Vol. 66: 171–173, 2005 | DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org | Published September 5

NOTE

First record of ostracods as natural intermediate hosts of *Anguillicola crassus*, a pathogenic swimbladder parasite of eels *Anguilla* spp.

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ABSTRACT: The ostracod *Physocypria nipponica* (Ostracoda: Candonidae) was found (prevalence 14.2%) to be the only intermediate host of the nematode *Anguillicola crassus* (Nematoda: Anguillicolidae), a pathogenic swimbladder parasite of eels, in a greenhouse-heated culture pond at Isshiki, Aichi Prefecture, Japan. Japanese eels *Anguilla japonica* from the same pond were found to be infected by adult *A. crassus* (prevalence 71.8%, intensity 1 to 6). This indicates that *A. crassus* could complete its life cycle under conditions of modern eel-culture technology where copepods were absent due to the unfavorable water quality for them, by utilizing ostracods as the intermediate host.

KEY WORDS: Ostracoda · *Physocypria nipponica* · Intermediate host · Parasitic nematode · *Anguillicola crassus* · Japanese eel · *Anguilla japonica* · Japan

INTRODUCTION

The nematode *Anguillicola crassus* (Nematoda: Anguillicolidae) is a widely distributed swimbladder parasite of eels, which was originally described from the cultured Japanese eel *Anguilla japonica* Temminck et Schlegel and the introduced European eel *Anguilla anguilla* (L.) in Japan (Kuwahara et al. 1974), and later it was also reported from both cultured and wild eels from China and Korea (see Nagasawa et al. 1994). In the 1980s, the nematode was introduced from East Asia into Europe, where it quickly spread in many countries (e.g. Peters & Hartmann 1986, Moravec 1992), causing serious problems in eel farms and wild eel populations. This highly pathogenic parasite was later also introduced into North Africa (Egypt, Morocco), where it infects *A. anguilla* (El Hilali et al. 1996, H. Koops pers. comm.), and into North America, occurring there in the American eel *Anguilla rostrata* (Lesueur) (Johnson et al. 1995, Barse et al. 2001). Mass mortalities of eels due to *A. crassus* infection have been described, for example, in Lake Balaton in Hungary (Molnár et al. 1991), Vranov and Orlík dam lakes in the Czech Republic (Baruš 1994, Moravec 1997) and in eel aquaculture farms in Taiwan (Ooi et al. 1996).

The life cycle of *Anguillicola crassus* was studied experimentally by many authors in both East Asia and Europe (e.g. Hirose et al. 1976, Egusa 1979, De Charleroy et al. 1987, 1990, Kim et al. 1989, Petter et al. 1989, 1990, Moravec et al. 1993). A total of 17 species of copepod (Copepoda) have been found to serve as

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experimental or natural intermediate hosts for this parasite, in the haemocoel of which the nematode third-stage larva develops within several days; this is already infective for the fish host.

The infected copepods are generally considered to be the main source of *Anguillicola crassus* infection for both cultured and wild eels, although different aquatic invertebrates (snails, insect larvae) and vertebrates (fishes, amphibians) may serve as paratenic hosts (e.g. Petter et al. 1989, De Charleroy et al. 1990, Moravec 1996, Moravec & Škoríková 1998). In Japan, Hirose et al. (1976) found the copepod *Eucyclops serrulatus* to be a suitable experimental intermediate host of *A. crassus*, whereas *Eucyclops euacanthus* and *Thermocyclops hyalinus* were found as natural intermediate hosts in the pond of cultured American eels infected with *A. crassus* in Taiwan (Ooi et al. 1997) and in the irrigation canal near fish farms in Korea (prevalence 3.6%, intensity 1 to 2 larvae) (Kim et al. 1989), respectively.

However, Petter et al. (1990) and Moravec & Konecny (1994) found that the European ostracods *Cypria ophthalmica* and *Notodromas monacha* may serve, in addition to copepods, as suitable intermediate hosts of *Anguillicola crassus* under experimental conditions; however, no ostracods have so far been reported as natural intermediate hosts of this nematode.

MATERIALS AND METHODS

On 21 June 1995, zooplankton sampling was carried out with a hand-net in a greenhouse-heated culture pond (660 m^2, 0.9 m depth, water temperature 27 to 28°C) at Isshiki (44° 48′ N, 137° 02′ E), Aichi Prefecture, one of the most productive eel culture areas in Japan. The sample was fixed in 5% formaldehyde solution, brought to the laboratory, and examined for the presence of *Anguillicola crassus* larvae. Japanese eels (48 to 54 cm total length) were also sampled from the same pond and examined for *A. crassus*.

RESULTS AND DISCUSSION

The zooplankton sample consisted of only 1 ostracod species, *Physocypria nipponica* Okubo, 1990 (Candonidae: Cyclocyprininae). A total of 33 out of 232 *P. nipponica* (prevalence 14.2%) were found to be infected by third-stage larvae of *Anguillicola crassus* (Fig. 1), with an intensity from 1 to 2 (mean 1.2) larvae per ostracod. Of 110 Japanese eels *Anguilla japonica* examined, 79 (prevalence 71.8%) were infected with 1 to 6 (mean 1.3) *A. crassus*; gravid females with larvated eggs in uteri were present.

The third-stage larvae of *Anguillicola crassus* (10 specimens measured) from ostracods (Fig. 2) were 728 to 1012 µm long and had a 32 to 36 µm maximum width. The cephalic end was rounded, the mouth was provided with 2 tiny lateral sclerotized teeth; each was followed posteriorly by a sclerotized apparatus situated at the level of the anterior end of oesophagus, which appeared as bifurcate in lateral view; this apparatus was 8 to 10 µm long and 12 to 16 µm wide. The oesophagus was 204 to 276 µm long and had a 26 to 32 µm maximum width. The nerve ring and the excretory pore were 48 to 72 and 78 to 92 µm, respectively, from the anterior extremity. The intestine contained numerous brownish granulae. The tail was conical, 36 to 68 µm long, with a small spike at the tip.

The present record of ostracods as natural intermediate hosts of *Anguillicola crassus* shows that this pathogenic parasite is capable of reproducing even under the conditions of modern fish culture technology, where copepods may be absent due to the bad quality of water in which, however, ostracods survive and become the principle source of *A. crassus* infection for the cultured eels.

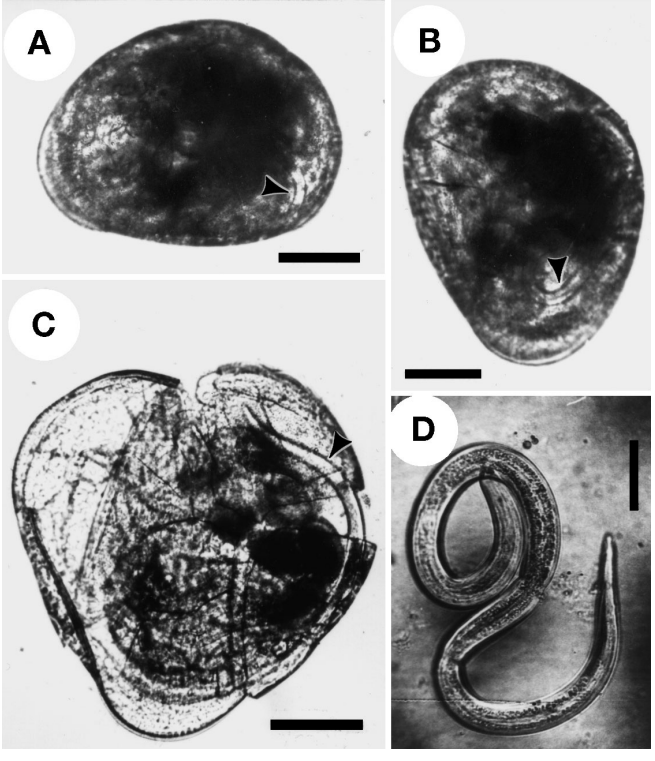

Fig. 1. *Physocypria nipponica* infected with *Anguillicola crassus* third-stage larvae. (A,B) Specimens harboring the larva (arrowheads). (C) Broken specimen containing the larva (arrowhead). (D) Larva expressed from the body of the ostracod (native). Scale bars: A to C = 200 µm; D = 70 µm

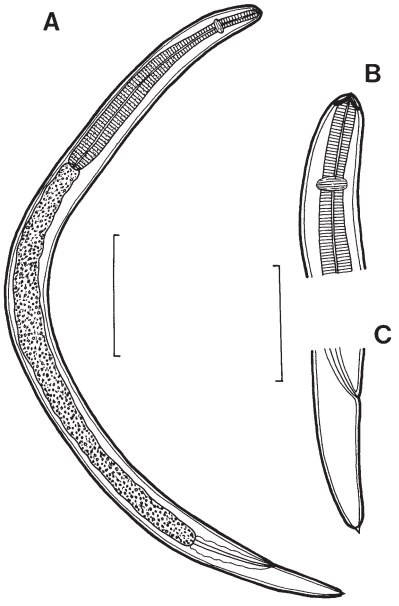

Fig. 2. *Anguillicola crassus* third-stage larva from the haemocoel of *Physocypria nipponica*. (A) General view. (B,C) Anterior and posterior ends, respectively, lateral views. Scale bars: A = 100 µm; B,C = 50 µm

Acknowledgements. The authors thank Dr. Ichiro Okubo, Shujitsu Joshi University, who kindly identified the ostracods. Thanks are also due to Mrs I. Husáková, Institute of Parasitology, Academy of Sciences of the Czech Republic (ASCR), for her help with the preparation of illustrations. This study was supported by Grant No. 524/03/0061 from the Grant Agency of the Czech Republic and the research project of the Institute of Parasitology, ASCR (No. Z60220518).

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Editorial responsibility: Wolfgang Körting, Hannover, Germany

Submitted: February 16, 2005; Accepted: May 31, 2005
Proofs received from author(s): August 23, 2005