be found on the outer surface of the swimbladder. Similar observations were made by De Charleroy et al. (1990) with experimentally infected carp (*C. carpio*) and ide (*L. idas*) in which *A. crassus* third-stage larvae remained free in the body cavity of all fish examined 15 and 60 days p.i. Petter et al. (1989) mention that in guppies (*P. reticulata*) *A. crassus* third-stage larvae penetrate through the digestive tract into the body cavity of fish and after a month, most of the larvae were alive, surrounded by a thick fibrous sheath. In our experiments, usually the larvae localized on the external intestinal surface were found to be encapsulated inside a thin-walled envelope produced by host's tissues, being usually less motile than free larvae from the body cavity and, sometimes, these were even dead or destroyed by the host's reaction. No morphological or biometrical development of third-stage larvae was observed in the fish paratenic hosts under study.

In contrast to the above experimental observations, De Charleroy et al. (1990) found the third-stage larvae of *A. crassus* in carp (*C. carpio*) samples taken from Belgian ponds and rivers both in the body cavity and in the swimbladder. In perch (*P. fluviatilis*) and pumpkinseed (*L. gibbosus*) from field samples taken in Belgium, third- and fourth-stage larvae and even preadult stages of *A. crassus* were found in the swimbladder (De Charleroy et al. 1990, Thomas and Ollevier 1992). Belpaire et al. (1989) found 3-spined sticklebacks (*G. aculeatus*) infected with *A. crassus* larvae in the swimbladder wall in the Yser River, Belgium.

These data suggest that there are distinct differences in the forms of paratenic parasitism among the individual fish species serving as paratenic hosts of *A. crassus*: While most fish species, paratenic hosts of this nematode (e.g., all cyprinids or guppy), may be assigned to the category of the so called euparatenic (astadiogenous) hosts (see e.g., Odening 1976, Moravec 1984), *Perca fluviatilis, Lepomis gibbosus* and possibly *Gasterosteus aculeatus* can be evaluated as metaparatenic (stadiogenus) hosts or even paradogetic hosts in the concept and terminology of Odening (1976). Undoubtedly, all these facultative hosts may play an important role in the transmission of *Anguillicola crassus* in eel populations. The capability of *A. crassus* third-stage larvae from naturally infected paratenic hosts (smelt, ruffe, black goby) to infect eels was experimentally demonstrated by Haenen and van Banning (1991), Höglund and Thomas (1992) and Thomas and Ollevier (1992).

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